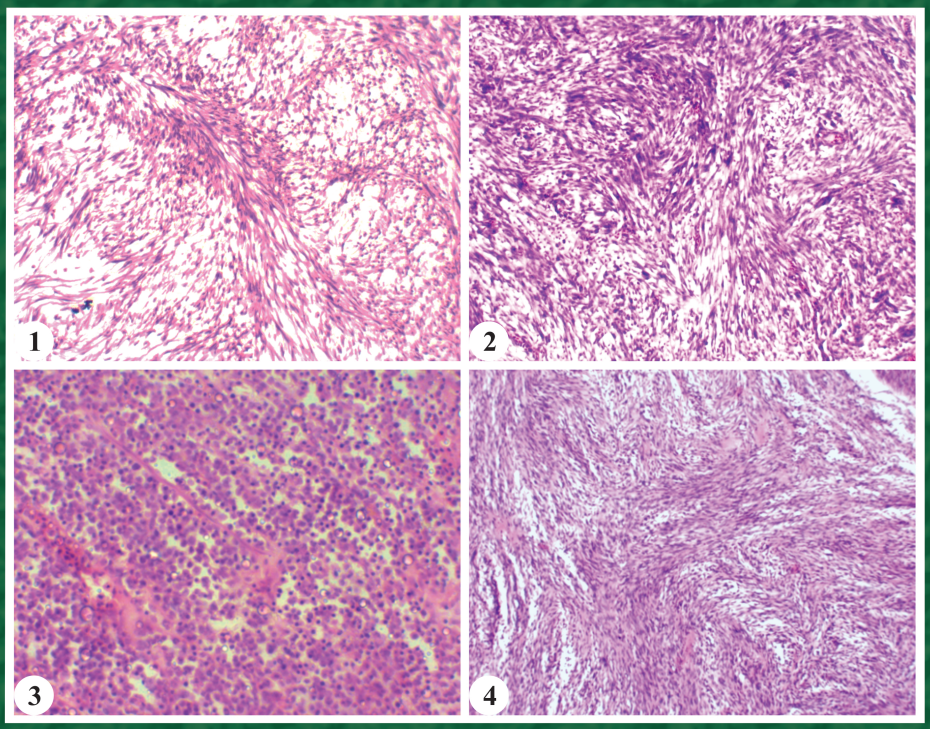
Vol.: 48(3)
September, 2024
ISSN: 0250-4758
Online ISSN: 0973-970X

IJVP-2024

INDIAN JOURNAL OF VETERINARY PATHOLOGY



INDIAN ASSOCIATION OF VETERINARY PATHOLOGISTS
(Registered under article 21 of Societies Act 1860)

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Vol. 48 (3)
September, 2024
ISSN: 0250-4758

INDIAN JOURNAL OF VETERINARY PATHOLOGY

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INDIAN JOURNAL OF VETERINARY PATHOLOGY

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INDIAN JOURNAL OF VETERINARY PATHOLOGY

INDIAN ASSOCIATION OF VETERINARY PATHOLOGISTS
(Estd. 1974)

Cover Page Photo (Clockwise) : Cutaneous neoplasm in avian species showing fibroma with loosely arranged fibroblasts running in different directions, fibrosarcoma having criss-cross pattern of fibroblasts with hyper chromatic nuclei, neurofibroma with collagen strands amidst myxoid material and lymphoma with uniform sheet of small lymphocytes having scanty cytoplasm.

IAVP Golden Jubilee (1974-2024)

Glorius Fifty Years of Indian Association of Veterinary Pathologists (1974-2024): A Brief Overview

PROF. R. SOMVANSHI
Former President, IAVP

Retired Head, Division of Pathology, Former Acting Joint Director, CADRAD, Ex. ICAR-Emeritus Scientist, Emeritus Professor, Ex. National Fellow, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly-243 122 Uttar Pradesh; E-mail: dr.rsomvanshi@gmail.com

On March, 1974, the Indian Association of Veterinary Pathologists, (IAVP) was established under the Chairmanship of Dr S. Damodaran, Professor and Head, Department of Pathology, Veterinary College, Madras, during a scientific get together of 37 eminent Veterinary Pathologists at the Division of Pathology, Indian Veterinary Research Institute, Izatnagar, UP. The association soon framed its constitution and extended the drive for membership amongst Veterinary Pathologists from different Veterinary and Animal Husbandry institutions and other laboratories. The assembly of pathologists in the form of Annual Conventions and Symposia on the current problems in Veterinary Science was first held at Veterinary College, Bangalore in 1983 but became an annual feature only after 1986.

Objectives

The initial objectives of IAVP laid were: (i) Cooperation for advancement of Veterinary Pathology, (ii) Publishing Indian Journal of Veterinary Pathology, (iii) Organizing scientific meetings, seminar, symposium, conferences, exhibitions and get together, (iv) Exchanging scientific information among members, (v) Attainment and dissemination of knowledge and (vi) To encourage young pathologists.

With growth of association objectives were later re-defined. The main objectives of the association are advancement and dissemination of knowledge of pathology. However, to achieve them with distinction, emphasis is largely laid upon:

1. Attainment and dissemination of knowledge and to encourage young pathologists for establishing their professional skills more vividly.
2. To organize annual conventions and symposia for encouraging the academic pursuits of pathologists by arranging presentation of research papers and invited lectures from pathologists and other scientists of eminence.
3. To encourage the members for their research aptitude and academic pursuit, the Association also publishes a journal Indian Journal of Veterinary Pathology.

Office Bearers (1974-2024)

Till date the association was led by following eminent Veterinary Pathologists:

Presidents

• Dr S. Damodaran	1974-1975*	• Dr R.N.S. Gowda	2004-2005
• Dr B.S. Rajya	1976-1979*	• Dr Lal Krishna	2006-2011
• Dr Balwant Singh	1980-1982	• Dr B. Murali Manohar	2012**
• Dr B.S. Rajya	1983-1986	• Dr R. Somvanshi	2013-2019
• Dr M.K. Nair	1987-1993	• Dr B.N. Tripathi	2020-Cont.
• Dr G.C. Mohanty	1994-1997	*Initially term Chairman was used	
• Dr J.L. Vegad	1997-2003	**Resigned	

Vice Presidents

- Dr P.K.R. Iyer 1980-1982
- Dr M. Krishnan Nair 1983-1986
- Dr S.J. Sheshadri 1987-1993
- Dr J.L. Vegad 1994-1996
  Dr Gopal Yadgir
- Dr A.T. Rao 1997-1998
  Dr O.P. Paliwal
- Dr Lal Krishna 1999-2000
  Dr P.P. Gupta
- Dr O.P. Paliwal 2001-2003
  Dr P.P. Gupta
- Dr P.S. Lonkar 2003-2005
  Dr P.P. Gupta
- Dr P.L. Kaul 2006-2007
  Dr B. Murali Manohar
- Dr B. Murali Manohar 2008-2011
  Dr R.S. Chauhan
- Dr T.V. Anilkumar 2012
  Dr V.K. Gupta
- Dr M.V. Joshi 2013-2016
  Dr C. Balachandran
- Dr N.K. Sood 2017-2019
  Dr R.C. Ghosh
- Dr D.V. Joshi 2020-2022
  Dr Amarjit Singh
- Dr K.P. Singh 2023-Cont.
  Dr S.K. Mukhopadhyay

Secretary Generals

- Dr P.K.R. Iyer 1976-1979
- Dr G.C. Mohanty 1980-1982
- Dr S.J. Seshadri 1983-1987
- Dr A. Rajan 1987-1993
- Dr R.N.S. Gowda 1994-2003
- Dr V.K. Gupta 2004-2008
- Dr T.V. Anilkumar 2008-2011
- Dr B.N. Tripathi 2013-2016
- Dr K.P. Singh 2017-2023
- Dr G.A. Balasubramanian 2024-Cont.

Web Manager

- Dr T.V. Anilkumar 2004-2008
- Dr Amarjit Singh 2009-2016
- Dr N.V. Kurkure 2017-2019
- Dr R. Somvanshi 2020-Cont.

Treasures

- Dr S.C. Gupta 1976-1979
- Dr R.L. Sah 1980-1988
- Dr N.S. Parihar 1989-1997
- Dr A.K. Sharma 1997-2005
- Dr K.P. Singh 2006-2007
- Dr Rajendra Singh 2008-2019
- Dr M. Palanivelu 2020-2021
- Dr Pawan Kumar 2021- Cont.

Editor/Chief Editor*/Associate Editor/Managing Editor*****

- Dr P.K.R. Iyer 1976
- Dr H.V.S. Chauhan 1977
- Dr G.C. Mohanty 1979-1982
- Dr N.S. Parihar 1983-1985
- Dr M.C. Prasad 1987-1998
- Drs P. Dwivedi/A.K. Sharma**/Rajendra Singh** 1999-2006
- Drs R.S. Chauhan*/B.N. Tripathi** 2006-2008
- Drs B.N. Tripathi*/Dr. R.V.S. Pawaiya 2009
- Drs B.N. Tripathi*/K.P. Singh/N.P. Kurade 2010-2011
- Drs K.P. Singh*/N.P. Kurade/Kuldip Gupta 2012-2014
- Drs R.V.S. Pawaiya*/N.P. Kurade/N. Shivsharnappa 2014-2019
- Drs M.R. Reddy/A. Anand Kumar/Vidya Singh*** 2020-2022
- Drs A. Anand Kumar*/K.S. Prasanna/Vidya Singh*** 2023-Cont.

Possibly First Central Committee (1977)

• Chairman	Dr B.S. Rajya
• Secretary	Prof. P.K.R. Iyer
• Treasurer	Dr S.C. Gupta
• Editor	Dr H.V.S. Chauhan
• Members	Dr S. Damodaran
	Dr J.L. Vegad
	Dr M.K. Nair
	Dr Balwant Singh

IAVP Constitution

After taking a decision of establishing IAVP on 24 March 1974 at IVRI, Izatnagar, UP IAVP Constitution was drafted and published in first issue of IJVP in 1976. It is having detailed guidelines and bylaws for office bearers and members. As per constitution IAVP had Central Council (Now Executive Committee) comprised of President, Vice President, General Secretary, Treasurer, Editor and four Members. Association had honorary members, members and life members. Honorary Members were admitted on recommendation of Central Council, Fee for member was Rs 15/- and Life Member 250/- only. IJVP had Editor and four members of Editorial Board. Persons paying Rs 1000/- were accepted as Patron. Price of IJVP was Rs 15/- for individual subscribers and Rs 20/- for Libraries in India and USD 20/- for libraries in abroad. Since beginning IAVP had given great importance to State Units. Guidelines were available for formation and functioning of local Units. Functions of each Office Bearers were well defined. In due course of time IAVP Constitution was amended several times and it is uploaded in IAVP website.

Current Executive Committee of IAVP (2023-2025)		
President	:	Dr B.N. Tripathi, Jammu, J & K
Vice-Presidents	:	Dr K.P. Singh, Bareilly, UP
		Dr S.K. Mukhopadhyay, Kolkata, West Bengal
Secretary General	:	Dr G.A. Balasubramaniam, Namakkal, Tamil Nadu
Joint-Secretary*	:	Dr M. Saminathan, Bareilly, UP
Treasurer	:	Dr Pawan Kumar, Bareilly, UP
Chief Editor	:	Dr A. Anand Kumar, Tirupati, AP
Editor	:	Dr K.S. Prasanna, Mannuthy, Thrissur, Kerala
Managing Editor*	:	Dr Vidya Singh, Bareilly, UP
Web Manager	:	Dr R. Somvanshi, Bareilly, UP
Zonal Secretaries	:	Dr R.D. Patil, Palampur, HP (North)
		Dr Arvind Ingle, Mumbai, MS (West)
		Dr S.K. Panda, Bhubaneshwar, Odisha (East)
		Dr Seema Rani Pegu, Guwahati, Assam (North-East)
		Dr R.C. Ghosh, Durg, CG (Central)
		Dr Manjunatha, S.S., Shivamogga, Karnataka (South)
Executive Members	:	Dr Pankaj Goswami, Jammu, J & K
		Dr C.K. Jana, Bareilly, UP
		Dr Kamal Purohit, Navania, Udaipur, Rajasthan
		Dr Rajeev Ranjan, Bhubaneshwar, Odisha
		Dr Asok Kumar M., Bareilly, UP
		Dr Ashwani Kumar Singh, Baghpat, UP
Ex-Officio ICVP Office Bearers		
President	:	Dr Vyas M. Shingatgeri, Gurugram, Haryana
Secretary	:	Dr R.V.S. Pawaiya, Bareilly, UP

*Nominated

IAVP Website

IAVP Website (www.iavp.org) was designed and established in 2004 by Dr T.V. Anilkumar the founder Web Master He served as webmaster during 2004-2008 which was followed by Dr Amarjit Singh (2008-2016). They made significant contributions in dissemination of association information to members. During 2017-2019, IAVP website was redesigned and it was made attractive and more informative through help of an IT Firm. Its menu was added certain new features viz. archival, image gallery, personalia etc. Recent FIAVP citations were uploaded while images of all IAVP President and IAVP Fellows were collected and uploaded. Website was uploaded with revised IAVP constitution, modified awards, proforma etc. in such a manner that its text can be used as reference and archival material. It was regularly updated with IVAP/ICVP conference news, proceedings, glimpses, appointments, award and honor news to members, superannuation biodata and obituaries. Current Web Manager Dr R. Somvanshi played a key role in upgrading and giving a new look to IAVP website.

IAVP Annual Conferences, National Symposium, Satellite Seminars, International Seminars and ICVP Meetings and Veterinary Pathology Congress

IAVP Conferences are attractive annual features for Indian Veterinary Pathologists particularly to students and young scholars and veterinary pathologists. So far 40th Annual Conventions and National Symposium are organized in different states of country. During 2005-2013, Satellite Seminar in association with Dr C.L. Davis DVM Foundation, USA were organized. From 2009 up to present day ICVP Annual Meeting has become constant feature of IAVP Conference. From 2013 onwards term Veterinary Pathology Congress was used. IAVP has organized more than six International Conferences at Chennai, 1992; Izatnagar, 2008. Ludhiana 2009, Bengaluru 2017, Bikaner, 2020 and Hyderabad, 2021. At Bengaluru VIII Biannual Meeting of Asian Society of Veterinary Pathology was organized which was attended by delegates from more than half dozen delegates from South East Asian countries.

IAVP Conferences (1983-2024)

Organizing Secretary	Date & Year	Place	Conference Details	Topic
S.J. Seshadri	March 9-11, 1983	Bangalore	1st AC & NS	Dairy cattle diseases
G.C. Mohanty	Dec. 10-12, 1984	Izatnagar	2nd AC & NS	Experimental and molecular pathology in diagnosis of diseases in animals
B.V. Jalnapurkar	March 5-7, 1986	Bombay	3rd AC & NS	Immuno-deficiency diseases of livestock and poultry
A. Rajan	Aug. 17-19, 1987	Trichur	4th AC & NS	Immunopathology of responses and diagnosis of emerging diseases of livestock and poultry
B. Chaudhary	Nov. 17-19, 1988	Guwahati	5th AC & NS	Pathology of environmental pollutants and mycotoxicosis in livestock and poultry
G.C. Mohanty	Sept. 7-9, 1989	Izatnagar	6th AC & NS	Pathology and biotechnology in diagnosis of diseases of livestock and poultry
P. Rama Rao	Sept. 17-19, 1990	Tirupati	7th AG & NS	Immunopathology and biomolecular research in diseases of livestock and poultry
G.J. Jha	Aug. 24-26, 1991	Ranchi	8th AC & NS	Clinical pathology and biotechnology in diagnosis of livestock and poultry diseases
A. Sundraraj	Aug. 6-8, 1992	Madras	9th AC & IS	Problems of emerging poultry diseases and International Seminar on advances on animal cancer research
U.K. Vyas	Oct. 8-10, 1993	Bikaner	10th AC & NS	Pathology and biotechnology in the diagnosis of liver diseases of livestock and poultry
K.S. Prajapati	Dec. 1-3, 1994	Anand	11th AC & NS	Recent advances in animal pathology and poultry diseases
A.T. Rao	Nov. 9-11, 1995	Bhubaneshwar	12th AC & NS	Impact of disease research on livestock, poultry production and wildlife conservation
S.P. Singh	Nov. 4-6, 1996	Pantnagar	13th AC & NS	Impact of environmental pollution on health and production of livestock, poultry and wild life
M.C. Prasad	Dec. 4-6, 1997	Izatnagar	14th AC & NS	Advances in Veterinary Pathology in post independent era
K.M. Ramachandran	Nov. 9-11, 1998	Trichur	15th AC & NS	Advances in diagnostic Veterinary Pathology
S.K. Vijayasarthy	Nov. 25-27, 1999	Bangalore	16th AC & NS	Topics of current interest in veterinary pathology of animal and poultry diseases: The millennium approach
M.V. Joshi	Nov. 11-13, 2000	Akola	17th AC & NS	Current concept in animal and poultry diseases: New millennium approach
P.L. Kaul	Oct. 11-13, 2001	S.K. Nagar	18th AC & NS	Current trends and challenges in the livestock and poultry diseases including wildlife in 21st century
V.K. Gupta	Sept. 26-28, 2002	Palampur	19th AC & NS	Recent advances in diagnosis of diseases of livestock, poultry and wildlife with special references to hill area diseases
A.K. Katiyar	Nov. 12-14, 2003	Jabalpur	20th AC & NS	Basic pathology and animal diseases: a need for fresh approach in Indian scenario
S.K. Mukhopadhayay	Nov. 23-25, 2004	Kolkata	21st AC & NS	Advances in pathological techniques in diagnosis of animal, bird and fish diseases
B.K. More	Nov. 25-27, 2005	Shirwal (held at Pune)	22nd AC, NS & Ist SS	Newer concepts in animal and avian diseases- A farmer, industry and institutional dialogue and SS on principals of histopathological basis of gross pathology
C. Balachandran	Dec. 27-29, 2006	Chennai	23rd AC, NS & IInd SS	Newer concepts in diagnostic veterinary pathology and toxicopathology with special emphasis on Indian systems of medicine and SS on Pathology of emerging zoonosis and transboundary diseases in animals
Chi. Srilatha	Oct. 1-3, 2007	Tirupati	24th AC, NS & IIIrd SS	Immunopathology and molecular approaches for disease diagnosis in livestock and poultry including wildlife and SS on Lab. animal pathology: influence of nature and nurture on pathology and research results

Rajendra Singh	Nov. 10-12, 2008	Izatnagar	25th AC, IS & IVth SS	Quality assurance in pathology and disease diagnosis and SS on microscopic veterinary pathology in necropsy, biopsy and certification examination
N.K. Sood	Oct. 28-30, 2009	Ludhiana	27th AC, IS & Vth SS	Advanced descriptive technique, ultrastructure, cytology and immuno-histochemistry and International Symposium on philosophy of disease diagnosis through morphological to biomolecular approaches and core theme diagnostic pathology
G.K. Baruah	Nov. 25-27, 2010	Guwahati	28th AC, NS & VIth SS	Recent trend in diagnosis and pathology of emerging diseases of livestock and poultry
R. Sridhar	Dec. 28-30, 2011	Chennai	29th AC, NS & 3rd ICVP	Innovative research approaches for diagnostic pathology
R.P. Gupta	Nov. 5-8, 2012	Hisar	XXIXth AC, NS & VIIth SS	Challenges in diagnostic pathology in domestic, pet, wild and aquatic animals and National Seminar on Emerging trends in diagnosis and control of poultry diseases
S.K. Panda	Nov. 21-23, 2013	Bhubaneswar	VPC, 30th AC, NS, VIIIth SS & 4th ICVP	Advances and applications of diagnostic pathology for disease management in live-livestock, poultry, pet, fish, laboratory animal and wildlife
K.S. Prajapati	Nov. 13-15, 2014	Anand	VPC 31st AC, NS & 5th ICVP	National Symposium on Impact of climate change on pathobiology of diseases of animals, poultry and fish
V. Rama Devi	Dec. 3-5, 2015	Gannavaram	VPC, 32nd AC, NS & 6th ICVP	Challenges and advances in disease diagnosis of livestock, poultry and fish: redefining the role of Veterinary Pathologists
R.C. Ghosh	Nov. 9-11, 2016	Durg	VPC, 33rd AC, NS & 7th ICVP	Innovative approaches for diagnosis and control of emerging and re-emerging diseases of livestock, poultry and fish
H.D. Narayana Swamy	Nov. 9-11, 2017	Bengaluru	IVPC, 34th AC, NS 8th ICVP & VIII ASVP Meeting	Emerging horizons in diagnosis of animal and poultry diseases: Towards sustainable production in Asian countries
B.J. Patel	Oct. 22-24, 2018	S.K. Nagar	VPC, 35th AC, NS & 9th ICVP	Recent advances in Veterinary Pathology and disease diagnosis for sustainable livestock and poultry production
T.K. Rajkhowa	Nov. 6-8, 2019	Selesih-Aizawl	VPC, 36th AC, NS & 10th ICVP	Advances in Veterinary Pathology for fostering one health, food security and environment protection
N.V. Kurkure	Dec. 26-29, 2020	Nagpur	IVPC, 37th, AC, NS & 11th ICVP	Role of Veterinary Pathology in controlling emerging and re-emerging diseases of livestock and poultry: An One Health approach
Hemant Dadhich	Dec. 17-19, 2021	Bikaner	IVPC, 38th AC, NS & 12th ICVP	Advances in Veterinary Pathology for diagnosis and control of emerging and re-emerging diseases of livestock, wild animals and poultry
M. Laksmanan	Nov. 17-20, 2022	Hyderabad	IVPC, 39th AC, NS & 13th ICVP	Global challenges in rapid diagnosis and management of animal and poultry diseases for improved health and productivity
K.P. Singh	Dec. 20-22, 2023	Izatnagar	VPC, 40th AC, NS & 14th ICVP	Advances in Veterinary Pathology for diagnosis and control of emerging diseases of livestock and poultry
Pankaj Goswami*	Nov. 28-30, 2024	Jammu	VPC, 41st AC, NS & 15th ICVP	Exploring Veterinary Pathology and diagnostic innovations in animal and poultry diseases amidst climatic challenges

*To be held

The Indian Journal of Veterinary Pathology

The Indian Journal of Veterinary Pathology (IJVP) is the official publication of the Association intended for publishing scientific review articles, research papers, short communications, thesis abstracts, book reviews and association news (conference proceedings, awards and honors, new appointments, promotions, obituaries etc.) for advancing the knowledge of pathology. IJVP publication was started in 1976 and its founder editor was Prof. P.K.R. Iyer. From 1976-90, every year one issue was brought out. Since 1991, two issues / year (June and December) were published by efforts of Dr M.C. Prasad. In 2013, Dr R.V.S. Pawaiya, new Chief Editor took a bold step to bringing out four issues of IJVP annually (March, June, September and December). He gave new look to Journal and online publication was also started. The issues from 2004 are uploaded and available in IAVP website. In the year 2010 NAAS impact factor of journal was 4.6 (S. No. 704, J. Id I050). NAAS rating w.e.f. 2017 is 5.48. Its Indian Citation Index Research Impact Indicator is 0.038.

IAVP News Letter-The Lesion

Initially, The Lesion was a biannual Newsletter of IAVP. It publishes association news and activities along with achievement of members with multicolor pictures. It also publishes cases reports and personalia. Its first issue was published in January 2009. Dr B.N. Tripathi was its founding editor. For a few issues, Drs C. Balachandran and T.V Anilkumar also served as Editor, Currently, the Lesion is annual publication and it is published before IAVP Conference. Present Chief Editor is Dr R. Somvanshi. He gave new look to the Lesion by publishing certain new columns-like features on- Conference Venue, Meet Your Organizing Secretary, Superannuation News, Obituaries, Appointments and Promotions etc. Details of issues may be visited under publication menu of IAVP website.

IAVP Annual Awards

The Indian Association of Veterinary Pathologist confers a number of awards every year to encourage different age groups of Veterinary Pathologists who have excelled in professional competence and contributed to the advancement of knowledge in Veterinary Pathology. The revised awards in the year 2016 are listed below and for rules and for regulations the applicants may log on to www.iavp.org. These awards are only for IAVP life members.

1. IAVP-Young Scientist Awards (Four Nos.)

IAVP-Dr Balwant Singh Memorial Young Scientist Award for Best Oral Presentation

IAVP-Dr S.K. Nigam Young Scientist Award for Second Best Oral Presentation

IAVP-Prof. S. Ramachandran Memorial Best Molecular Oncologist Presentation Award

IAVP-Prof. C. Balachandran Molecular Pathology Best Oral Presentation

2. IAVP-Poster Presentation Awards (Three Nos.)

IAVP-Best Poster Presentation Award

IAVP-Organizing Secretary Second Best-Poster Presentation Award

IAVP-Savithree Jibachch Sinha Third Best Poster Presentation Award

3. IAVP-Journal Awards (Four Nos.)

IAVP-Dr C.M. Singh Award for Best Full Research Article (Non-Pack Animals)

IAVP-Dr S. Damodaran Award for Best Oncology Case Report/Full Paper

IAVP Dr B.S. Rajya Award for Best Non-Oncology Short Communication

IAVP-Gang-Mana Sharma Award for Best Article/Case Report on Pack Animals

4. IAVP-Best Post Graduate Thesis Awards (Four Nos.)

IAVP-Prof. P.K.R. Iyer Memorial Best MVSc Thesis Award

IAVP-Dr Ram Raksha-Kiran Shukla Award for Second Best MVSc Thesis

IAVP-Best PhD Thesis Award

IAVP-Dr Patri Rama Rao Second Best PhD Thesis Award

5. IAVP Achievement Awards in Specialty Subjects

IAVP-Best Farm Animals Pathologist Award

IAVP-Best Poultry Pathologist Award

IAVP-Dr B.L. Purohit Memorial Best Toxicologist Pathologist Award

IAVP-Wildlife Pathologist Award (Biannual Award-Odd Year)

6. IAVP-Special Encouragement Awards

IAVP-Best Veterinary Pathology Teacher Award (Annual Award)

IAVP-Best Women Veterinary Pathologist Award (Biannual Award-Odd Year)

7. Fellow of Indian Association of Veterinary Pathologists (FIAVP)

For outstanding pathologists based on their contribution to the advancement of the Veterinary Pathology, Research and Education

8. IAVP Appreciations/Activities/Recognitions

Dr P.P. Gupta Oration*

IAVP-Veterinary Pathology Congress-Thematic Lecture*

IAVP-Veterinary Pathology Congress-Continuing Veterinary Pathology Education Lecture*

IAVP-Appreciation to Organizing Secretary and Team for Best EC Worker/Zone/Chapter*

NB: On nomination and invitation of EC-IAVP

Discontinued IAVP Awards

In past following awards were given but now discontinued for want of sponsorship from concerned firms/ individuals:

I. Young Scientists Awards (Two Nos.)

A. SKM Animal Feeds and Foods (India) Ltd. Award

B. M/S Narmada Hatcheries Award

II. Dr Nemi Chand Jain and Jawahar Lal Vegad Award: For outstanding research in Veterinary Pathology

III. Dr Nemi Chand Jain Life Time Achievement Award in Veterinary Pathology

Recently Instituted Awards and Sponsorship

1. IAVP-Dr S.K. Nigam Memorial Young Scientist Award for Second Best Oral Presentation

Dr Suresh Kumar Nigam (b5-6-1936-d12-11-2015) was eminent Toxicopathologist, Senior Deputy Director and Emeritus Medical Scientist at National Institute of Occupational Health (NIOH), Ahmedabad, Gujarat. Dr Vijay Kale (vetvijay@gmail.com) donated Rs 1,00,000/- (Rs One lakh only) to IAVP sponsor award. First Award was given in 2019.

2. IAVP-Dr Balwant Singh Memorial Young Scientist Award for Best Oral Presentation

Dr Balwant Singh (b27-01-1939-d29-12-2014) was eminent Veterinary Pathologist and educationist. He retired as Dean, Veterinary College, GADVASU, Ludhiana, Punjab. Dr Balroop Singh (balroopsingh@gmail.com) S/O late Dr Balwant Singh donated Rs 1,00,000/- (Rs One lakh only) to IAVP sponsor award. First Award was given in 2016.

3. IAVP-Prof. P.K.R. Iyer Memorial Best MVSc Thesis Award

Prof. P.K.R. Iyer (b29-01-1929-d20-08-2017) was eminent teacher, Veterinary Pathologist and animal disease diagnostician, former Professor and Head, Division of Pathology, IVRI, Izatnagar, UP. Award was sponsored by family members and students of late Prof. Iyer by donating Rs 1,20,000/- (Rs One lakh twenty thousand only). Of this amount, Major General P.R. Venkatesh, New Delhi, Mr P.R. Krishna Kumar, Thiruvallaur (sons) and Mrs Chitra Murti (daughter), Texas, USA donated Rs 45,000/-) and his students (Prof. Utpal Sengupta, Agra, Rs 30,000/; Prof. A.T. Rao, Bhubaneswar; Dr Lal Krishna, New Delhi; Dr R.K.S. Dogra, Lucknow; Dr K.N. Gowal, Gurgaon; Dr G.S. Pandey, USA; Dr R. Somvanshi, Bareilly; Dr K.C. Varshney, Bengaluru; Dr Taibur Rahman, Guwahati and Dr G. Saikumar, Bareilly (by donating Rs 5000 each) and his admirer Dr C. Balachandran, Chennai (by donating Rs 5000/). First Award was given in 2017.

4. IAVP-Dr B.L. Purohit Memorial Best Toxicologist Pathologist Award

Dr Balkrishna Lakshmikant Purohit (b2-9-1924-2-12-d1980) was Professor and Head, Veterinary Pathology, Nagpur and Dean, Faculty of Veterinary Science, Nagpur and Akola. Mrs. Shanta Purohit wife of late Prof. B.L. Purohit and his five students donated Rs 45,000/- (Rs Forty-five thousand only) for starting this Award to IAVP. Details of donors

were: Mrs. Shanta Balkrishna Purohit, Pune- Rs 20,000/; Dr Subhash Shankar Bhagwat, Mumbai-Rs 7500/; Dr A.G. Bhandarkar, Nagpur- Rs 3500; Dr Shrikant Gharote, Wardha- Rs 3000; Dr Madan Vasant Joshi, Akola-Rs 5000 and Dr Ashok K. Thatoo, Pantnagar-Rs 6000. First Award was given in 2016.

5. IAVP-Prof. C. Balachandran Molecular Pathology Best Oral Presentation

Prof. C. Balachandran, Ex. Vice Chancellor of Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai, Tamil Nadu is eminent Veterinary Pathologist and very active member/office bearer of IAVP and ICVP. EC-IAVP and GB-IAVP meeting held at Hyderabad in November, 2022 approved the proposal of Prof. C. Balachandran to start IAVP-Prof. C. Balachandran Young Scientist Best Oral Molecular Pathology Presentation Award. For this Prof. C. Balachandran donated Rs 1,00,000/- (Rupees One lakh only) under NEFT (UTR No. UBINK23193041514) to Treasurer, IAVP on 12.07.2023 vide Receipt No. IAVP/R.No. 4963 Dated: 12.07.2023. The annual award contains certificate (to all authors) and memento (to young author). First award was proposed given in 2023.

6. IAVP-Sessional Specialty Poster Award (2 Awards Per Session)

In online Meeting of GB-IAVP held during Bikaner VPC-2021 and earlier occasions too; several members demanded that two Poster Presentation Awards in each session should be given. Therefore, IAVP-Sessional Specialty Poster Award (2 Awards per Session) were started in 2022 and given for first time in IAVP Conference held at Hyderabad. In this award only certificates will be given to authors.

Fellowship of IAVP

Fellowship of IAVP is awarded to outstanding Veterinary Pathologists based on their contribution to the development of Pathology or Veterinary Education/Research. The list of FIAVP awardees of IAVP is as follows:

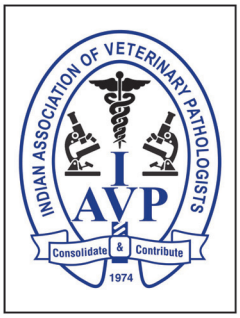
M.Y. Mangrulkar, 1988	R.S. Chauhan, 2005	G.A. Balasubramaniam, 2016
C.M. Singh, 1990	B. Murali Manohar, 2007	R.V.S. Pawaiya, 2016
G.L. Sharma, 1990	P. Dwivedi, 2007	Ch. Srilatha, 2016
S. Damodaran, 1990	T.V. Anilkumar, 2007	R.P. Gupta, 2017
P.B. Kuppuswamy, 1991	R. Somvanshi, 2008	Nitin Virmani, 2017
B.S. Rajya, 1992	B.N. Tripathi, 2008	V. Rama Devi, 2017
K.P.C. Nair, 1992	C. Balachandran, 2009	B.P. Joshi, 2018
P.R.K. Iyer, 1993	V.K. Gupta, 2009	Arvind D. Ingle, 2018
M.K. Nair, 1997	Madan V. Joshi, 2010	H.D. Narayanswamy, 2018
G.C. Mohanty, 1997	K.P. Singh, 2010	S.K. Mukhopadhayay, 2019
M.C. Prasad, 1997	R. Singh, 2011	N.V. Kurkure, 2019
P.P. Gupta, 1997	Amarjit Singh, 2011	A. Anand Kumar, 2019
S. Ramchandran, 1999	N.K. Sood, 2012	Col. C. Churamani, 2020
A. Rajan, 1999	R.C. Ghosh, 2012	Hemant Dadhich, 2020
Lal Krishna, 1999	K.S. Prajapati, 2013	Susen K. Panda, 2020
R.N.S. Gowda, 1999	A.K. Sharma, 2013	Kuldip Gupta, 2021
S.J. Seshadri, 2000	D.V. Joshi, 2014	Mekala Lakshman, 2021
J.L. Vegad, 2000	C.K. Singh, 2014	K. Sujatha, 2021
A.T. Rao, 2000	R.N. Sharma, 2015	S.D. Moregaonkar, 2022
O.P. Paliwal, 2000	A. Chakraborti, 2015	N. Pazhanivel, 2022
L.N. Acharjyo, 2001	P.K. Sahoo, 2015	T.K. Rajkhowa, 2023
P.L. Kaul, 2004	Hony. Fellowship: Maj Gen N.S. Kanwar, 2012	

Indian College of Veterinary Pathologists (ICVP)
(https://www.icvp.in)

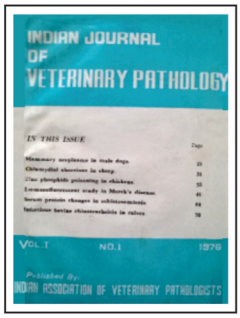
Indian College of Veterinary Pathologists (ICVP) is an affiliated body of IAVP which was established in November 2008 as an independent certification organization for personal accreditation at Division of Pathology, IVRI, Izatnagar, UP. during the Presidentship of Dr Lal Krishna. It aims for the advancement of Veterinary Pathology and its sub-specializations including Clinical Pathology, Avian Pathology, Aquatic Animal Pathology, Toxicologic Pathology, Laboratory Animal Pathology etc. by establishing standards of training and experience. The organization was pioneered, conceived and developed by a group of 22 life members of the Indian Association of Veterinary Pathologists having recognizable stature in academia, research, industry and practice, identified as Charter Members.

Historical Pictorial Glimpses of IAVP

Old Logo of IAVP



New Logo of IAVP

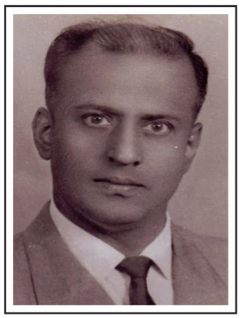


First issue of IJVP, 1976

First Issue of The Lesion, 2009

Prof. S. Damodaran, Founder Chairman, IAVP, 1974

Dr B.S. Rajya, First President, IAVP, 1976-1979

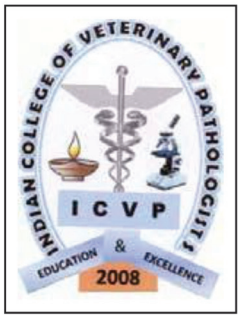


Prof. P.K.R. Iyer, First Vice President, IAVP, 1980-1982 & First Editor, IJVP, 1976

Dr J.L. Vegad, Three Tenure IAVP President, 1997-2003

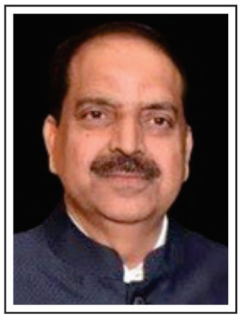
Dr M.Y. Mangrulkar, First Fellow, IAVP, 1988

Dr S.J. Sheshadri, First IAVP Conference Organizing Secretary, 1983



ICVP Logo, 2008

Dr Lal Krishna, Founder President, ICVP, 2008



Dr B.N. Tripathi, Present President, IAVP, 2020 Cont.

Dr K.P. Singh, Present Vice President, 2023 Cont.

Dr S.K. Mukhopadhyay, Present V.P., 2023 Cont.

Dr G.A. Balasubramaniam, Present S.G., 2023 Cont.

ICVP eventually aims for harmonization of professional and ethical standards in Veterinary Pathology practice on an international platform for the benefit of the society in general. Dr Lal Krishna was its founder President, Dr V.K. Gupta was first Secretary and Dr Anilkumar, Chairman Examination Committee. Dr T.V. Anilkumar and Dr V.K. Gupta played a key role in formulation of its constitution and establishing ICVP. Till 2023, ICVP is having 84 qualified diplomats. Presently, Dr V.M. Shingatgeri is President, Dr Amarjit Singh is Vice President and Dr R.V.S. Pawaiya is Secretary of ICVP.

Major Achievements of IAVP

1. Indian Association of Veterinary Pathologists (IAVP) is one of premier scientific society of Veterinary Science in India in specific discipline of Veterinary Pathology with moto of contribute and consolidate (as depicted in IAVP logo) in its discipline. The main objectives of the association are advancement and dissemination of knowledge of Veterinary Pathology.
2. IAVP has provided such a national forum where Veterinary Pathologists from each part of country are meeting regularly frequently and interacting with each other and in turn resulting in very strong cooperation and national integration. IAVP social media forums have also promoted this strongly.
3. So far IAVP has organized 40 Annual National/International Conferences and around two dozen Zonal Conferences in different parts of country and professional scientific information were exchanged amongst fellow Veterinary Pathologists.
4. It published 48 volumes of The Indian Journal of Veterinary Pathology and 12 volumes of newsletter The Lesion. Journal published researches in pathology and pathogenesis and animal diseases conducted in different parts of country which helped in control of dreaded animal diseases.
5. IAVP is having around 1500 life members who are participating regularly in its scientific activities and getting benefits in their academic pursuits.
6. IAVP is maintaining an active website (www.iavp.org) which made significant contributions in dissemination of association related information to its members. Website was uploaded with revised IAVP constitution, modified awards, new proforma etc. in such a manner that its text can be used as reference and archival material. It was regularly updated with IVAP/ICVP conference news, proceedings, glimpses, appointments, award and honor news of members, superannuation profile and obituaries.
7. IAVP is awarding 8 categories of a total of 35 annual awards to its members. More than 50 percent awards are given to post graduate and doctoral students and young scientists who are getting benefits in their career advancement.
8. So far IAVP has awarded its Fellowship (FIAVP) to 65 eminent Veterinary Pathologists based on their contribution to the advancement of the Veterinary Pathology research and education.
9. IAVP was instrumental in establishing its affiliated body Indian College of Veterinary Pathologists (ICVP) an independent certification organization for personal accreditation of practicing Veterinary Pathologists. ICVP also aims for harmonization of professional and ethical standards in Veterinary Pathology practice on an international platform for the benefit of the society in general.
10. IAVP is instrumental in promoting primitive knowledge of Veterinary Pathology of 1970 decade of radio to present day era of artificial intelligence. In 1970s research and animal disease diagnosis was confined to clinical pathology, necropsy, pathogen isolation and histopathology while presently conventional gold standards (gross and histopathology), molecular techniques and immunohistochemistry is applied by every Indian Veterinary Pathologists. They have created respectful place in global scientific community.

Conclusions

To conclude it may be re-emphasized that Veterinary Pathology is the back bone of modern Veterinary Science as regards understanding of pathogenesis of disease, research and its early diagnosis is concerned. IAVP which was established in 1974 under visionary leadership of Drs S. Damodaran, B.S. Rajya, P.K.R. Iyer and others at IVRI is continuously guiding professionals in befitting manner. The Indian Journal of Veterinary Pathology was started publishing in 1976 and since then it is serving profession without any break with quality research publications. ICVP was founded in 2008 at IVRI, Izatnagar with an aim for personal accreditation of practicing Veterinary Pathologists. It is producing new generation of perfectly trained and skilled diplomats. To conclude it may be stated that IAVP is growing fast and in right direction with innovative ideas. It is taking care to keep up interest of academia and industry. Steps are on to establish links with national and international sister professional organizations.

Indian J. Vet. Pathol., 48(3) : 205-225, 2024: DOI: 10.5958/0973-970X.2024.00036.1

REVIEW ARTICLE

Unveiling the Canine Mammary Gland: From Development to Neoplasm

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Received: 16.4.2024; Accepted: 16.6.2024

ABSTRACT

In the present review, the development of mammary gland in dogs, along with its anatomy and histology followed by epidemiology and risk factors of canine mammary tumors is discussed. Next section of this review is comprised of various molecular mechanisms of canine mammary tumors development by discussing various genes and proteins involved in sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, reprogramming of energy metabolism, tumor promoting inflammation, and evading immune destruction. The last section covered classification, grading, and prognosis of canine mammary tumors.

Keywords: Canine tumors, development of mammary gland, receptors, signaling

INTRODUCTION

The mammary glands, an epidermal append ages that possibly evolved over 300 million years ago from ancient apocrine sweat glands[1], are unique organ that distinguishes mammals from all other animals, and secretes milk for the nourishment of the newborn[2,3]. The development of this gland is unique, as the last stages of development occur in the adult female only during pregnancy. Mammary glands development begins prenatally with formation of rudimentary ductal structure of the gland[2-4]. Puberty initiates ductal elongation, branching, and lobuloalveolar development with stroma[4]. During pregnancy there is proliferation of the ductal tissue, differentiation to milk-producing acini, secretion of milk by the acinar cells, and, at the end of lactation, involution of the secretory component of the gland with preservation of the ductal structures[5].

Mammary cancer is most common cancer in women, and about 1 in 8 (12%) women in the US will develop invasive breast cancer during their lifetime. According to National Cancer Institute, USA, only in 2016, about 2,46,660 (14.6% of all new cancer cases) of new cases of breast cancer will be diagnosed in women and about 40,450 (6.8% of all cancer deaths) women will die from breast cancer[6].

In animals, mammary gland tumors are rare except dog and cat[7]. Canine mammary tumors (CMTs) are the most common neoplasms in the female dog with 0.2% prevalence[8]. The annual incidence rate has been estimated at 205/100,000[9]. The incidence of naturally occurring CMTs is three times greater than the level reported in human populations[10,11]. In female dog, the incidence of mammary tumors is estimated at 50% of all neoplasms in this species, of which approximately 60% are benign and 40% malignant[7].

Previous studies have identified many similarities between CMT and breast cancer in women, in terms of epidemiology, biology, dietary risk factors, and clinical behaviour, as well as hormonal dependence. Despite having different lifespan, the average age at onset of mammary tumors is approximately the same for humans (after 40 years) and dogs (after 6 years), in addition the reported peak incidence of the disease is also comparable between the two species (humans 50-58 years) and dogs (8-11 years)[12]. CMTs seem to mimic human breast cancer, as a range of similarities are found at the molecular level concerning the over expression of steroid receptors, proliferation markers, epidermal growth factor, p53 suppressor gene mutations, metalloproteinases, cyclooxygenases, among many others[12]. Due to spontaneous occurrence, similarities and differences in their molecular pathogenesis, CMT has considered a spontaneous animal model of human breast cancer[13].

In this review, we first describe pre and post-natal development of mammary gland followed by anatomy and histology, epidemiology and risk factors of CMTs, hallmarks of canine mammary tumors, classification and grading of CMTs and

How to cite this article : Raval, S.H., Parmar, R.S., Pandya, M.M. and Joshi, D.V. 2024. Unveiling the Canine Mammary Gland: From Development to Neoplasm. Indian J. Vet. Pathol., 48(3) : 205-225.

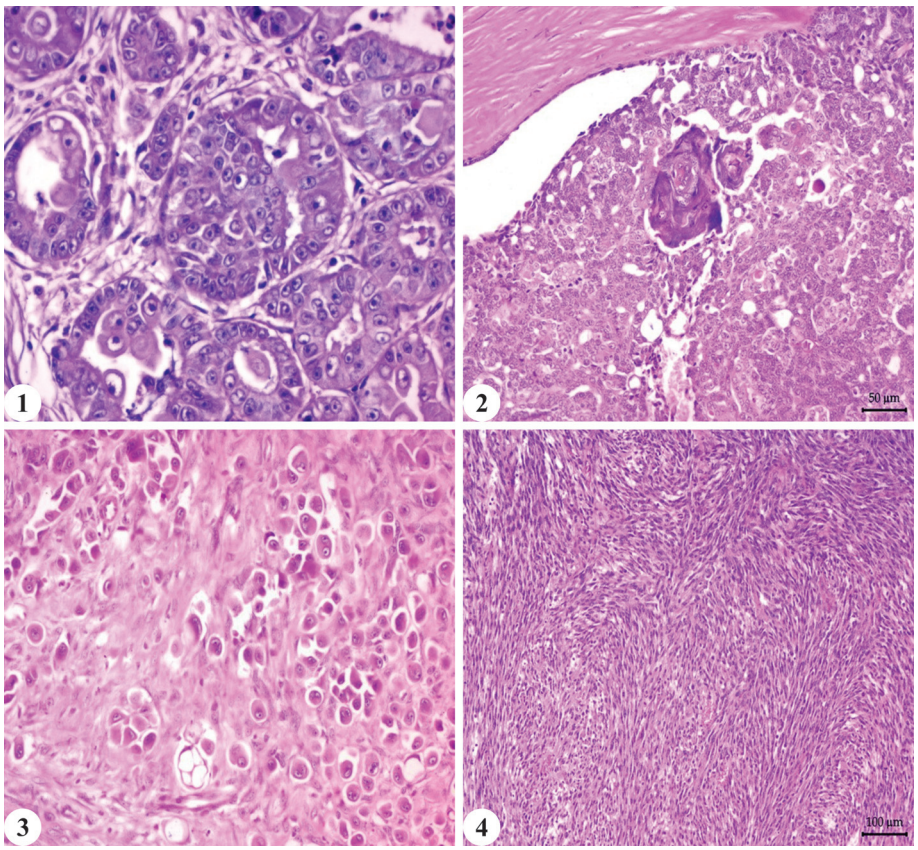

Fig. 1. Carcinoma-tubular. Neoplastic cells predominantly arranged in a tubular fashion and supported by fibrovascular stroma. Neoplastic cells showing anisocytosis and anisokaryosis (H&E ×400); **Fig. 2.** Ductal carcinoma. Neoplastic cells arranged in irregular tubules and showing focal areas of squamous differentiation with presence of intracytoplasmic keratohyaline granules (H&E ×200); **Fig. 3.** Carcinoma-anaplastic. Individualized neoplastic cells showing marked pleomorphism and binucleation (H&E ×200); **Fig. 4.** Fibrosarcoma. Neoplasm composed of spindle-shaped cells arranged in long interlacing bundles (H&E ×100).

lastly molecular classification.

Development of the mammary gland

Published reports emphasised on development of the canine mammary gland are rare in English literature, and most of our knowledge of mammopoiesis comes from mice hence murine mammary gland development is discussed here. There are three major stages of breast development - embryonic, pubertal, and reproductive. Upto puberty, mammary gland development occurs hormone - independent, while thereafter mammary gland development is hormone - dependent[14]. Epithelial and mesenchymal tissue component of mammary gland are derived embryologically from ectoderm and mesoderm, respectively. Development of mammary gland begins with formation of milk lines, a columnar multilayered ectoderm, that run between the fore (anterior) and hind limbs (posterior), on the ventral surface of the embryo[2,15,16]. The ectodermal cells symmetrically migrate along each milk line and coalesce to form a placode, which eventually becomes individual mammary glands[5]. Placode location and number will decide the location and number (normal or supernumerary) of mammary glands in animals. Formation of placode occurs asynchronously and in specific sequence with pair 3 emerging first, followed by pair 4, then pair 1 and 5 simultaneously, followed finally by pair 2[2,16]. In dog, at 25 days of gestation, mammary ridge is well developed and at 30 days of gestation, distinct papillae have appeared along the former mammary ridge[17]. Placodes transform into bulbs of epithelial cells that descends into the underlying mesenchyme to become the mammary buds. The mesenchymal cells that surround the buds condense to become the mammary mesenchyme. The mammary buds subsequently branch to form a mammary sprout, which develop a lumen with an opening to the skin, marked by the formation of the nipple sheath. The parathyroid hormone-related protein plays a central role in regulating the transition from budding to branching. Each mammary sprout will eventually form the papillary duct of the adult mammary gland[2,5,15,16,18]. Epithelium and mesenchymal component reciprocal interaction play crucial role in embryologically development of mammary gland. The embryonic mammary mesenchyme beneath the mammary line gives signals to mammary epithelial cell for differentiation[19], while the epithelium influences mammary mesenchyme to condensed and form a

few layers of fibroblast-rich cells, closely surrounding the epithelial rudiment[20]. In mice, mammary gland development ceases at 18.5 day of gestation and does not commence again until puberty[15]. In the male Beagle dog fetus, 1 week prior to birth, development of prepuce results in early involution and loss of the inguinal mammary gland[17]. After birth, the mammary glands continue to grow in proportion to the rest of the body[5].

After puberty, mammary gland development is under hormones influence. Estrogen and progesterone secreted by ovaries, growth hormone and prolactin secreted by pituitary, and glucocorticoids secreted by adrenal cortex play crucial role in post-pubertal mammary gland development[21]. Recurrent estrous cycles in adulthood trigger side branching, while pregnancy enhances side branching and induces development of alveoli with lactational differentiation followed by involution at weaning[22,23]. At puberty, insulin-like growth factor-1 (IGF1), growth hormone along with estrogen induces epithelial cell proliferation at the tips of the ducts[5,15]. Proliferation within the terminal end buds (TEBs), a club-shaped structures comprising an outer layer of cap cells (differentiate into myoepithelial cells) and a multilayered inner core of cells called body cells, results in ductal elongation, and clefting of the TEBs results in bifurcation of the ducts to generate branches. Apoptosis has been detected in the body cells and could be the mechanism for lumen formation[2,15,24].

At the time of pregnancy, under influence of progesterone, receptor activator of nuclear factor-kappa B ligand (RANKL) and prolactin, mammary gland matures and alveoli formation occurs[2,15,25]. Under influence of these hormones there are extensive side-branching and proliferating epithelial cells generate alveolar buds that progressively cleave and differentiate into distinct alveoli, which become milk-secreting lobules during lactation[2]. Thus, at parturition, the mammary gland consists of a ductular-lobular-alveolar structure.

Weaning, forced involution and glucocorticoid administration, leads to apoptosis of secretory epithelium and remodeling the ductular-lobular-alveolar structure back to a simple ductal architecture with replacement by adipocytes[2,26]. In mice mammary gland involution is occurs in two phases. In first phase, within hours of end of suckling, milk accumulates in alveoli and mammary gland. Stagnation of milk initiates the process of secretory epitheliumapoptosis, controlled by local factors [leukemia inhibitory factor (LIF) and transforming growth factor (TGF) β3] secreted by mammary gland[26,27]. 48 hours of first phase is reversible. Thus, if pups are return to dam for suckling in 48 hours, apoptosis stops and lactation recommences[26]. After 48 hours irreversible mammary gland involution begins and alveoli start to collapse[2]. Apoptosis and detachment of luminal cells collapses the alveoli and start of proteolytic degradation of the mammary gland

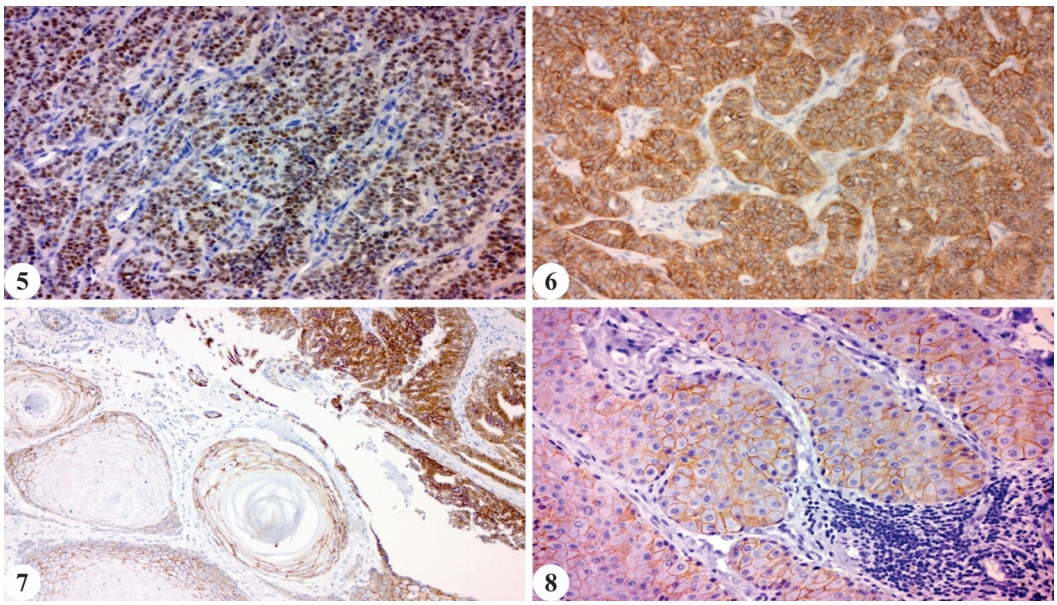

Fig. 5. Estrogen Receptor (ER). Neoplastic cells showing diffuse strong nuclear immunoreactivity with anti ER antibody. Immunoperoxidase staining, DAB chromogen (Mayer's hematoxylin counterstain ×200); **Fig. 6.** HER-2 overexpression. Neoplastic cells showing diffuse complete membranous immunoreactivity with anti HER-2 antibody. Immunoperoxidase staining, DAB chromogen (Mayer's hematoxylin counterstain ×200); **Fig. 7.** Adenosquamous carcinoma: In right side of the image, neoplastic cells showing strong membranous labelling, while left side of the image, squamous cell carcinoma cells showing moderate membranous labelling with E-Cadherin. Immunohistochemistry, DAB chromogen (Gill hematoxylin counterstain 100X); **Fig. 8.** Squamous cell carcinoma. Neoplastic cells showing diffuse complete membranous immunoreactivity with anti beta-catenin antibody. Immunoperoxidase staining, DAB chromogen (Mayer's hematoxylin counterstain ×200).

basement membrane. Mesenchymal-epithelial interaction induces synthesis of proteinases from fibroblast-like cells in the periductal stroma and stromal cells surrounding the collapsed alveoli. Expression of the matrix metallo protein asesgelatinase A and stromelysin-1 and the serine proteinase urokinase-type plasminogen activator, which was low during lactation, was strongly upregulated in parallel starting at day 4 after weaning[28]. These proteinases destroy the extra cellular matrix of alveoli and induces second phase of apoptosis. Action of matrix metalloproteases 3 and plasmin, re-differentiate the adipocytes that refill the space[2]. Thus, mammary gland returns to pre-pregnant state.

Anatomy and histology of the mammary gland

The number of glands in any given species and the positions in which they are located is genetically determined[18]. Most dogs have 2 thoracic, 2 abdominal, and 1 inguinal pair of mammary glands, although 4 or 6 pairs of mammary glands reported infrequently[5,18]. In one study of 24 canine fetuses, 2 thoracic, 2 abdominal, and 1 inguinal pair was found only in 12 fetuses, while in other fetuses numbers of pairs vary[29].

Two thoracic mammary glands receive their arterial blood supply from the perforating branches of the internal thoracic arteries, cutaneous branches of intercostal arteries and lateral thoracic arteries. The cranial superficial epigastric arteries and cutaneous branches of the caudal intercostal arteries supply the cranial abdominal gland. The caudal superficial epigastric arteries supply the caudal abdominal and inguinal glands. The venous drainage is parallel to the arterial supply[18]. However, the veins are more voluminous and have more anastomoses compared with the arteries[30]. The internal thoracic veins, the cranial superficial epigastric veins and intercostal veins drained the venous blood from thoracic glands. The caudal superficial epigastricvein drains the abdominal and inguinal glands. Craniocaudal anastomoses and frequent crossing of midline by small veins facilitates deposition of malignant tumor cells in adjacent and contralateral mammary glands[18,30].

Lateral cutaneous branches of the thoracic nerve innervate the thoracic mammary glands. The 4th, 5th and 6th thoracic nerve ventral branches (intercostal) innervate cranial while 6th and 7th innervate caudal mammary glands. Genitofemoral nerve and the ventral cutaneous branches of the first three lumbar nerves (cranial ilio-hypogastric, caudal ilio-hypogastric, and ilio-inguinal) innervate the abdominal and inguinal mammary glands[17].

Cranial thoracic mammary gland usually drains by the axillary node of same side, and, in few cases by the axillary and superficial cervical nodes simultaneously while caudal thoracic mammary gland drains by the axillary node of same side[31]. In neoplastic condition, cranial thoracic and caudal thoracic neoplastic mammary glands usually drain into the ipsilateral axillary lymph nodes and rarely superficial cervical or ventral thoracic lymph centers can be involved[32-34]. Cranial abdominal mammary gland usually drains by the axillary and superficial inguinal nodes simultaneously, but in some cases it drains only cranially to the axillary nodes or rarely, drains only caudally to the superficial inguinal nodes[35]. In neoplastic condition, cranial abdominal mammary gland drains ipsilateral axillary and superficial inguinal lymph nodes simultaneously, but sometimes only cranially into the ipsilateral axillary lymph nodes or rarely, it drains only caudally into the ipsilateral superficial inguinal and medial iliac lymph nodes, simultaneously[33]. The caudal abdominal mammary gland usually drains by the superficial inguinal nodes, but it may, rarely, drain to the superficial inguinal and medial iliac nodes simultaneously. In neoplastic condition, caudal abdominal mammary gland drains by inguinofemoral and popliteal lymph centers[32]. The inguinal mammary gland drains to the superficial inguinal nodes[31]. In neoplastic condition, inguinal mammary glands drains by ipsilateral superficial inguinal lymph nodes but rarely, does it also drain into the ipsilateral popliteal lymph node and into a lymphatic plexus at the medial aspect of the ipsilateral thigh[32,33].

Microscopically, epithelium and mesenchymal tissues form mammary glands. Mesenchymal component of mammary gland is made up of fibrous connective tissue, adipose tissue, blood vessels, nerves, lymphatics, and leukocytes that support epithelium components, ductular-lobular-alveolar structure, of mammary glands. Depending upon the age of bitch, stage of estrus cycle, and pregnancy, there is changes in the glandular morphology and proportion of epithelium and mesenchymal tissue. Subcutaneous tissue contain lobules of compound tubulo-alveolar glands and intralobular ducts[36]. Each teat has between 7 and 16 duct openings, and each of these ducts will eventually form a lobe of the adult gland and act as an independent functional unit within the gland[5]. Within each lobe of the breast, the main duct branches repeatedly to form a number of terminal ducts, each of which leads to a lobule consisting of multiple acini. Each terminal duct and its associated lobule is called a terminal duct-lobular unit[37]. These lobules are separated by adipose and collagenous connective tissue. Lactating and non-lactating glands have different glandular morphology. In lactating animals, alveoli are composed of cuboidal to columnar luminal cells surrounded by a star-shaped myoepithelial cells and they rest on basement membrane, principally composed of type IV collagen, laminin, nidogen, and heparin sulfate proteoglycan[5]. A luminal cell contains variable numbers of intracellular

fat droplets. Lumen of alveoli may fill with pretentious secretion and fat droplet. Through small and large ducts, secreted material of luminal cells of alveoli is taken away to teat sinus. Small ducts have similar structure like alveoli and are lined by single layer of cuboidal epithelium and fusiform myoepithelial cells. Bi-layered cuboidal epithelium surrounded by fusiform myoepithelial cells lined the larger ducts. Teat sinuses are lined by bi-layered columnar epithelium, surrounded by fusiform myoepithelial cells. From teat sinuses milk is push in to teat ducts. These ducts are lined by stratified squamous epithelium and have smooth muscle sphincter. A 7 to 16 teat ducts open in to teat. The skin surface of the teatis lined by stratified squamous epithelium (epidermis). Dermis of teat region lack most adnexal structure except number of fine hairs with their associated sebaceous systems[18]. Such fine hairs are not present on tip of the teat. In non-lactating animals, mammary glands have similar architecture as lactating mammary gland, except that they are smaller and without developed alveoli[38].

Effects of estrus cycle and related hormones on mammary gland morphology are extensively studied elsewhere[39-42]. In this review, we summarized histological changes occur in mammary gland during different stage of estrus cycle. In bitch, estrus cycle is divide in to four phases: proestrus, estrus, diestrus, and anestrus. The mammary gland is in inactive in the late anestrus, the proestrus, and the estrous phase of the cycle. In proestrus and estrus phases, mammary gland is composed of ducts and small lobular structures with high proportion of stromal tissue then glandular tissue. The duration of diestrus phase in bitches last for up to 75 days with functional corpus luteum[40,43]. During diestrus phase, mammary gland proliferates, differentiate, and regress. In early diestrus phase, plump fibroblasts proliferate around basophilic ductular structures and forms myxomatousstroma. Mitotic activity increased in epithelium cells of ducts and distended with eosinophilic secretions (from the previous cycle). In pubertal dogs, rather than distended duct, foci of undifferentiated, branching ducts surrounded by myxomatousstroma is evident[40]. In mid diestrus, due to proliferation of duct and formation of alveoli, relatively equal proportions of mesenchymal and glandular tissue are evident. Mid to late diestrus characterized by abundance of glandular tissue with lobules composed of closely packed acini with eosinophilic secretions in lumen and ducts distended with secretions. In late diestrus, early regression characterized by apoptosis of epithelium cells and atrophy of lobules is evident. Interlobular connective tissue is increased with distended ducts[40,42]. The anestrus phase may last up to 10 months in bitches. In early anestrus with regressing corpus luteum, regressive changes similar to those of the previous phase are seen. In late anestrus with completely regressed corpus luteum, shows prominent regressive features like marked lobular architecture and collapsed ducts. Proportion of stromal tissue is markedly increased and histologically this phase is difficult to differentiate from proestrus and estrus[40].

Epidemiology and risk factors of canine mammary tumors

National Cancer Institute (NCI), and GLOBOCAN regularly published human cancer incidence data worldwide. There are few agencies like California Animal Neoplasm Registry (ANR), USA Veterinary Medical Data Program (VMDP), and Animal Tumor Registry of Genoa (ATR) in Italy providing animal neoplasm epidemiological data in their respective tertiary. India has very large population of livestock and pets animals, yet information on animal onco-epidemiology is very meager due to unviability of reporting and registry system.

The exact incidences of mammary tumors are difficult to determine because actual dog population and tumor incidences are not reported and registered. Sometime, small benign tumors are either not brought to the veterinarian's attention or are not surgically removed. Still some studies are undertaken to know the incidences of canine mammary glands tumors. The annual incidence rate of canine mammary glands tumors has been estimated at 198/100000 by Schneider[44]. According to[9] the standardised incidence rate of canine mammary glands tumors were 205/100000 dogs per year. In Italy, incidences of all cancers was 272.1/100,000 in female dogs, and among these, highest incidence rates were detected for mammary cancer (191.8/100000)[45]. According to geographical area and considering some other factors, incidences of all cancers and mammary cancers per 100000 population of dogs were derived, but results are quite variable[46-48]. Among of all cancer incidences, ratio of canine mammary gland tumors is range from 16.8 to 46.87%[45,49-53]. Mammary glands tumors are also reported in male dogs[54-56].

As age advances, incidences of benign and malignant CMTs are increased. Highest incidences reported between 8 to 12 years of age[5,49,50,53,57,58], however somewhat older age (> 10 years) has been also reported[59-61]. In bitches, incidences of CMTs below 2 years of age are rare, but a sharp increase begins at approximately 6 years of age[50,52]. In one recent study stated that, there is statistically significant difference between age and malignancy. A mean age of 8.5 years suffer from benign tumors as compared to mean age of 9.5 years suffer from malignant tumors[62]. Younger dogs are more likely to have benign tumors than older dogs[5,57,59,63-65].

Breed-specific incidence rates of CMTs were differed across studies and geographical areas. Purebred and inbred bitches are at high risk for mammary glands

tumors[44,66]. According to geographical location, various breeds have increased risk of developing a mammary tumor. These breeds include; English Springer Spaniel, Brittany Spaniel, Cocker Spaniel, English Setter, Pointer, Afghan Hound, German Shepherd, Labrador Retriever, Boxer in addition to the typical smaller breeds such as Miniature and Toy Poodle, Maltese, Chihuahua, Beagle, Dachshund, West Highland White Terrier, Yorkshire Terrier, Shih Tzu, and Bichon Frise[5,30,49,52,64,65,67,68]. In India, breed have increased risk of developing a mammary tumor are: German Shepherd, Pomeranian, Spitz, Labrador, Boxer, Doberman, Cocker Spaniel, Bhutia, Great Daneand non-decrepit breed[53,55,58,61,69,70]. Earlier study[64] revealed that the incidence for mammary tumors varied by breed, from 319 dogs per 10,000 DYAR in the English springer spaniel to 5 dogs per 10,000 dog-years at risk (DYAR) in the rough-haired collie. As compared to other breed, English Springer Spaniel median age of onset of mammary gland tumors is 7 years and at 10 years of age about 32% female dogs are affected. This early onset mimics that of familial breast cancer in humans and indicates that inherited risk factors influence canine mammary tumors development. In genome-wide association analysis for canine mammary tumors in the English Springer Spaniel, research found significant peak on chromosome 11 and the candidate region includes a regulator of cyclin-dependent kinase 5 (CDK5RAP2) play important role in molecular pathogenesis of mammary tumors[71].

Another well-recognized risk factor for mammary tumor development is exposure of mammary tissue to ovarian hormones during first 2 years of life. Significant correlation between spaying, number of estrus cycle and incidence of mammary tumors was established[72]. There is an approximately 0.5% of the mammary cancer risk, if bitches are spayed before first estrus. If bitches are spayed between the first and the second estrus, mammary cancer risk is approximately 8%. Bitches spayed after second or more estrus cycle has 26% mammary cancer risk. There is equivocal effect of spaying after fourth estrus cycles on mammary cancer risk[57,63,72,73]. One study findings suggest that ovariohysterectomy when mammary tumors are removed does not have a significant effect on the progression of malignant disease and that about one in four bitches with a benign mammary tumor is likely to develop a further tumor in another gland[74]. Although, another study favors ovariohysterectomy at the time of mammary tumor excision, because this reduced the risk of new tumors by about 50% among dogs with benign mammary tumors[75].

Pathogenesis of canine pseudocyesis (pseudo-pregnancy) is not completely understood, although it is believed that prolactin plays a central role[76]. Effect of pseudocyesis and prolactin on development of CMTs is still under investigation. In bitches, it is hypothesized that estrus irregularity, pseudo-pregnancy, pregnancy, number of litters, number of puppies and their size do not have significantly influence mammary tumor risk[44,72,77]. However, in recent studies prolactin level is altered in bitches suffered from mammary tumors[78-80], thus more studies are required to elucidate role of prolactin in pathogenesis of mammary tumors.

Sex steroids, growth hormones, and growth factors play pivotal role in mammary gland proliferation, differentiation, and involution[81,82]. Progesterone and 17β-estradiol have significant effect on mammary gland carcinogenesis. It is hypothesized that progesterone increase growth hormones production from mammary glands tissue which might directly stimulate local or systemic IGF-I secretion. These hormones stimulate the proliferation of normal epithelium and increase in the number of susceptible cells which ultimately develop CMTs[81-86]. Administration of progesterone and estradiol increased the incidences of mammary tumors in canines[87-91].

Various studies advocating that, some nutritional factors and obesity predispose the mammary gland for cancer. Nutritional factors operating early in life may be of etiologic importance in canine mammary tumors[73]. Older age, obesity at 1 year of age, homemade meals (compared to that of commercial foods) and a high red meat (beef and pork, and a low intake of chicken) intake are increased the risk of mammary tumor[92,93]. Obesity is consider as risk factor for the development of mammary cancer in human and dogs. Obesity systemically and locally elevate estrogen levels and alter insulin levels and the insulin-like growth factor-1 (IGF-1) axis, thus influence development of mammary tumors[94,95]. In recent studies, researcher found that, mean age of CMTs onset was lower in the overweight or obese group than in the lean or ideal body weight group[96,97]. They also found that, in obese animals, expression of aromatase, estrogen and progesterone receptors, macrophages infiltrating within and around tumoral areas were increased while adiponectin expression was decreased.

Hallmarks of canine mammary tumors

When normal cells progressively evolve to malignant cells, many gene expressions and signaling pathways alter, resulting in heterogeneous tissue that acquired hallmark features of cancer. Cancer cells features distinguish them from normal cells, and these includes; sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, reprogramming of energy metabolism, evading immune destruction, and tumor promoting inflammation[98]. In this part of review, we will discuss these hallmarks features in context to CMTs.

Sustaining proliferative signaling

In normal cells, proliferation is strictly regulated hence normal number of cells and tissue architecture maintained. To maintain homeostasis, tissues carefully control the production and release of growth-promoting signals. In cancer cells, number of proto-oncogenes and oncogenes are dysregulated, which favors the uncontrolled growth of tumors cells.

Well-organized interaction of cyclins, cyclin-dependent kinases (Cdks) and Cdk inhibitors (CKIs) control the cell cycle. Cyclin proteins are important subunits do not have enzymatic activity of their own but activate CDKs by binding to them. In the cell cycle, cyclin D, cyclins E and A, and cyclins B and A, are associated with G1, S, and M phase of cell cycle, respectively[99]. Cyclin-dependent kinases contain a serine/threonine-specific catalytic core, whose activity requires association with specific cyclin subunits and have more than 20 members[100]. Based on the classical model of cell cycle regulation, D-type cyclins along with CDK4 or CDK6 oversee early events in the G1 phase. Cyclin E-CDK2 initiates the S phase, cyclin A-CDK2 and cyclin A-CDK1 control the progression through the S phase, and CDK1-cyclin B orchestrates mitosis[101]. A meager information is available on role of cyclin and CDK proteins in CMT development. Cyclin D1 over expression was found largely independent of the type of proliferative anomaly and was expressed in 60% of the pre-cancerous lesions and in 44% of cancerous lesions in mammary gland of bitch[102]. Increased expression of cyclin D1 in CMTs was reported by[103,104]. In few canine mammary tumors, cyclin A expression or amplification was noted[104,105].

The epidermal growth factor receptors are transmembrane receptors belong to family of receptor tyrosine kinases that initially recognized in avian erythroblastosis tumor virus and include four members: EGFR/ErbB-1, ErbB-2/HER-2/neu, ErbB-3/HER-3, and ErbB-4/HER-4[106]. Over expressions of these receptors activate downstream signals that responsible for oncogenic proliferation of cells. Among these four, HER-2 received great attention because similar to human over expressions have been reported in CMTs. HER-2 over-expressive tumors associated with poor clinical features and survival[107]. HER-2 over-expressive tumors are treated with monoclonal antibody therapy, Trastuzumab. The significance of HER-2 over-expression is unclear in CMTs. Although in many studies, HER-2 over-expression is detected by immunohistochemistry (IHC) but result show great variability[108-113]. To minimize the interlaboratory variation, researches recommended the use of recent guideline of American Society of Clinical Oncology/College of American Pathologists for detection of HER-2 expression by IHC[114,115]. Advocating HER-2 expressive CMTs should be considered a spontaneous animal model of HER-2 over-expressive human breast cancer because they found 92% amino acid homology for ErbB-2 between canine and human molecules and almost identical trastuzumab binding site in human and canine ErbB-2[116]. Although[117] more studies are required to consider CMT as model for HER-2 over expressive human breast cancer as they found that FDA-approved antibody in canine mammary tumors lack of specificity. The epidermal growth factor receptor (EGFR) expression in CMTs is seem to be associated with increased angiogenesis and metastasis[118-120].

Hormones play very crucial role in mammary gland development and lactation. Reproductive state and the endocrine milieu seem to play a pivotal role in the development of CMTs. Serum or tissue levels of estrogens, progesterone, prolactin, growth hormone, androstenedione, oestrone sulphate, dehydroepi and rosterone, and testosterone were higher in dogs with malignant neoplasms compared to those without tumors[80,121-123]. In humans, expressions of hormones receptors have better survival than tumors with low or no hormones receptors. In CMTs, role of estrogen and progesterone receptors expression as prognostic marker is still unclear. Estrogen and progesterone receptors expression are decreased in malignant CMTs[114] although variable percentages of positive estrogen and progesterone receptor tumors have been recorded[108,109,111,124-129].

Ki-67 protein (MKI67, marker of proliferation Ki-67) is strictly associated with cell proliferation with half-life of only - 1-1.5 hours. Ki-67 protein exclusively found in nucleus during G1, S and G2 phase of cell cycle, relocated to the surface of the chromosomes during mitosis, sharply decline during anaphase and telophase, but not expressed in resting cells (G0)[130,131]. Proliferating cell nuclear antigen (PCNA) is a ring like protein with central hole, belongs to DNA sliding clamp family and play important role in DNA repair, DNA synthesis, cell cycle regulation[132,133]. Biochemical analyses indicate the half-life of PCNA is 20 times longer than the half-life of Ki-67[134], hence PCNA pick expression found in G1 and S phase[135], and it may also found in the cells that have recently concluded the M phase and can be detected in noncycling cells because it is also involved in DNA repair[8]. Ki-67 and PCNA are excellent marker for determining the so-called growth fraction of a given cell population and tumor aggressiveness. Ki-67 and PCNA expression is significantly lower in the benign tumors and dysplasias than in the malignant tumors[136]. High index values of Ki-67 were positively correlated with metastasis[136], death from neoplasia, low disease-free survival rates, and low overall survival rates, while PCNA index had a positive correlation with the histologic malignant grade and nuclear grade. In recent study, proliferation index

of Ki-67 and PCNA of adjacent nontumoral mammary glands were associated with clinicopathological features of tumor aggressiveness and shorter overall survival[137].

Evading growth suppressors

The p53 gene, "Guardian of the genome" prevents neoplastic transformation by temporary or permanent cell cycle arrest and/or triggering apoptosis. The p53 gene is most commonly mutated genes in human cancers. In canine mammary tumors, mutations were detected in exons 4, 5, 6, 7 and 8 of the p53 gene and consisted of nonsense, splicing, and frame shift mutations[138-140]. Due to short half-life and relatively unstable, wild-type p53 protein is undetectable by immunohistochemistry[141]. In contrast, mutant p53 has a much longer half-life, and therefore, accumulates in the nucleus creating a stable target for immunohistochemical detection[142]. In many studies, p53 expression was detected in benign and malignant CMTs with help of IHC[143-149]. The varied expression of TP53 in canine mammary tumors indicates that TP53 gene expression alone cannot be regarded as a marker for tumor aggressiveness in canine mammary carcinomas[150].

Cyclin-dependent kinases inhibitors (CKIs) play important role in cell cycle regulation by controlling cyclin-CDK activity, differentiation, development, and acts as tumor suppressor. Mutation in the CKIs gene leads to malignant transformation. Role of CKIs is need to be elucidated in canine mammary tumors.

GATA-3 exhibits a multifaceted role in breast cancer. While it functions as a tumor suppressor by inhibiting TGF-β mediated epithelial-to-mesenchymal transition (EMT), a process crucial for metastasis, it can also interact with the estrogen receptor (ER) through both ER-dependent and independent pathways, suggesting a potential tumor-promoting function[151]. In CMTs expression of GATA-3 in ≥79.4% of cells showed significantly higher survival rates[151].

Resisting cell death

Apart from physiological role, apoptosis, a programmed cell death, is important mechanism that prevents cancer initiation and/or progression. Over-expression of regulatory gene or under expression of effector gene favors the cancer growth. Caspase-3 is an important member of execution-phase of cell apoptosis. Active caspase-3 was detected immunohistochemically in CMTs[152] and they found that, caspase-3 positive cells were significantly higher in benign tumors as compared to their malignant counterparts. BAX and BAK are important pro-apoptotic gene that activate apoptosis while BCL_2 and BCL-X_L are anti-apoptotic gene that inhibit the apoptosis[153]. In CMTs, BCL_2 expression was significantly increased in few studies[154-156]. Another anti-apoptotic gene Derlin-1 transport misfolded or unfolded proteins from the endoplasmic reticulum (ER) lumen to the cytoplasm of cells for further degradation and prevents endoplasmic reticulum stress-induced apoptosis[157]. Derlin-1 was detected immunohistochemically in CMTs[158] and they found that, adenocarcinomas and adenocarcinomas with lymph node metastasis showed moderate to marked expression of Derlin-1 while non-neoplastic gland and mammary adenomas showed weak expression. In CMTs, expression of anti-apoptotic gene, secreted frizzled-related proteins 2 were significantly increased as compared to normal mammary glands[103,159].

The Ataxia-Telangiectasia Mutated (ATM) gene belongs to a protein family known as the Phosphoinositide 3-Kinase related kinases (PIKKs)[160]. Some important function of ATM include, DNA double-strand breaks repair, recognition of damaged DNA, recruitment of repair proteins, and activation of apoptosis[160,161]. In recent study of Raposo-Ferreira *et al*.,[162] conclude that, the ATM gene was down-regulated in benign mammary tumors and carcinomas compared with normal canine mammary glands.

Heat-shock proteins (HSPs), also known as chaperones, play crucial roles in proteins folding/unfolding, cell-cycle control and signaling, and prevention of apoptosis[163]. Heat-shock proteins expressions were altered in several human cancer when compared with normal cells. In CMTs, expression of HSP 27[164,165], 70[156,164,166], 72[164,165], 90[156,164,165], were increased, while HSP 73[164,165] was decreased.

Enabling replicative immortality

Ceratin numbers of cell division, somatic cells lose the capacity to divide due to progressive shortening of telomeres at the ends of chromosomes. Somatic cells with short telomeres is either undergo senescence or undergo mitotic catastrophe and death[167]. Telomerase is an RNA-dependent DNA polymerase enzyme responsible for the maintenance of telomeres[168]. Activity of telomerase enzyme is a highly regulated and confined to cells like the germline, embryonic tissues, and stem cell populations of different organs[169]. In other cells, telomerase is inactivated during gestation, thereby restricting the proliferation program[170]. In humans 85% to 95% of cancers cells, telomere length is maintained due to upregulation of the enzyme telomerase[167]. Telomerase consists of two essential components: one is the functional RNA component, which serves as a template for telomeric DNA synthesis; the other is a catalytic protein with reverse transcriptase activity[171].

Earlier studies[172] reported the telomerase reverse transcriptase expression in normal and malignant mammary tissues by IHC and RT-PCR. Immunohistochemical expression was detected in 46/50 malignant tumors, 26/50 adjacent to the tumor mammary tissues

while healthy mammary tissue did not show any expression. Immunohistochemically, telomerase activity was detectable in adenomas, benign mixed tumors, and adenocarcinomas as examined by[173] but not in normal mammary glands, hyperplasia, and malignant mixed tumors. Telomerase activity was measured by the telomeric repeat amplification protocol assay[174]. They found that dog healthy somatic tissues showed little or no telomerase activity, while 21 mammary gland tumors and some other tumors showed telomerase activity. Further in another experiment they found that, telomere length was maintained in canine mammary gland tumors regardless of the age of the affected dog[175].

Inducing angiogenesis

Without blood supply, tumor cannot grow beyond 1 to 2 mm in diameter[167]. During tumor progression, new blood vessels are continuously form by angiogenesis, or vasculogenes is to sustain expand in neoplastic growths[98,167]. In health and probably in diseases vascular endothelial growth factor (VEGF) and thrombospondin-1 (TSP-1) are important angiogenesis inducer and inhibitor, respectively. Angiogenesis in tumor parenchyma increase chance of metastasis. In response to low level of oxygen, hypoxia-inducible factor 1α (HIF-1α) is secreted, which activates the transcription of VEGF, that promotes angiogenesis[176]. In CMTs, expression of VEGF is more pronounced in malignant tumors and less differentiated tumors as compared to benign tumors[177-181]. Among the canine malignant carcinoma, inflammatory mammary carcinoma showed more pronounced expression of VEGF than non-inflammatory mammary carcinoma[182-185]. As compared with the healthy dogs, plasma and serum level of VEGF were high in dogs with mammary gland tumors[176,186]. In CMTs, expression of HIF-1α is increase in carcinoma as compared to adenoma[187,188]. The gene expression of VEGF and HIF-1α by quantitative real-time PCR (qPCR) and the serum levels by ELISA was studied by[176]. They found that, serum levels of VEGF in the dog with mammary tumors was high as compared to healthy dogs and its highly correlated between abundant vascularization, lymph node involvement, metastasis, death rate and low survival. The serum level of HIF-1α in female dogs with metastasizing mammary neoplasia was higher than that in the healthy dogs. They also reported that, VEGFA gene was overexpressed in female dogs with poor outcome, in contrast to the gene HIF-1A was under expressed.

Vascular endothelial growth factor binds with specific endothelial receptors termed VEGFR-1 (Flt-1), VEGFR-2 (Flk-1) and VEGFR-3 to angiogenesis. Flk-1 expression was evaluated immunohistochemically in CMTs by[189]. They reported that, Flk-1 expression was higher in malignant than in benign tumors. Moreover, in the malignant tumors, expression of Flk-1 increased from well to less differentiated phenotypes (grade 1-3). Similar findings was reported by[190].

Activating invasion and metastasis

Local invagination and distant metastasis are two important features of malignant tumors. To achieve these features, the associated cancer cells typically developed alterations inthere, shape, attachment to other cells, tumor micro environment and alterations in the metastatic target site[98,191]. Multistep process of metastatic cascade start with local infiltration of tumor cells into the adjacent tissue (invasion) followed by entry into the vasculature followed by extravasation of cancer cells and subsequent proliferation colonization at the distal sites[192,193]. Epithelial-mesenchymal transition (EMT) is a cellular mechanism that allows a polarized epithelial cell to undergo multiple biochemical changes that enable it to assume a mesenchymal cell phenotype that includes enhanced migratory capacity, invasiveness, elevated resistance to apoptosis, and greatly increased production of ECM components[194]. Molecularly, EMT is characterized by loss of epithelial characteristics and the concomitant gain of a mesenchymal gene expression program[192].

A critical molecular feature of EMT is the down regulation of E-cadherin[195]. E-cadherin is a calcium-dependent cell adhesion molecule that localized on plasma membrane of most normal epithelial cells in regions of cell-cell contact known as adherens junctions[192,196]. E-cadherin acts as a tumor suppressor inhibiting invasion and metastasis, and it is frequently repressed or degraded during transformation[195]. In CMTs, expression of E-cadherin decreased with the increase in the neoplasm malignancy and proliferative activity[197-205]. E-cadherin expression was higher in normal and benign tumors as compared with carcinomas but lower in solid or poorly differentiated carcinomas as compared with simple, tubulo-papillary, complex or well differentiated carcinomas[198,200,204,205]. In CMTs, E-cadherin down regulation is might be induced by transcriptional repression mediated by the transcription factors SNAIL, and TWIST, whose primary function is to promote epithelial-to-mesenchymal transition (EMT)[167,206]. The investigations of[206] revealed E-cadherin expression and its relationship to those transcriptional repressors (i.e. SNAIL, and TWIST in spontaneously arising canine invasive micropapillary carcinoma. In normal and hyperplastic canine mammary glands, P-cadherin is restricted to myoepithelial cells, however in tumor tissues, P-cadherin expression was increased in both epithelial and myoepithelial cells, with a cytoplasmic pattern of cellular distribution[207]. Expression of beta 1 integrin, a adhesion receptors, in normal, dysplastic and neoplastic canine mammary glands by immunohistochemistry was evaluated[208]. They reported that, in malignant neoplasms, expression of beta 1 integrin was decreased

and redistributed along the entire cell membrane.

Matrix metalloproteinases (MMPs), collectively called matrixins, are zinc-dependent proteinases that play a pivotal role in tumor growth, invasion and metastasis, including proteolytic degradation of ECM, alteration of the cell-cell and cell-ECM interactions, migration and angiogenesis[209,210]. Matrix metalloproteinases includes, collagenases (MMP - 1, 8, 13, and 18), gelatinases (MMP-2, and 9), stromelysins (MMP-3, 10, and 11), matrilysins (MMP-7 and 26), membrane-type (MT) MMPs (MT MMP-14, 15, 16, 17, 24 and 25) and other MMPs[209]. Among all, gelatinases (MMP-2 and 9) are extensively studied in CMTs. In various immunohistochemical detection, mRNA expression and zymography studies on CMTs, expression of MMP-2[211-215], MMP-9[201,212,213,215-218], MT1-MMPs[214] are found to be significantly higher in malignant tumors than benign neoplasms or normal mammary tissue. These studies advocating that, MMP-2 and MMP-9 are probably produced by neoplastic and tumor-adjacent stromal cells contributed to digestion of ECM and facilitate invasion and metastasis. MMP3 may be useful as malignancy biomarkers as MMP3 were significantly overexpressed in malignant CMTs compared to benign CMTs[219].

Reprogramming of energy metabolism

Neoplastic cells are actively dividing cells that's requires high energy in form ATP for various cellular process and synthesis of new proteins. To meet high demand of energy, cancer cells acquire alterations to the energy metabolism. The best characterized alterations to the energy metabolism is Warburg effect, in which neoplastic cells shift from ATP generation through oxidative phosphorylation to ATP generation through glycolysis, even under normal oxygen concentrations (aerobic glycolysis)[220]. The Warburg effect is less efficient in terms of ATP generated per unit of glucose consumed than oxidative phosphorylation, thus required abnormally high rate of glucose uptake[221].To meet the high glucose demand neoplastic cells upregulate glucose transporters, notably GLUT1, which substantially increases glucose import into the cytoplasm[98]. Hypoxia present in all cancerous growth, is another condition which increased GLUT1 in neopsatic cells[221]. Hypoxia marker, pimonidazole to dogs affected by spontaneous mammary carcinoma was administered and compared with immunohistochemical staining for GLUT1 and 3, and other proteins[222]. They found statistically significant correlation between pimonidazole staining and GLUT1-expression. The experiment of[223] suggested that GLUT1 is more highly expressed in neoplastic epithelium and mesenchyme than normal canine mammary glands. The activities of D-glucose transport (D-GT) in adenocarcinoma were over three-and-a-half times higher than in mammary gland from normal dogs[224]. They also reported that, the activities of hexokinase and pyruvate kinase, in the adenocarcinoma were also more than three-and-a-half times higher than in the controls.

Tumor promoting inflammation

Most tumors are variable infiltrated with varieties of inflammatory/immune cells that either help in eradication of tumors or benefits the proliferation and survival of malignant cells, promoting angiogenesis and metastasis. Among all leukocyte population, macrophages are predominant in number, consider as "tumor-associated macrophages (TAMs)". Tumor-associated macrophages secret variety of substance, that play prominent role in breast cancer progression via angiogenesis, migration, metastasis, and immune evasion[225]. Few studies have been undertaken to characterized role of TAMs in CMTs. TAMs expression in CMTs by immunohistochemistry was evaluated by[226]. They found that, TAMs value was significantly higher in malignant CMTs than benign CMT as well as higher levels of TAMs associated with decreased overall survival. According to[227] the numbers of TMAs, MCSF-R (macrophages colony stimulating factor receptor) and CD14 are related to the cancer ability to metastasize. TAMs were significantly associated with malignant CMT and VEGF positive tumors and also associated with VEGF expression within malignant CMT[228]. The degree of T and B lymphocyte infiltration was significantly higher in malignant CMTs with lymphatic invasion and high histologic grade than in those without lymphatic invasion and lower histologic grade[229]. Animals with high proportions of CD4+ and low CD8+ T-cells had lower survival rates was demonstrated by[230].

Prostaglandins, the chemical mediators of inflammation, generated by the actions of two cyclooxgenases, called COX-1 (constitutive) and COX-2 (inducible) from arachidonic acid present in membrane phospholipids[231]. In comparison to COX-1, COX-2 is extensively studied in CMTs. COX-2 expression affects mammary tumorigenesis by promoting angiogenesis and cell proliferation, encouraging metastatic spread and tumor-associated inflammation[232]. In many studies, upregulation of COX-2 has been reported in CMTs[232-238]. In CMTs, increased Cox-2 expression is related with angiogenesis, malignancy, worse prognosis and shorter overall survival[181,183,232,236,239,240]. According to reports of[234] Cox-1 immunoexpression did not differ significantly between benign and malignant lesions.

Evading immune destruction

Increased incidences of some cancers in immuno deficient patients indicate the roll of host immunity in development and progression of cancers. Immune system continuously identified and removes many cancer cells from the body before they form a neoplasm. Even though, cancers occur in immunocompetent patients indicate

Table 1. Histopathologic Classification[252].

1. Malignant Epithelial Neoplasms
- Carcinoma - in situ
- Carcinoma - simple
  a. Tubular
  b. Tubulo-papillary
  c. Cystic-papillary
  d. Cribriform
- Carcinoma - micropapillary invasive
- Carcinoma - solid
- Comedocarcinoma
- Carcinoma - anaplastic
- Carcinoma arising in a complex adenoma/mixed tumor
- Carcinoma - complex type
- Carcinoma and malignant myoepithelioma
- Carcinoma - mixed type
- Ductal carcinoma
- Intraductal papillary carcinoma

2. Malignant Epithelial Neoplasms - Special Types
- Squamous cell carcinoma
- Adenosquamous carcinoma
- Mucinous carcinoma
- Lipid-rich (secretory) carcinoma
- Spindle cell carcinomas
- Malignant myoepithelioma
- Squamous cell carcinoma - spindle cell variant
- Carcinoma - spindle cell variant
- Inflammatory carcinoma

3. Malignant Mesenchymal Neoplasms - Sarcomas
- Osteosarcoma
- Chondrosarcoma
- Fibrosarcoma
- Hemangiosarcoma
- Other sarcomas

4. Carcinosarcoma - Malignant Mixed Mammary Tumor

5. Benign Neoplasms
- Adenoma - simple
- Intraductal papillary adenoma (duct papilloma)
- Ductal adenoma (basaloid adenoma)
  o With squamous differentiation (keratohyaline granules)
- Fibroadenoma
- Myoepithelioma
- Complex adenoma (adenomyoepithelioma)
- Benign mixed tumor

6. Hyperplasia/Dysplasia
- Duct ectasia
- Lobular hyperplasia (adenosis)
  o Regular
  o With secretory activity (lactational)
  o With fibrosis - interlobular fibrous connective tissue
  o With atypia
- Epitheliosis
- Papillomatosis
- Fibroadenomatous change
- Gynecomastia

7. Neoplasms of the Nipple
- Adenoma
- Carcinoma
- Carcinoma with epidermal infiltration (Paget like disease)

8. Hyperplasia/Dysplasia of the Nipple
- Melanosis of the skin of the nipple

that neoplasms have host immuno-editing capacity in which they become invisible to the host immune system or induced immunosuppression. Studies that explain how CMTs evade immune destruction are limited. Major histocompatibility complex (MHC) molecules are key players of anti-tumoral immunity. Down regulation of MHC class I & II expression has been reported in breast cancer[241]. Loss of MHC class I expression from canine mammary gland carcinomas was significantly correlated with poor prognosis as found by[242]. Dendritic cells (DCs) are the most potent antigen-presenting cells (APCs) and are able to stimulate naive T lymphocytes and to generate memory T lymphocytes. Mouse models demonstrate that the generation of protective anti-tumor immunity depends on the presentation of tumor antigens by DCs[243]. Many neoplasms secret IL-10, thus inhibit DC maturation or tumor-derived factors can alter the maturation of DCs[243,244] have evaluated the presence of immature and mature myeloid DCs, plasmacytoid DCs and MHC-II in CMTs. They reported that immature DCs were predominate in CMTs as compared to normal mammary glands. Predominance of immature DCs in the tumor group is possibly related to an inefficient immune response, promoting the development and survival of tumor cells. They also reported that immuno detection of MHC-II was not significant when comparing the groups. RCAS1 (receptor-binding cancer antigen expressed on SiSo cells), one of novel cancer cell-surface antigens that strongly expressed in invasive cancers. RCAS1 inhibited the invitro growth of receptor-expressing cells and induced apoptotic cell death. Due to this, tumor cells may evade immune surveillance by expression of RCAS1, which would suppress clonal expansion and induce apoptosis in RCAS1 receptor-positive immune cells[245,246] have evaluated expression of the RCAS1 in CMTs. Immunohistochemically, RCAS1 was negative in 100% of normal mammary glands, but all malignant CMTs showed cytoplasmic expression of RCAS1 with no polarity of expression, while in benign mammary tumors, it was detected on the luminal surface of the tumor cell. They concluded that RCAS1 expression or localization was significantly correlated with malignancy.

Programmed cell death protein 1 (PD-1), found on tumor-infiltrating lymphocytes, and programmed death ligand 1 (PD-L1), expressed by tumor cells, are immune checkpoint proteins. These proteins act like a regulatory switch, controlling the immune system response to cancer cells. In one study, PD-L1 significantly overexpressed in malignant CMTs compared to benign CMTs, suggesting it may be useful as malignancy biomarker[219].

Classification of canine mammary tumors

In 1974, the World Health Organization published the first ''International Histological Classification of Tumors of Domestic Animals'' which included tumors and dysplasia of the mammary gland. Other several classifications of CMTs had been proposed in past[52,247-251]. Recently[252] have proposed modifications to the WHO classification criteria to create a more morphologically diverse and detailed classification system for CMTs. Recent classification is listed in Table 1. This system describes and proposes a nomenclature for tumors and hyperplasia/dysplasia. Some neoplasms (carcinoma-solid and carcinoma-anaplastic) retain the same names, while others (carcinoma-micropapillary invasive and ductal carcinoma) are separated from their previously assigned types. In addition, this classification adds new types such as inflammatory carcinoma. Many studies have been undertaken for histopathological classification of CMTs in India[55,58,61,253-260] and abroad[45,111,198,261].

A 2-year study of[262] validated the 2011 histologic classification as an independent prognostic indicator in 229 female dogs. They reported that anaplastic carcinoma and carcinosarcoma had the worst prognosis (3 months median survival) and the highest metastasis rates (89% and 100%, respectively), while adenosquamous carcinoma was most likely to recur locally (50%). Dogs with complex carcinoma and simple tubular carcinoma experienced longer survival times. In contrast, those with simple tubulopapillary carcinoma, intraductal papillary carcinoma, and carcinoma with malignant myoepithelioma faced a more than tenfold higher risk of tumor-related death. The study found an excellent prognosis for all dogs with benign tumors, even those where a carcinoma arose within a previously benign

Table 2. Histopathologic malignancy grade of canine mammary gland carcinoma[265].

Methodology

1. Tubule and gland formation are assessed and given 1 to 3 points, as follows:

- One point : Tubules or glands formed in >75% of the tumor.
- Two points : Tubules or glands formed in 10% to 75% of the tumor.
- Three points : Few, if any, tubules formed, accounting for <10% of the tumor.
  - In complex and mixed tumors, the percentage of tubular formation will be scored considering only epithelial areas.
  - In malignant myoepithelioma, tubular formation will be 2.
  - In heterogeneous canine mammary carcinomas, tubular scoring shall be assessed in the most representative malignant area.

2. Nuclear pleomorphism is assessed and given 1 to 3 points, as follows:

- One point : Uniform or regular small nucleus and occasional nucleoli.
- Two points : Moderate degree of variation in nuclear size and shape, hyperchromatic nucleus, presence of nucleoli (some of which can be prominent).
- Three points : Marked variation in nuclear size, hyperchromatic nucleus, often with prominent nucleoli.
  - In complex and mixed tumors, nuclear pleomorphism will be evaluated in all the malignant components.

3. Mitotic figures are counted, and the scores are converted into 1 to 3 points, as follows:

- Field diameter (0.55 mm)
- One point : 0-9 mitoses/10 HPF
- Two points : 10-19 mitoses/10 HPF
- Three points : ≥ 20 mitoses/10 HPF
  - The fields will be selected at the periphery or the most mitotically active parts of the sample (not only epithelial cells).

The final grade of malignancy, combining values of the above 3 features, will be calculated as follows:

- Grade 1 or Low grade or Well-differentiated carcinoma : 3 to 5 points.
- Grade 2 or Intermediate grade or Moderately differentiated carcinoma : 6 to 7 points.
- Grade 3 or High grade or Poorly differentiated carcinoma : 8 to 9 points.

mixed tumor.

Grading of canine mammary tumors

Histological diversity of canine mammary tumors makes their diagnosis difficult and provides little prognostic information. The use of a histological grading system may be helpful for classification and prognosis. Among various suggested grading systems in humans, the most prevalent system used worldwide is the Elston and Ellis numeric method (Nottingham method) for grading human breast cancer[263]. This grading system is based on the assessment of the following three morphological features : tubule formation, nuclear pleomorphism, and mitotic counts. Each of these features is scored from 1 to 3, and this grading system is mostly directed at invasive (simple) adenocarcinomas.

In veterinary science, 2 slightly different Misdorp[10] and Peña[264] systems (modifications of the human method) are used for histological grading of the canine mammary gland carcinoma. This previous study showed that both histological grading systems are significant predictors of the risk of lymphatic vessel invasion and regional lymph node metastases at the time of diagnosis. However, the Peña system has been shown to have a better predictive ability[265]. Recently Peña *et al*.,[265] evaluated Pena system as a prognostic indicator in canine mammary carcinomas. They found that the tumor size, clinical stage, histological diagnosis, presence/absence of myoepithelial proliferation, and regional lymph node metastases at diagnosis were significantly associated with histological grade. A Peña system is listed in Table 2.

Molecular classification of canine mammary tumors

Using gene expression analysis on DNA microarrays, molecular classification of breast cancer was done by[266]. Gene expression profiling with breast carcinomas has allowed further classification of these tumors into 5 distinct subtypes (luminal A, luminal B, HER2-overexpression, basal-like, and normal-like) with unique clinical outcomes[267]. Among five types luminal A tumors shows most favourable clinical features; luminal B tumors shows less favourable clinical features than luminal A; Basal like and HER2-overexpression tumors associated with poor clinical features and survival[107]. Identification of molecular type of tumors helps to determine the treatment protocol in human. Positive staining for ER or PR recommends the patients for endocrine treatment, while in HER2-overexpression tumors monoclonal antibody therapy, Trastuzumab.

Table 3. Molecular Classification[109].

- Luminal like A : ER+ and/or PR+, HER2-, any CK5/6 or CK14
- Luminal like B : ER+ and/or PR+, HER2+, any CK5/6 or CK14
- ERB-B2 expressing : ER-, PR-, HER2+, any CK5/6 or CK14
- Basal like (triple negative) : ER- and PR-, HER2-, CK5/6+ and/or CK14+
- Unclassified or normal like : Negative for all markers

Although guidelines for the treatment of human breast cancers are based on the molecular subtype, application of gene expression profiling is not easy for routine diagnosis. Given the simplicity and the clinical relevance of the molecular classification, it appears that immunohistochemical markers can be used to identify molecular subtypes in routine diagnostic use[268]. In dogs, few studies have been undertaken using the above immunohistochemical classification, and the results are not consensual[108-111,113,269]. As proposed by Im *et al*.,[111] and Sassi *et al*.,[109], CMTs can be classified by immunohistochemistry (IHC) in luminal A, luminal B, HER2-overexpression, basal-like, and normal-like. Table 3 showing criteria used for molecular subtyping, however, the application of molecular subtyping is still uncertain. According to Im *et al*.,[111] and Ribeiro *et al*.,[110] most frequently occurring molecular subtype was luminal A, while according to Sassi *et al*.,[109] most frequently occurring molecular subtype was luminal B.

FUTURE PROSPECTIVE

CMTs are the most frequently reported tumors of dogs, even though their prenatal development and molecular pathogenesis is not clearly understood. Due to spontaneous occurrence, similarities and differences in their molecular pathogenesis, CMT is considered a spontaneous animal model of human breast cancer. In India and many other countries, tumor reporting and registry system need to be strengthened. Immunohistochemistry is very essential tool and widely used in diagnostic issues, estimating prognosis or predicting response to therapy, even though many factors *viz.*, fixation, antigen retrieval methods, selection of clone are need to be harmonized across the veterinary pathology laboratories. Many more molecules *viz.*, Von hippellindau protein, Thrombospondin-1 (TSP-1), PMSD-10 are need to be evaluated to get more information on molecular carcinogenesis of CMTs.

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Indian J. Vet. Pathol., 48(3) : 226-230, 2024: DOI: 10.5958/0973-970X.2024.00037.8

RESEARCH ARTICLE

Comparison of diagnostic tests for detection of Canine Parvo Viral Enteritis

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Received: 19.12.2022; Accepted: 3.4.2024

ABSTRACT

The canine parvovirus enteritis was diagnosed in the present study using the lateral flow assay (LFA), polymerase chain reaction (PCR) for VP-2 gene amplification and fluorescent antibody test (FAT). For this study, faeces samples were taken from 50 canine parvovirus suspected dogs of any breed and sex but age between 3 months to 1 year, presented at Multispeciality Veterinary hospital, GADVASU, Ludhiana from April 2021 to April 2022. Results from LFA, PCR, and FAT revealed that 44% (22/50), 48% (24/50), and 40% (20/50) of canine parvoviral enteritis cases were positive, respectively. In comparison to PCR, ICT and FAT demonstrated 83.3% and 66.7% sensitivity as well as 92.3% and 84.6% specificity, respectively. Further, the canine parvovirus was also identified as CPV-2c using phylogenetic analysis, indicating 100% similarity with QBQ84353.1 strains from China based on a partial VP2 gene. This study demonstrated the field applicability of ICT, a highly sensitive and specific test, and the applicability of FAT, a backup test, in the absence of PCR and ICT.

Keywords: Canine parvovirus, FAT, ICT, PCR

INTRODUCTION

Canine parvovirus disease is highly contagious and causes severe acute haemorrhagic gastroenteritis and myocarditis in dogs of any breed, age or sex but puppies between 6 weeks and 6 months of age appear to be more susceptible. Canine parvovirus was first time identified as a cause of gastrointestinal and respiratory disease in canines in 1967. It is non-enveloped, single stranded DNA virus belonging to the family *Parvoviridae.* Three strains of canine parvovirus-2 are strains 2a, 2b and 2c. The virus can survive at ambient temperature for up to three months. Virus mainly infects rapidly proliferating cells such as intestinal epithelium, lymphatic tissues and bone marrow[1]. About 27% of canine diarrhoea cases are caused by CPV[2]. Various factors predispose the puppies to parvoviral infection *viz.* lack of protective immunity, intestinal parasites, overcrowding, unsanitary and stressful environmental condition. Intestinal form and cardiac form are the two main manifestations of the disease. Four to eight week old puppies experienced the cardiac form. Because most dams are immune and pups are shielded from perinatal CPV infection, parvoviral myocarditis is now extremely rare. The intestinal form is seen in puppies that are 8 to 12 weeks old. The disease's usual clinical symptoms include nausea, vomiting, and diarrhoea that is foul-smelling, mucoid or haemorrhagic and causes dehydration, hypovolemic shock, septicaemia, endotoxemia, and death[3]. Faecal excretions from infected dogs contain >10^9/gm virus particles during the acute phase and is the most suitable sample for detection of canine parvovirus in the enteric form of the disease. Since, clinical signs of the disease resemble other enteric diseases, so rapid and reliable diagnosis is critical to initiate the treatment. Currently, a wide variety of diagnostic tools are in use for specific viral diagnosis. PCR is most sensitive and specific method for detection of canine parvovius[4]. In recent years rapid and cost effective pen side diagnostic kits based on the principle of lateral flow assay are widely used[5]. The objective of the study is to compare the diagnostic accuracy and clinical utility of PCR, lateral flow assay (LFA) kits, and fluorescent antibody test (FAT) for the detection of canine parvovirus (CPV) in clinical settings. This comparison aims to identify the strengths and limitations of each diagnostic method, facilitating informed decision-making regarding their application in routine veterinary practice and outbreak management strategies.

How to cite this article : Saini, A., Mahajan, V., Kaur, G. and Sandhu, B.S. 2024. Comparison of diagnostic tests for detection of Canine Parvo Viral Enteritis. Indian J. Vet. Pathol., 48 (3) : 226-230.

MATERIALS AND METHODS

This work was conducted at department of veterinary

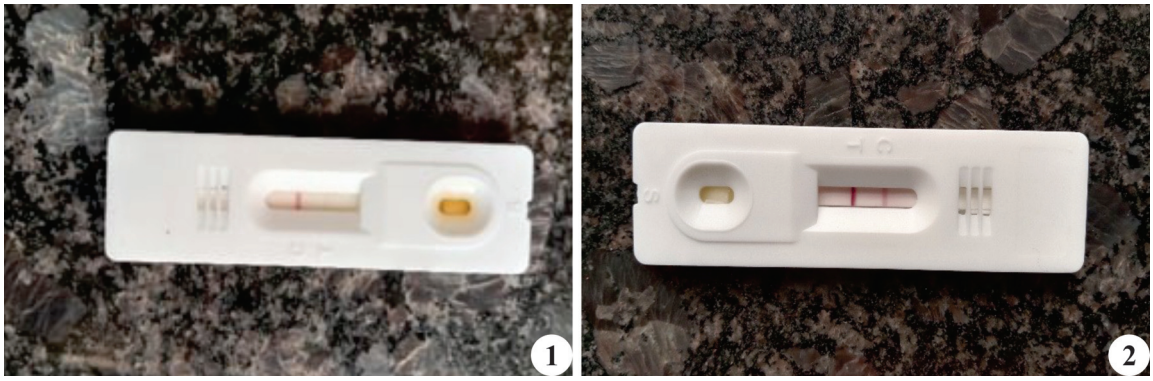

Fig. 1. Canine parvovirus Ubio quick VET kit showing negative result; **Fig. 2.** Canine parvovirus Ubio quick VET kit showing positive result.

pathology and animal disease research centre, college of veterinary science, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, Punjab from April 2021 to April 2022. For the purpose of detecting canine parvovirus infection, several diagnostic techniques, including PCR, FAT and ICT, were performed and for this, total 50 faecal samples were collected from CPV suspected dogs with clinical signs of fever, depression, vomiting and haemorrhagic foul-smelling diarrhoea and of different breeds in age group between 3 months to 1 year, presented to multispeciality veterinary hospital, GADVASU, Ludhiana. Immunochromatographic studies were carried out first for detection of canine parvoviral antigen in fecal samples. Fecal samples were collected with the help of sterile swab dipped in CPV diluent and antigen was detected by Ubio quickVET rapid antigen test kit (Ubio Biotechnology Systems Pvt Ltd, Kerala). Polymerase chain reaction was used for further confirmation of CPV. For this, approximately 250 mg of fecal samples were used for DNA extraction using commercially available QIAamp® PowerFecal® DNA kit by Qiagen as per manufacturer's guidelines. PCR was standardized by the use of a set of published primers[5] VP2 Forward: 5'-CAGGTGATGAATTTGCTACA-3' and Reverse: 5'-CATTTGGATAAACTGGTGGT-3'. Reaction mixture (25 µl) contained master mix 13 µl, forward primer 1 µl, reverse primer 1 µl, template DNA 5 µl and nuclease-free water 5 µl. PCR was performed for the amplification of 611 bp fragment of VP-2 gene in Veriti 96 Well Thermal Cycler with initial denaturation at 94^{O}C for 3 min and then 40 cycles consisting of denaturation at 94^{O}C for 30 sec, annealing at 57^{O}C for 1 min and extension at 72^{O}C for 1 min and final extension at 72^{O}C for 7 min. This reaction product 6 µl along with 1 µl loading dye were analysed electrophoretically in 1.5% agarose gel in 1X TBE buffer after staining with ethidium bromide. To determine the size of the amplified product, a DNA ladder (3 µl) and a known positive control (6 µl) were also run. DNA bands were seen using a UV light and photographed using gel documentation system (Bio Rad). Sequencing was also done by sending amplified VP2 gene of canine parvovirus to Barcode biosciences, Bangalore. The phylogenetic tree was constructed by neighbour-joining method using MEGA 6.0 software[6]. Fluorescent antibody test was performed for the first time on 50 CPV suspected dogs after preparation of smear on slide and fixation with cold acetone and then the standard procedure was used for the detection of CPV antigen in fecal sample.

RESULTS

In this investigation, out of 50 suspected CPV cases, 28 (56%) samples were found to be negative (Fig. 1) and 22 (44%) samples were found to be positive (Fig. 2) using the Ubio quickVET rapid Ag test kit. Further validation through PCR on 50 samples, 24 (48%) were found to be positive (Fig. 3) and 26 (52%) were negative using PCR. Based on a partial VP2 gene, a phylogenetic study of canine parvovirus indicated that it is CPV-2c, showing 100% identity with QBQ84353. 1 strains from China. The virus has displayed 97.8% identity with CPV-2a strains and 98.4% identity with CPV-2b strains. Phylogenetic tree shown in Fig. 4. FAT revealed 20 samples showed green birefringence, indicating a positive result when compared to a positive control (VMRD, USA). Positive control and sample results are shown in Figs. 5-8.

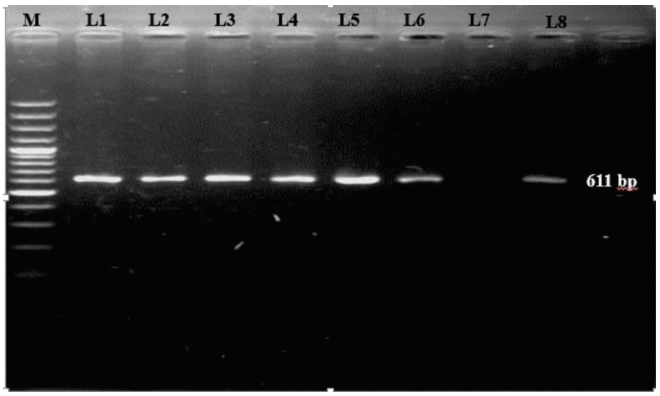

Fig. 3. PCR amplification product (VP2 gene). M : Ladder; L1, L2, L3, L4, L5, L6 : Samples showing positive result; L7 : Negative control; L8 : Positive control.

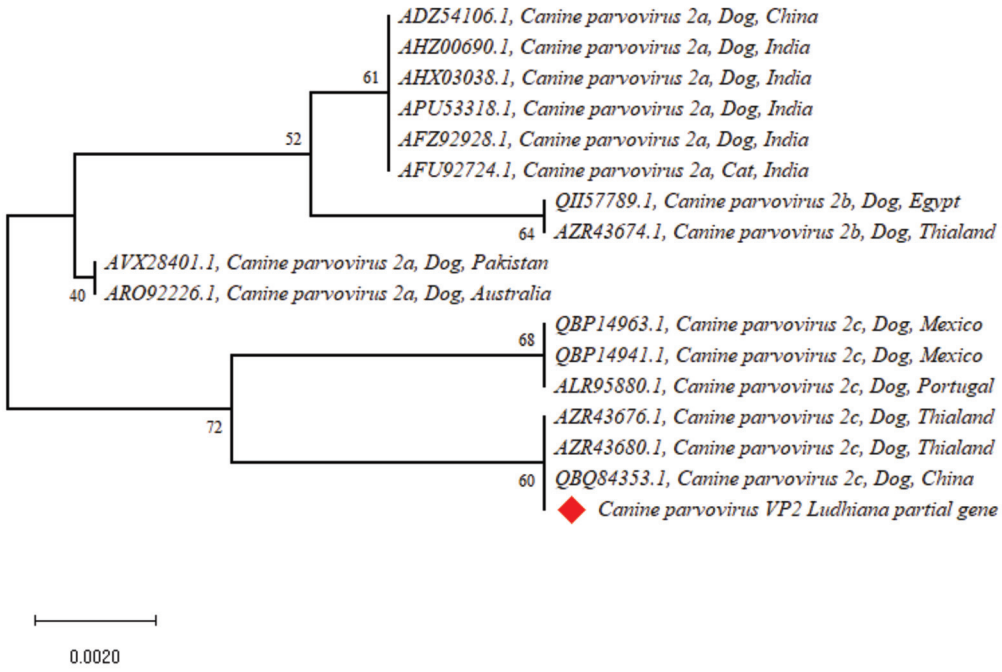

Fig. 4. Neighbour Joining tree showing clustering of present study canine parvovirus strain based on nucleotide sequence of VP2 gene using MEGA-X program. The bootstrap value are shown on the nodes of the tree and genetic distance is shown in 0.0020 scale bar. Sequence obtained in this study is shown with highlighted taxon marker.

In comparing, ICT and PCR for cases where CPV was suspected, PCR was considered the gold standard test. While PCR detected four positive samples, the immunochromatographic testing showed them as negative. Consequently, sensitivity of the immunochromatographic test was 83.33% when compared to PCR. Only 2 cases had immunochromatography results that were positive but PCR results that were negative. As a result, when compared to PCR, the specificity of the immunochromatographic test was 92.3%. The two tests had an 88% overall agreement. This comparison is presented in Table 1. The kappa value represents the degree of agreement between two tests. When the two tests were compared, the Kappa value was 0.759,

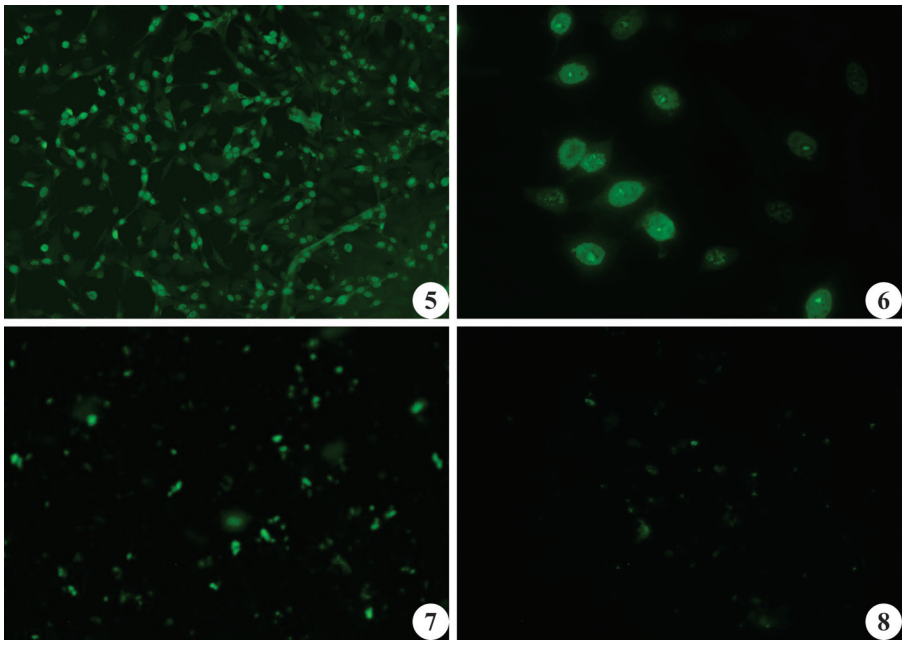

Fig. 5. Positive control slide at low power (FAT); **Fig. 6.** Positive control slide at high power (FAT); **Fig. 7.** Sample showing positive result on FAT; **Fig. 8.** Sample showing mild positive result on FAT.

Table 1. Sensitivity and specificity of immunochromatography Ag test kit in comparison to polymerase chain reaction.

TEST	Polymerase Chain Reaction (PCR)			Sensitivity	Specificity	Overall Agreement	Kappa Value (Agreement)
Ubio Quick VET test kit	Positive	20	2	20/24*100 = 83.33%	24/26*100 = 92.3%	88%	0.759 (Substantial) (0.477 - 0.889, CI = 95%)
	Negative	4	24				

Table 2. Sensitivity and specificity of Fluorescent Antibody Test in comparison to Polymerase Chain Reaction.

TEST	Polymerase Chain Reaction (PCR)			Sensitivity	Specificity	Overall Agreement	Kappa Value (Strength)
Fluorescent Antibody Test (FAT)	Positive	16	4	16/24*100 = 66.67%	22/26*100 = 84.61%	76%	0.516 (Moderate) (0.209 - 0.719, CI = 95%)
	Negative	8	22				

indicating that there was substantial degree of agreement between the two tests.

Moreover, comparisons between the PCR and FAT tests were also made. Both the FAT and PCR results for 16 samples were positive. There were 8 cases where the PCR was positive but the FAT was negative. Therefore, the sensitivity of FAT was 66.67% as compared to PCR, and 4 cases that tested positive with FAT but not PCR. As a result, the specificity of FAT was 84.61% when compared with PCR. The overall agreement between the two tests was 76% (Table 2). By employing PCR as a reference test, the agreement between PCR and FAT was also determined using the kappa value. The Kappa value when the two tests were compared was 0.516 (CI = 95%), indicating moderate agreement between the two tests. Following the testing, comparisons between ICT and FAT were made. Eight cases were positive with ICT but negative with FAT, while 14 samples were both FAT and IC positive. Therefore, the sensitivity of FAT was 63.63% as compared to IC and 6 cases were positive with FAT but negative with IC. Therefore, the specificity of FAT was 78.57% as compared to IC. There was 72% overall agreement between the two tests (Table 3). Kappa value was also used to find out the agreement between ICT and FAT using PCR as standardized test. When the two tests compared, Kappa value was 0.426 which indicated moderate agreement between both the tests.

Depends on the age of CPV suspected dogs, they were divided into 3 groups to study age wise prevalence. These groups were divided as ≤3 months, 3-6 months and >6 months. The age wise prevalence recorded for ≤3 months of age was 59.09% (13 out of 22 examined dogs), followed by 3-6 months of age 47.06% (8 out of 17 dogs) and >6 months of age 27.27% (3 out of 11) by Ubio quickVET rapid antigen test kit. Thus the highest prevalence was reported in the age group of ≤3 months as given in Table 4.

DISCUSSION

Rapid and cost effective pen side diagnostic kits based on the principle of lateral flow assay are widely used in recent years[6]. According to some previous study[7], the RapiGEN Canine Parvo Ag test was one of the most reliable, simple, quick (5-10 minutes to complete), and one-step tests based on the immunochromatographic detection of canine parvovirus antigen in canine faeces. In this study also, ICT was used and 44% positive samples were found as similar with previous finding[8]. PCR is most sensitive and specific method for detection of canine parvovius[4]. When comparing the prevalence rates of the different CPV strains, one previous study[9] found that CPV-2c was highly prevalent (51.8%), while another study[10] found that CPV-2b was the more common strain (50%) and one more study[11] found that 96.43% of all cases were CPV-2a strain positive cases. Previous study[12] suggested the following ranges for strength of agreement for the kappa coefficient: ≤0 = poor, 0.01-0.20 = slight, 0.21-0.40 = fair, 0.41-0.60 = moderate, 0.61-0.80 = substantial, and 0.81-1 = almost perfect. Sensitivity and specificity of immunochromatographic testing compared to PCR were 83.33% and 92.3% respectively, with an overall agreement of 88% and a Kappa value of 0.759, indicating substantial agreement which is almost similar with the previous studies[13] who found 73.68% of sensitivity and

Table 3. Sensitivity and specificity of Fluorescent Antibody Test in comparison to Lateral flow assay.

TEST	Immunochromatographic Test			Sensitivity	Specificity	Overall Agreement	Kappa Value (Strength)
Fluorescent Antibody Test (FAT)	Positive	14	6	14/22*100 = 63.63%	2/28*100 = 78.57%	72%	0.426 (Moderate) (0.110 - 0.670, CI = 95%)
	Negative	8	22				

Table 4. Age wise prevalence of canine parvovirus in dogs.

S. No.	Age in months	No. of Dog examined	No. of CPV affected dogs	Prevalence (%)
1.	≤3	22	13	59.09%
2.	3-6	17	8	47.06%
3.	>6	11	3	27.27%
	Total	50	24	48%
	Chi-Sq		2.983	
	df		2	
	P value		0.225	

100% of specificity when Ubio fast VET rapid Ag test kit was compared with PCR. The false negative results could be caused by low viral antigen levels in the faeces at a later stage of infection or by high antibody titres in the intestinal lumen at the time of sample collection. Due to the possibility of PCR inhibitors present at the time of testing, two samples that tested negative on PCR but positive on IC. Comparisons between PCR and FAT revealed 16 positive results for both, with a sensitivity of FAT of 66.67% and a specificity of 84.61%, resulting in a moderate agreement (Kappa value = 0.516). Almost similar results were made by one previous study[14] who discovered that all cases that tested positive for canine and feline parvovirus (CPV) in PCR tests also tested positive in FAT samples taken from the tongue.

In the present study, age wise prevalence was high in ≤3 months old dogs which agreed with the previous studies[15,16] who also showed high prevalence rates of CPV in 0-3 month pups or pups below 6 months age and prevalence rates recorded were 77.08% and 65.67% respectively. The reason for high prevalence of CPV in young pups is because of their habit to eat garbage, foreign objects etc., and their weak immune response[21] or lack of maternal antibodies in pups after 6 weeks and in pups of unvaccinated mother or non-availability of vaccines or vaccination failure. Since enterocytes in intestinal crypts have a higher mitotic index and young puppies (less than 8 weeks) exhibit higher intestinal and cardiac mitotic activity than older puppies, it may also be one of the reason that young puppies have a high prevalence of CPV infection.

This study demonstrates that ICT is a sensitive and specific diagnostic tool suitable for field settings in identifying canine parvovirus infection. Additionally, FAT can serve as a reliable backup test in situations where PCR and ICT are unavailable. Furthermore, phylogenetic analysis based on a partial VP2 gene revealed that the canine parvovirus strain detected belongs to CPV-2c.

ACKNOWLEDGEMENTS

Authors are highly grateful to Dr B.S. Sandhu, Head of Pathology Department, College of Veterinary Sciences, GADVASU, Ludhiana for providing necessary support to conduct the research programme.

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Indian J. Vet. Pathol., 48(3) : 231-235, 2024: DOI: 10.5958/0973-970X.2024.00038.4

RESEARCH ARTICLE

Histomorphological and immunohistochemical studies on Canine ovarian tumours

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Received: 28.11.2023; Accepted: 7.12.2023

ABSTRACT

The present study was carried out to know the occurrence and pathomorphology of different ovarian tumors in dogs. A total of 124 canine ovaries were screened for the presence of tumors based on gross and histopatholgy. The gross lesions ranged from slight enlargement of the ovaries to presence of lobulated, solid to cystic masses on the surface of ovary. On histological diagnosis, the ovarian tumours were classified as papillary cystadenoma, granulosa cell tumor, thecoma and dysgerminoma. Immunohistochemical studies for inhibin alpha revealed that sex - cord stromal tumors *viz.* granulosa cell tumors and thecoma showed cytoplasmic expression, while the epithelial and germ cell tumors showed negative expression.

Keywords: Cystadenoma, dog, dysgerminoma, granulosa, inhibin, ovary, thecoma, tumors

INTRODUCTION

Animals frequently develop ovarian tumours, with female dogs being the most commonly affected[1]. The uncontrolled and disorderly proliferation of cells within the ovary is the source of these tumours, and potential causes include trauma, congenital factors, nutrition, viruses, hormones, irradiation, transplantation of intact cancer cells, parasites, and carcinogens[2,3]. Since there are no definite screening tests for ovarian tumors and due to the difficulty in definite distinguishing features of ovarian tumors based on clinical, radiological or gross appearances, histopathological and immunohistochemical studies play an important role in diagnosis and to achieve the optimum treatment response. The ovarian tumours originate from one of the three ovarian components *viz.* epithelium (ovarian or rete ovarii), ovarian stroma and germ cells and therefore are classified as epithelial, sex - cord stromal, and germ cell tumours[1,2,4]. Epithelial and sex - cord stromal tumors are the most frequently recorded ovarian tumors in bitches[5,6]. Inhibin is a polypeptide hormone found in ovarian granulosa and lutein cells. Its primary role is to reduce pituitary gonadotropin production[7]. Inhibin immunohistochemistry helps in the crucial clinical distinction between sex - cord stromal and other primary ovarian neoplasms. In diagnostically challenging cases, inhibin immunohistochemistry is a very useful adjunct because granulosa and sertoli - stromal tumors are positive whereas other potential mimickers are negative[8].

How to cite this article : Pragathi, A., Chowdary, C.S.R., Samatha, V., Subhashini, N., Devi, V.R. and Vardhan, M.S. 2024. Histomorphological and immunohistochemical studies on Canine ovarian tumours. Indian J. Vet. Pathol., 48(3) : 231-235.

MATERIALS AND METHODS

The study was conducted during the period from April to October, 2023. Ovariohysterectomized samples from clinically normal and suspected cases of reproductive tract pathologies were included in the study. The ovaries were examined for gross morphology and representative tissue samples were collected in 10% neutral buffered formalin. The samples were subjected to routine tissue processing by paraffin embedding technique and stained with haematoxylin and eosin method[9].

Duplicate tissue sections were subjected to immunohistochemistry using ready to use inhibin alpha antibody and super sensitive polymer - HRP detection system (Bio Genex, USA). All the incubations were carried out in a humidity chamber. Four microns thick tissue sections were mounted on positively charged slides (Bio Genex, USA). The sections were pre-heated on a hot plate at 60^{O}C for 5 min, dewaxed in xylene, hydrated through a graded series of ethanol solutions and distilled water. The sections were washed three times in 0.1 M phosphate buffered saline (PBS; pH 7.4) and subjected to antigen retrieval by heat treating sections in 0.01M citrate buffer at pH 6.0

in a pressure cooker for 20 min. Tissue sections were allowed to cool down to room temperature and rinsed in PBS. Endogenous peroxidase in the tissues was blocked by applying Peroxide block™ reagent (HK111-5K, Bio Genex) containing 3% hydrogen peroxide to the sections for 20 min at room temperature. The sections were then washed in PBS three times. A proteinaceous blocking agent containing casein, Power block™ reagent (HK083-5K, Bio Genex) was applied to the sections and incubated for 10 min to prevent non - specific binding of antibodies to the highly charged sites. The power block was then tipped off and the tissue sections were incubated with the primary antibody at room temperature for 60 min. The sections were rinsed in PBS and incubated in Super enhancer™ (HK518-06K, Bio Genex) for 20 min at room temperature to enhance the signals. The sections were then rinsed in PBS buffer and incubated with Polymer - HRP reagent (HK519-06K, Bio Genex) containing anti mouse IgG labelled with enzyme polymer for 30 min. Sections were then rinsed in PBS buffer. The sections were incubated in liquid DAB chromogen *i.e.*, 3,3′- Diamino benzidine (HK124-5K, Bio Genex) mixed with DAB buffer (HK520-10K, Bio Genex) for 5 min. Sections were rinsed in PBS, washed with distilled water, counter stained with Harri's haematoxylin and mounted with DPX mountant. Negative control sections were also included in the staining procedure by omitting the primary antibody[10].

RESULTS

In the present study, gross and histopathological examination of 124 canine ovaries revealed tumors in 17 ovaries with an occurrence of 13.7%. Four types of ovarian tumors were recorded in the present study *viz.* papillary cystadenoma (6.25%), granulosa cell tumor (75%), thecoma (6.25%) and dysgerminoma (12.5%) (Table 1).

Grossly, the ovary affected with papillary cystadenoma was slightly enlarged and irregular in shape. Cut section revealed a multilocular cyst containing clear fluid. The inner surface was smooth in the most part with only few foci of papillation (Fig. 1). Histologically, the ovary revealed numerous, branched, papillary projections inside the cystic lumen (Fig. 2). The papillae were lined by a single or multiple layers of ciliated cuboidal epithelial cells. The cells had abundant eosinophilic cytoplasm, round to oval nuclei and multiple nucleoli.

Table 1. Types of canine ovarian tumors.

S. No.	Type of tumor	No. of cases
1.	Epithelial tumors	1
	i) Papillary cystadenoma	1
2.	Sex - cord stromal tumors	13
	i) Granulosa cell tumor	12
	ii) Thecoma	1
3.	Germ cell tumors	2
	i) Dysgerminoma	2
	Total	16

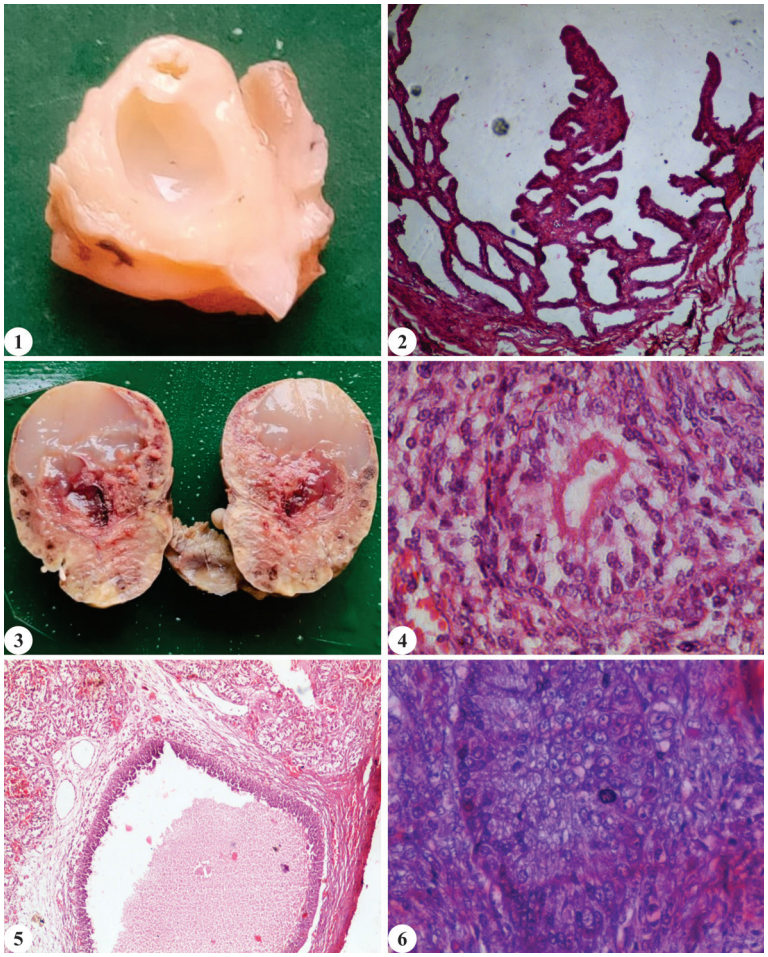

Fig. 1. Papillary cystadenoma: Tumor cut section showing a multilocular cyst; **Fig. 2.** Papillary cystadenoma: Multiple, branched papillary projections in the lumen of a cyst (H&E x40); **Fig. 3.** Granulosa cell tumor: Cut section showing solid to cystic areas with opaque and gelatinous contents; **Fig. 4.** Granulosa cell tumor: Microfollicular pattern showing Call - Exner bodies with a central space and radiating granulosa cells (H&E x400); **Fig. 5.** Granulosa cell tumor: Macrofollicular pattern showing a large cyst lined by multiple layers of granulosa cells, eosinophilic fluid and debris in the lumen (H&E x40); **Fig. 6.** Granulosa cell tumor: Insular pattern showing densely packed nests of granulosa cells (H&E x400).

Granulosa cell tumor affected ovaries were slightly enlarged, irregular, firm and lobulated with a smooth surface. The cut section was solid to cystic and yellowish in color. The cystic contents were clear to opaque and gelatinous in nature (Fig. 3). Histologically, different patterns of granulosa cell tumors *viz.* microfollicular, macrofollicular, insular, sertoli like cells and trabecular patterns were observed. Almost, all the affected ovaries showed a mixture

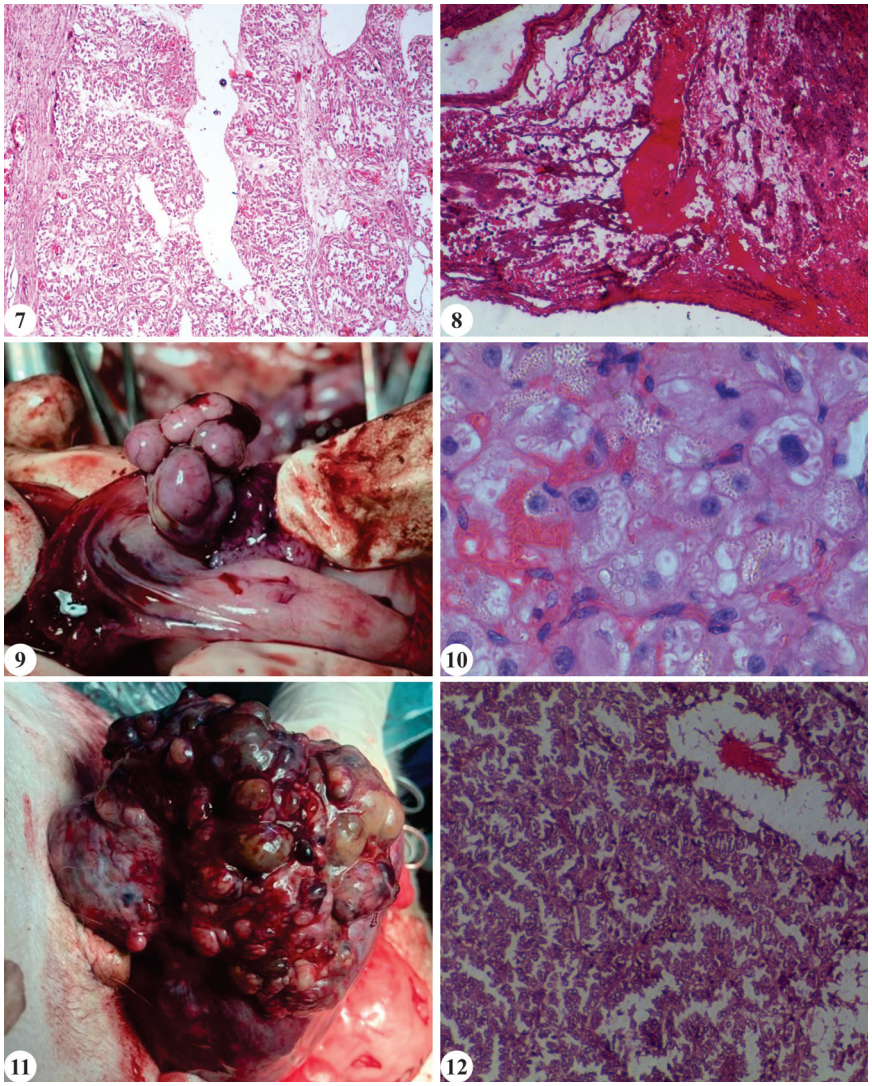

Fig. 7. Granulosa cell tumor: Solid to cystic lobules of sertoli like cells resting on a basement membrane (H&E x40); **Fig. 8.** Granulosa cell tumor: Trabecular pattern showing irregular cords of granulosa cells separated by a thin fibrovascular stroma along with congested blood vessels (H&E x40); **Fig. 9.** Thecoma: Variable sized round and smooth tumor masses on the ovary; **Fig. 10.** Thecoma: Section showing neoplastic thecal cells containing single to double nuclei and prominent nucleoli (H&E x400); **Fig. 11.** Dysgerminoma: Greatly enlarged ovary with numerous, solid to cystic nodules on the surface; **Fig. 12.** Dysgerminoma: Section showing large sheets of round to oval cells separated by a thin fibrous stroma. Note a cystic space with eosinophilic fluid (H&E x100).

of two or more patterns. The granulosa cells were small, cuboidal to polygonal and occasionally spindle shaped. The cells had scanty eosinophilic cytoplasm with a centrally placed vesicular nucleus containing single to multiple nucleoli. The stroma of the tumors was formed by variable amounts of fibroblasts and thecal cells. In few tumors, there was severe congestion of the stroma and infiltration of macrophages, lymphocytes and plasma cells. In the microfollicular pattern, Call - Exner bodies were typically observed. They were characterized by the presence of follicle - like spaces surrounded by radiating granulosa cells. Occasionally, the central space in the micro follicle was filled with eosinophilic fluid (Fig. 4). The macrofollicular pattern of granulosa cell tumors had variable sized, cystic spaces lined by several layers of moderately pleomorphic granulosa cells (Fig. 5). Few tumors showed papillary structures projecting into the lumen of the cysts. The cystic lumen contained eosinophilic fluid and debris. Occasionally, mitotic figures were observed in the lining granulosa cells. The insular pattern was characterized by the formation of well demarcated, densely packed nests of granulosa cells separated by a thin fibrovascular and thecal stroma (Fig. 6). The sertoli - like cell pattern was characterized by solid to cystic lobules of triangular to spindle shaped cells resting on a basement membrane (Fig. 7). Few tumors exhibited radiating cells on a central basement membrane resembling corona radiata. The trabecular pattern was characterized by two to three cells thick irregular cords of granulosa cells in a thin fibrovascular stroma (Fig. 8).

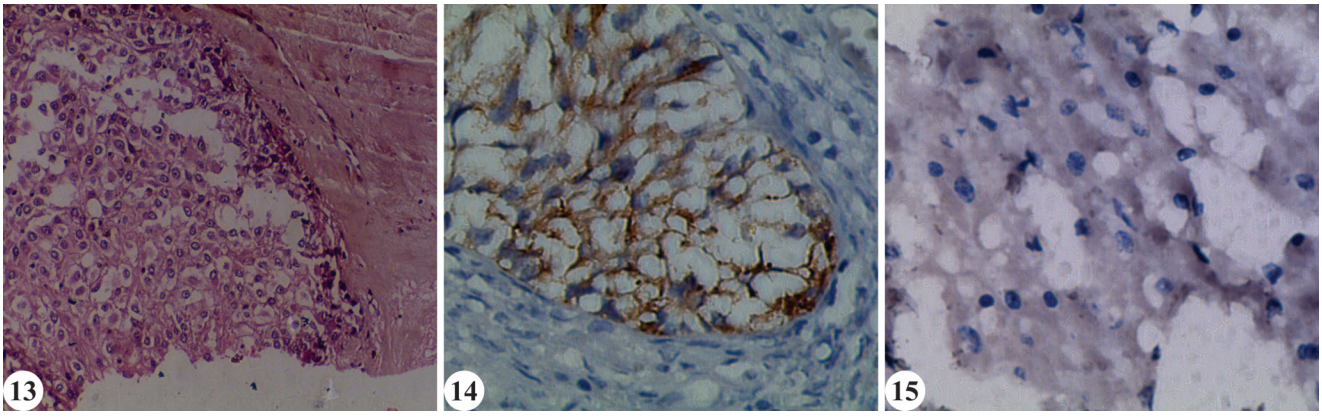

Fig. 13. Dysgerminoma: Section showing an island of tumor cells within a large desmoplastic stroma (H&E x100); **Fig. 14.** Granulosa cell tumor: IHC, Inhibin. Note intense cytoplasmic expression in the granulosa cells x400; **Fig. 15.** Thecoma: IHC, Inhibin. Note mild to moderate cytoplasmic expression in the theca cells x400.

Thecoma in the present study was observed bilaterally on the ovaries. The tumor masses were 0.3 - 0.5 cm in diameter, round, smooth and reddish brown in color (Fig. 9). The cut section was fleshy and slightly haemorrhagic. Histologically, the tumor was severely congested and composed of large, round to polyhedral luteinized thecal cells arranged in sheets with a thin fibrovascular stroma. The cells were pleomorphic with granular to fibrillar, pale eosinophilic cytoplasm and contained variable sized lipid droplets. The cells had one to two pleomorphic, central to eccentrically placed nuclei that were coarse to hyperchromatic with a single prominent nucleolus (Fig. 10). Occasionally, mitotic figures were also seen.

In the present study, dysgerminomas occurred unilaterally in the ovaries. Grossly, in one of the cases, the affected ovaries were severely enlarged and showed multiple, variable sized, solid to cystic nodules on the surface (Fig. 11). In the other case, there was diffuse enlargement of the ovary with a very hard consistency. The cut surfaces were greyish white, fleshy and showed focal areas of cystic degeneration. Microscopically, the tumor was composed of sheets of cells separated by a thin fibrous stroma in a single case (Fig. 12), while in the other case, there were islands of tumor cells with a severe desmoplasia (Fig. 13). The cells were large, round to oval and polygonal in shape with clear cell borders and high nucleus to cytoplasmic ratio. The cytoplasm was eosinophilic and the nuclei were vesicular to hyperchromatic with prominent nucleoli and a high mitotic activity. There was infiltration of lymphocytes, hemosiderin laden macrophages and a few neutrophils in the stroma.

In the present study, immunohistochemistry for inhibin marker revealed mild to moderate cytoplasmic positivity in granulosa cell tumors (Fig. 14) and thecoma (Fig. 15) and negative immunostaining in papillary cystadenoma and dysgerminoma.

DISCUSSION

In the present study, the occurrence of ovarian tumors was 13.7% comparable to the findings of other investigators[11]. Ovarian tumors in the present study identified were epithelial, sex - cord stromal and germ cell in origin. Of all the ovarian tumors, the occurrence of granulosa cell tumors was highest in the present study akin to the findings of previous reports[1,5]. However, in few studies[6,11], epithelial tumors were more common.

The gross morphology of papillary cystadenoma in the present study is comparable to the findings of previous studies[12,13]. In addition, another study recorded hemorrhagic spots to pustular lesions on the surface of ovary with papillary cystadenoma[14]. Histologically, the epithelium lining the papillae in papillary cystadenoma was cuboidal and ciliated in the present study. The surface epithelium, sub surface epithelial structures and the reteovarii in the ovary can give rise to papillary cystadenoma. Presence of ciliated epithelium suggests the origin of the tumor from reteovarii[15]. However, in previous reports on papillary cystadenoma, the epithelium was non - ciliated[12,16]. The origin of the tumors in those studies could be from other structures of the ovary.

The gross features of granulosa cell tumors in the present study are similar to the findings of previous reports[6,17]. Earlier, it was reported[6] that a large number of bitches had subclinical and microscopic granulosa cell tumors in the ovaries. In the present study too, majority of the granulosa cell tumors were not appreciable grossly and were presented only as slightly enlarged ovaries. Histologically, different patterns of granulosa cell tumors were observed in the present study similar to the findings of an earlier study[6] that reported different patterns of granulosa cells in different tumors and in different areas of the same tumor. Granulosa cell tumors produce estrogen, progesterone, testosterone and inhibin

hormones in higher amounts that leads to persistent estrus or proestrus, vaginal cornification, swollen mammary glands, pyometra, symmetrical alopecia, behavioral changes, estrogen myelotoxicity and ovarian atrophy[18-21].

The gross and histological features of leutinized thecoma in the present study are in line with the findings of a previous study[22]. Non - leutinized thecoma was also reported in an earlier study in which there were aggregations of individual spindle shaped cells with elongated nuclei[23].

In the present study, the germ cell tumors identified were dysgerminomas. The gross and microscopic findings of dysgerminoma in this study are comparable to the findings of other studies[24-27]. In addition, in the present study, there was severe desmoplastic reaction in a dysgerminoma which was not reported in earlier studies.

In the present study, immunohistochemistry revealed positive immunoreaction to inhibin alpha antibody in granulosa cell tumors and thecoma consistent with the findings of previous studies[22,28]. The ovarian stromal tissue is the source of inhibin. Inhibin positive immunostaining is a special feature of both normal and neoplastic granulosa cells and is related to sex - cord differentiation. Thus, inhibin can be regarded as a histogenetic marker of granulosa cell tumors and thecomas and a negative marker for ovarian non - stromal neoplasms[29].

In conclusion, the present study has put on record various ovarian tumors encountered in female dogs and the role of inhibin as an immunohistochemical marker for differentiating sex - cord stromal tumors from other ovarian tumors.

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Indian J. Vet. Pathol., 48(3) : 236-243, 2024: DOI: 10.5958/0973-970X.2024.00039.0

RESEARCH ARTICLE

Subacute toxicity of profenofos and its amelioration with curcumin in Swiss albino mice

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Received: 4.1.2024; Accepted: 23.1.2024

ABSTRACT

The present study was designed to assess subacute toxicity of profenofos and its amelioration with curcumin in Swiss albino mice. In this study, various parameters *i.e.* body weight, relative body weight gain, organ weight, relative organ weight gain, haematology and histopathology of various organs like liver, kidney and bone marrow, were assessed. This study was carried out in six groups, each comprised six mice. Group I, II, III, IV, V, VI were administered orally 2% gum acacia, 4.5 mg/kg b.wt. of profenofos, 9 mg/kg b.wt. of profenofos, 200 mg/kg b.wt. of curcumin, 4.5 mg/kg of profenofos + 200 mg/kg of curcumin and 9 mg/kg of profenofos + 200 mg/kg of curcumin, respectively daily for 28 days. No significant change in body weight, relative body weight gain, organ weight and relative organ weight was observed in this subacute toxicity study. In histopathological findings, profenofos produced degenerative changes in liver, kidney and bone marrow in a dose dependent manner. Curcumin showed ameliorative effect markedly by causing restoration of degenerative changes produced by profenofos. It was assessed that profenofos caused a dose dependent significant decrease in Hb and TEC in group III as compared to group I and curcumin has ameliorative effect in restoring these haematological parameters. On the basis of above results, it was concluded that profenofos produced mild dose-dependent toxic effects in Swiss albino mice at a dose of 9 mg/kg b.wt. administered daily orally for 28 days and curcumin (200 mg/kg b.wt., p.o.) has moderate ameliorative effect in profenofos-induced toxicity.

Keywords: Curcumin, haematology, histopathology, profenofos, relative body weight, relative organ weight, subacute toxicity, Swiss albino mice

INTRODUCTION

Pesticides are agrochemicals used in agricultural lands, urban green areas and public health programs in order to protect plants from pests, weeds or diseases and humans from vector-borne diseases, such as malaria, dengue fever, and schistosomiasis. Insecticides, fungicides, herbicides, rodenticides, and plant growth regulators are various types of pesticides[1].

Commonly used agricultural insecticides include organochlorines, organophosphates (OP), carbamates, pyrethroids and neonicotinoids[2]. The wide use of organophosphorus (OP) pesticides in agriculture has several interesting implications for environmental safety, such as their persistence and selective toxicity for insects rather than mammals[3]. In spite of the selectivity use against insects, OP pesticides are often toxic to humans and are responsible for most accidental intoxications in agriculture and the pesticide industry.

Profenofos is a broad-spectrum organophosphorus insecticide and acaricide used widely for agricultural and household purposes. In India, it is commonly used for pest control in mango, banana, cotton, and pineapple crops[4]. Its acts in the body by inhibiting acetylcholinesterase activity[5].

Curcumin [1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione], the active component of turmeric, a common spice used in the Indian cuisine and natural medicine[6]. It is obtained from the dried rhizome of the turmeric plant, which is a perennial herb that is cultivated extensively in south and southeast Asia. Major phytoconstituents of turmeric are diarylheptanoids, which occur in a mixture termed curcuminoids that generally make up approximately 1-6% of turmeric by dry weight. Most crude extracts prepared from turmeric, and even some refined "curcumin" materials, contain three major compounds: curcumin [1, (1E, 6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione, typically 60-70% of a crude extract], demethoxycurcumin (3, 20-27%), and bisdemethoxycurcumin (4, 10-15%), along with numerous and less abundant secondary metabolites[7,8].

In this context, the study was conducted with a goal to assess subacute toxicity of profenofos and its amelioration

How to cite this article : Devi, S., Kumar, V., Bagri, P. and Lather, D. 2024. Subacute toxicity of profenofos and its amelioration with curcumin in Swiss albino mice. Indian J. Vet. Pathol., 48(3) : 236-243.

Table 1. Effects of subacute oral exposure of mice to profenofos, curcumin and their combinations on haematological parameters.

Hematological Parameters	Treatment (mg/kg b.wt.)					
	Control (200)	Pro I (4.5)	Pro II (9)	Cur (200)	Pro I (4.5) + Cur (200)	Pro II (9) + Cur (200)
TEC (x 10^6/mm^3)	7.48 ± 0.37	6.36 ± 0.30	5.48[a] ± 0.56	7.37[c] ± 0.41	6.38 ± 0.56	6.69 ± 0.25
Hb (g/dl)	12.63 ± 0.28	11.38 ± 0.42	10.48[a] ± 0.44	12.58[c] ± 0.13	11.63 ± 0.26	11.26[a] ± 0.23
TLC (x 10^3/mm^3)	7.47 ± 0.21	6.68 ± 1.09	5.88 ± 0.79	7.22 ± 1.56	6.92 ± 0.59	6.16 ± 0.32

Data are presented as Mean ± SEM (n = 6 mice/group). Pro: profenofos; Cur: curcumin. The values were compared using one-way ANOVA followed by Tukey post-hoc test. Means bearing a and c superscripts differ significantly (P≤0.05) vs control, Pro II, respectively.

with curcumin in Swiss albino mice.

MATERIALS AND METHODS

Experimental animals

Healthy male Swiss albino mice weighing between 22-26 g were purchased from Disease Free Small Animal House (DFSAH), Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar, Haryana. Mice were housed in polypropylene cages under 12-12 h dark-light cycle with free access to water and freshly prepared feed. Temperature of animal house was maintained between 22^{O}C to 27^{O}C throughout the experiment. They were acclimatized for a period of one week in the animal house of the department before start of experiment. Bedding material (rice husk) of cages was changed on alternate days. Before the sacrifice, the animals were not fed for 12 h but water was provided *ad lib*. The present study was carried out after the approval from the Institutional Animal Ethics Committee (IAEC) (Approval No. VCC/IAEC/630-51 dated 25-03-2021).

Dose selection and chemical preparation

Two different oral doses of profenofos, i.e. 5% and 10% of maximum tolerated dose, MTD (4.5 and 9 mg/kg b.wt. respectively) were selected. Curcumin was administered at a dose rate of 200 mg/kg b.wt.[9] and freshly prepared suspension of curcumin in 2% aqueous gum acacia was prepared. In group I, 2% aqueous gum acacia suspension was administered.

Experimental protocol

This study was conducted in six treatment groups. The details of six treatment groups of each sub study are mentioned below:

Group I : Vehicle control : 2% gum acacia was given at the dose rate of 1 ml/100 g b.wt. orally daily for 28 days.

Group II : Profenofos (5% of MTD-4.5 mg/kg) : Profenofos in DW once daily orally for 28 days.

Group III : Profenofos (10% of MTD-9 mg/kg) : Profenofos in DW once daily orally for 28 days.

Group IV : Curcumin (200 mg/kg) : Curcumin in 2% gum acacia once daily orally for 28 days.

Group V : Profenofos (5% of MTD-4.5 mg/kg) and curcumin (200 mg/kg) : Curcumin in 2% gum acacia and Profenofos in DW were administered once daily orally for 28 days.

Group VI : Profenofos (10% of MTD-9 mg/kg) and curcumin (200 mg/kg) : Curcumin in 2% gum acacia and profenofos in DW were administered once daily orally for 28 days.

A gap of 12 hrs. was maintained between the dosing of profenofos and curcumin. Groups II and III mice were also administered 2% gum acacia at a gap of 12 hrs. of profenofos administration. After 28 days of treatment, animals were sacrificed on 29th day of first dosing and samples were collected for various studies.

Table 2. Effects of subacute oral exposure of mice to profenofos, curcumin and their combinations on differential leukocyte count (DLC).

Treatment (mg/kg b.wt.)	Relative DLC (%)				
	Lymphocyte	Neutrophil	Monocyte	Eosinophil	Basophil
Control (200)	76.50 ± 2.92	18.33 ± 2.74	1.67 ± 0.33	2.67 ± 0.21	0.83 ± 0.31
Pro I (4.5)	69.20 ± 1.49	26.80 ± 1.28	2.00 ± 0.26	1.40 ± 0.49	0.60 ± 0.20
Pro II (9)	67.00 ± 2.22	28.17 ± 2.27	2.33 ± 0.33	2.33 ± 0.21	0.17 ± 0.17
Cur (200)	78.20 ± 1.28	22.17 ± 2.32	1.50 ± 0.43	2.67 ± 0.21	0.83 ± 0.31
Pro I (4.5) + Cur (200)	70.83 ± 7.32	24.17 ± 7.14	2.00 ± 0.26	2.50 ± 0.22	0.50 ± 0.22
Pro II (9) + Cur (200)	70.20 ± 2.93	25.60 ± 3.15	2.20 ± 0.40	1.80 ± 0.40	0.20 ± 0.16

Data are presented as Mean ± SEM (n = 6 mice/group). Pro: profenofos; Cur: curcumin. The values were compared using one-way ANOVA followed by Tukey post-hoc test.

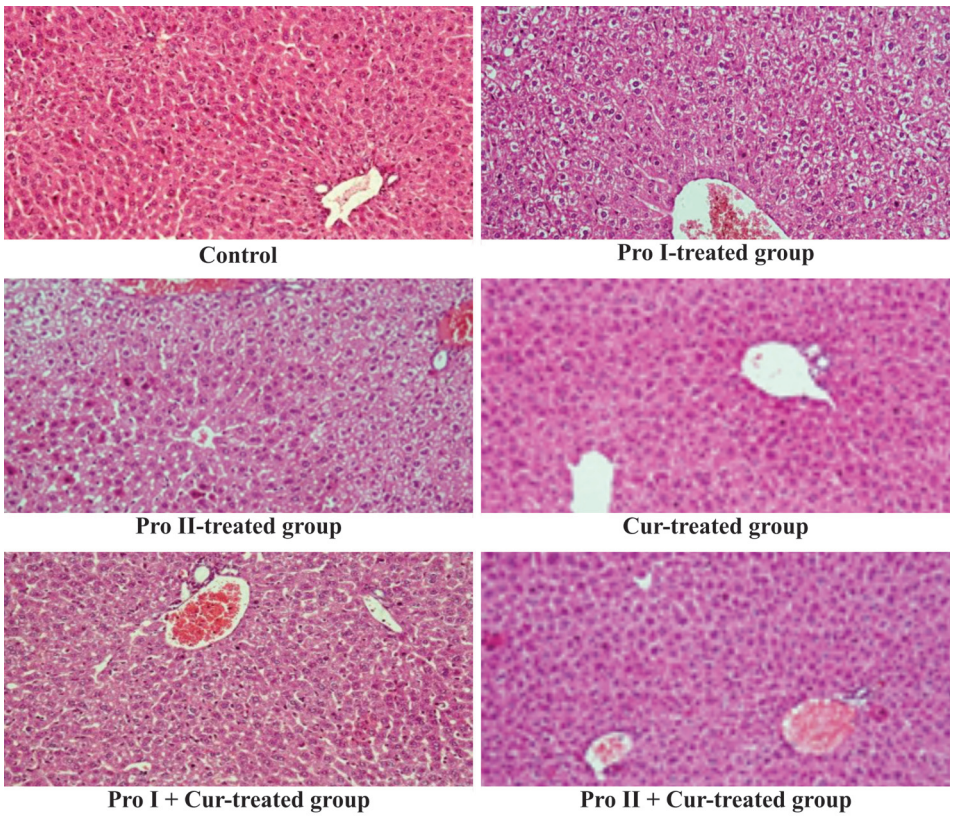

Fig. 1. Representative images (H&E stain, 200x magnification) showing effects of subacute oral exposure of profenofos, curcumin and their combinations in liver.

Procedure

Haematological Studies

Blood was collected through cardiac puncture in EDTA containing tubes and haematological parameters such as haemoglobin (Hb), total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC) were estimated using standard methods.

Histopathological evaluation

Liver, kidneys and bone marrow samples of mice from all the treated and control groups were collected and fixed in 10% buffered formalin for histopathological studies. After overnight washing, tissues were dehydrated in ascending grade of alcohol, cleared in benzene and embedded in paraffin wax to prepare paraffin blocks. Approximately, 5 µm thick sections were prepared and stained with hematoxylin and eosin (H&E) for assessment of the morphological changes under light microscopy[10].

Body weight

Body weight of each mouse was taken weekly during 28 days of treatment period.

Relative body weight gain

Relative body weight gain of each male mouse was determined weekly during 28 days of treatment period by the following formula and was expressed as g/100 g b.wt.

$$\text{Relative body weight gain} = \frac{\text{Final body weight (g) - Initial body weight (g)} \times 100}{\text{Initial body weight (g)}}$$

Relative organ weight

The relative organ weights of heart, liver, kidneys, spleen, testes and epididymis were expressed as g/100 g body weight of male mice.

$$\text{Relative organ weight} = \frac{\text{Organ weight (g)} \times 100}{\text{Body weight (g)}}$$

Statistical analysis

Data were analyzed using Graph Pad Prism version 5.03. Results were expressed as Mean ± SEM with 'n' equal to number of animals. Differences among the groups were compared by one-way analysis of variance (One-Way ANOVA) with Tukey post-hoc test. In all tests, p values less than 0.05 were considered statistically significant[11].

RESULTS

Effects of subacute oral exposure of mice to profenofos, curcumin and their combinations on haematological parameters

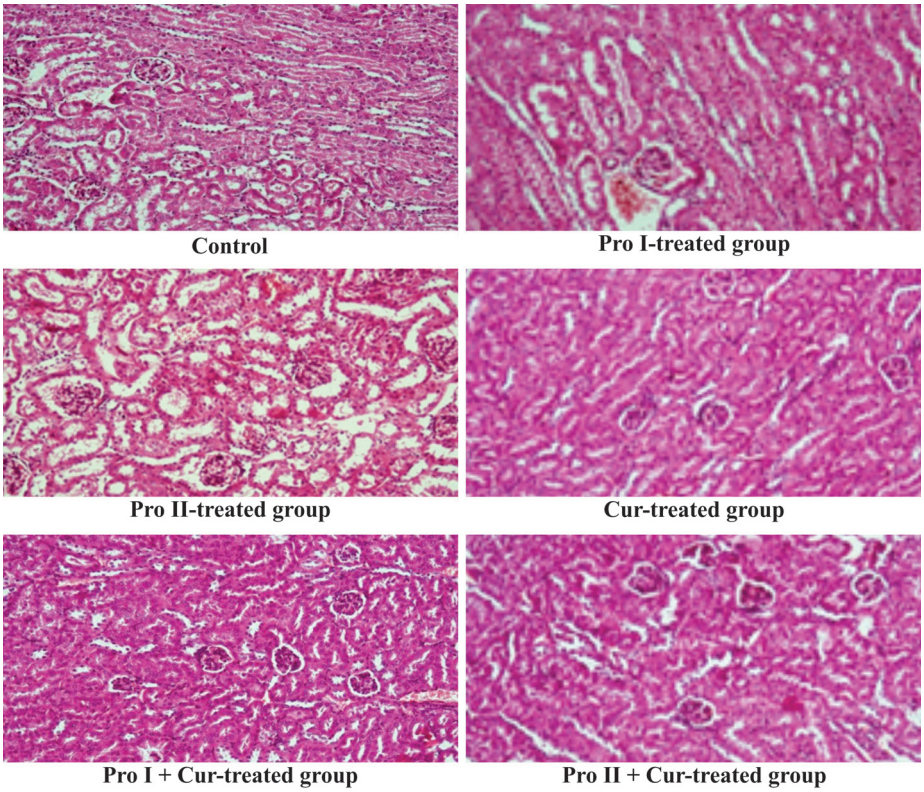

Fig. 2. Representative images (H&E stain, 200x magnification) of kidney showing effects of subacute oral exposure of profenofos, curcumin and their combinations.

The results of effects of subacute oral exposure of mice to profenofos, curcumin and their combinations are presented in Tables 1 and 2. There was a significant decrease in TEC value in Pro II-treated group as compared to control. There was a significant decrease in Hb value in Pro II-treated group as compared to control. In Pro II + Cur-treated group, it was still low as compared to control. A non-significant decrease in TLC was observed in Pro I and Pro II treated groups. There was non-significant increase in TEC, Hb and TLC values in Pro I + Cur and Pro II + Cur-treated groups as compared to Pro I and Pro II groups, respectively. No significant difference in differential leukocyte count (DLC) was observed in all treatment groups. A decreasing trend was observed in mean values of per cent lymphocyte, monocyte, eosinophil and basophils, whereas mean values of per cent neutrophils showed an increasing trend in Pro I and Pro II treated groups. There was non-significant increase in per cent lymphocyte and decrease in percent neutrophil after co-treatment with curcumin in Pro I and Pro II treated groups.

Effects of subacute oral exposure of mice to profenofos, curcumin and their combinations on histopathological examination

Histopathological lesions were studied in liver, kidney and bone marrow of mice in different groups (Control, Pro I, Pro II, Cur, Pro I + Cur and Pro II + Cur-treated groups).

Liver

Histological lesions in liver of control and treatment groups are presented in Fig. 1. Histopathological examination of liver of control group revealed normal histological structures. Group treated with Pro I showed congestion of portal veins, vacuolar degeneration in hepatocytes, centrilobular and midzonal necrosis of hepatocytes with dark pyknotic nuclei and deep eosinophillic cytoplasm. Group treated with Pro II showed similar lesions with more severity *i.e.* massive necrosis involving periportal and centrilobular hepatocytes characterized by dark condensed pyknotic nuclei with infiltration of leukocytes in parenchyma. Groups co-treated with Curcumin showed mild histological lesions in liver as compared to the groups treated with Pro I and Pro II, respectively. The Pro I + Curcumin and Pro II + Curcumin groups showed mild congestion in hepatic blood vessels with few small focal areas of necrosis in parenchyma.

Kidney

Histological lesions in kidneys of control and treatment groups after subacute exposure are presented in Fig. 2. Control group revealed normal histological structure of kidney cortex and medulla. Group treated with Pro I showed congestion in glomerular and

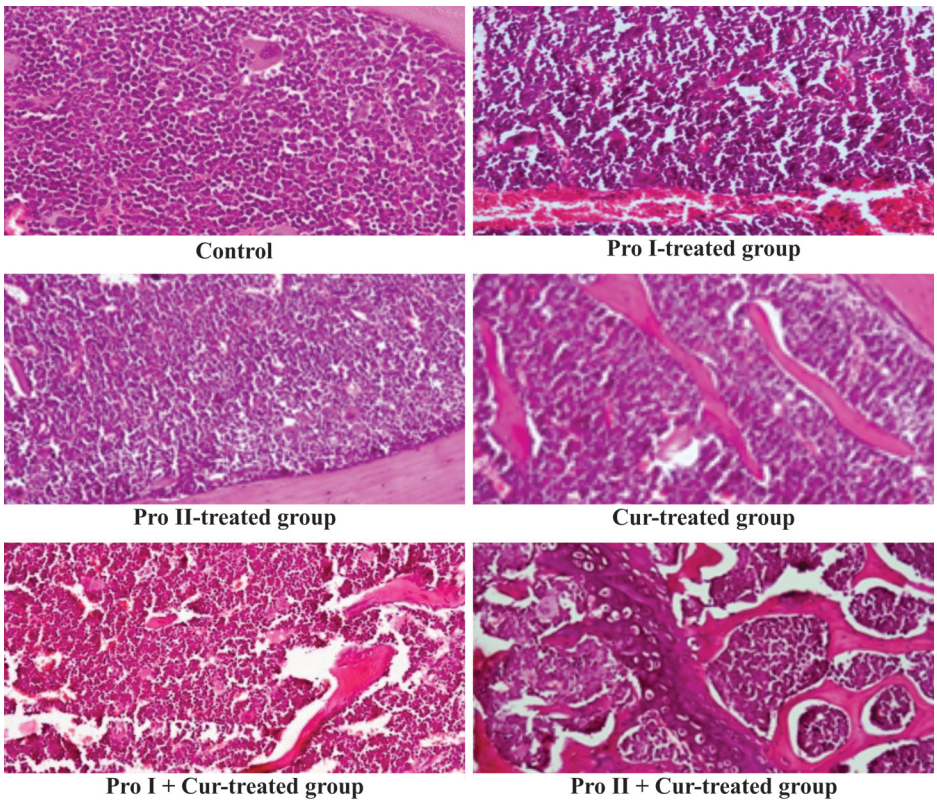

Fig. 3. Representative images (H&E stain, 200x magnification) showing effects of subacute oral exposure of profenofos, curcumin and their combinations in bone marrow.

Table 3. Effects of subacute oral exposure of mice to profenofos, curcumin and their combinations on body weight.

Treatment (mg/kg b.wt.)	Body Weight (Weeks)				
	0th	1st	2nd	3rd	4th
Control (200)	20.17 ± 1.14	23.08 ± 1.00	25.42 ± 1.03	27.67 ± 1.29	29.33 ± 1.18
Pro I (4.5)	19.60 ± 0.37	22.70 ± 0.58	25.60 ± 0.75	27.10 ± 0.83	28.20 ± 1.14
Pro II (9)	22.67 ± 1.60	23.42 ± 1.60	26.00 ± 1.65	27.42 ± 1.73	28.33 ± 1.76
Cur (200)	18.58 ± 0.55	20.83 ± 0.76	22.83 ± 0.85	25.92 ± 0.85	28.58 ± 0.75
Pro I (4.5) + Cur (200)	19.50 ± 0.89	23.17 ± 0.74	25.42 ± 0.77	26.50 ± 1.98	27.92 ± 1.90
Pro II (9) + Cur (200)	18.90 ± 0.80	22.80 ± 0.92	25.10 ± 0.99	26.80 ± 0.82	27.70 ± 0.78

Data are presented as Mean ± SEM (n = 6 mice/group). Pro: profenofos; Cur: curcumin. The mean values were compared using one-way ANOVA followed by Tukey post-hoc test.

intertubular capillaries, degenerative changes in renal tubular epithelial cells with presence of hyaline casts in lumen and proliferation of parietal epithelial cells in bowman's capsule. Pro II treated mice showed same lesions but with more severity. Curcumin treated group revealed almost normal histological structure in kidney. Only mild degeneration of the epithelial cells of tubules was evident in both the groups i.e. Pro I + Curcumin and

Table 4. Effects of subacute oral exposure of mice to profenofos, curcumin and their combinations on relative body weight gain (4th week).

Parameter	Treatment (mg/kg b.wt.)					
	Control (200)	Pro I (4.5)	Pro II (9)	Cur (200)	Pro I (4.5) + Cur (200)	Pro II (9) + Cur (200)
Relative body weight gain (g/100 g b.wt.)	47.06 ± 8.36	43.86 ± 4.10	25.94 ± 5.79	66.02c ± 6.31	42.33 ± 4.13	48.89 ± 7.65

Data are presented as Mean ± SEM (n = 6 mice/group). Pro : Profenofos; Cur : Curcumin. The values were compared using one-way ANOVA followed by Tukey post-hoc test. Means bearing c superscript differ significantly (P≤0.05) vs Pro II.

Table 5. Effects of subacute oral exposure of profenofos, curcumin and their combinations on organ weight of mice.

Treatment (mg/kg b.wt.)	Organ weight (g)								
	Heart	Liver	Left Kidney	Right Kidney	Spleen	Left Testis	Right Testis	Left Epididymis	Right Epididymis
Control (200)	0.14 ± 0.01	1.40 ± 0.10	0.20 ± 0.01	0.19 ± 0.02	0.16 ± 0.03	0.11 ± 0.01	0.11 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
Pro I (4.5)	0.14 ± 0.00	1.38 ± 0.13	0.19 ± 0.01	0.20 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.11 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
Pro II (9)	0.14 ± 0.01	1.35 ± 0.12	0.19 ± 0.02	0.20 ± 0.02	0.11 ± 0.02	0.10 ± 0.00	0.11 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
Cur (200)	0.12 ± 0.00	1.43 ± 0.07	0.18 ± 0.00	0.18 ± 0.01	0.11 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
Pro I (4.5) + Cur (200)	0.14 ± 0.01	1.46 ± 0.08	0.19 ± 0.02	0.20 ± 0.02	0.13 ± 0.02	0.10 ± 0.01	0.11 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
Pro II (9) + Cur (200)	0.12 ± 0.00	1.37 ± 0.06	0.18 ± 0.01	0.18 ± 0.00	0.08[a] ± 0.00	0.10 ± 0.00	0.11 ± 0.00	0.04 ± 0.00	0.04 ± 0.00

Data are presented as Mean ± SEM (n = 6 mice/group). Pro: profenofos; Cur: Curcumin. The values were compared using one-way ANOVA followed by Tukey post-hoc test. Mean bearing a superscript differ significantly (P≤0.05) vs control.

Table 6. Effects of subacute oral exposure of mice to Profenofos, curcumin and their combinations on relative organ weight gain of mice.

Treatment (mg/kg b.wt.)	Relative organ weight gain (g/100 g b.wt.)								
	Heart	Liver	Left Kidney	Right Kidney	Spleen	Left Testis	Right Testis	Left Epididymis	Right Epididymis
Control (200)	0.48 ± 0.02	4.77 ± 0.28	0.68 ± 0.04	0.65 ± 0.05	0.53 ± 0.10	0.38 ± 0.03	0.38 ± 0.01	0.14 ± 0.01	0.13 ± 0.01
Pro I (4.5)	0.47 ± 0.02	4.54 ± 0.31	0.64 ± 0.03	0.66 ± 0.02	0.33 ± 0.02	0.32 ± 0.02	0.37 ± 0.01	0.13 ± 0.01	0.13 ± 0.01
Pro II (9)	0.49 ± 0.01	4.72 ± 0.24	0.68 ± 0.03	0.69 ± 0.03	0.40 ± 0.05	0.38 ± 0.02	0.40 ± 0.02	0.14 ± 0.01	0.16 ± 0.00
Cur (200)	0.44 ± 0.01	5.00 ± 0.16	0.64 ± 0.01	0.62 ± 0.02	0.38 ± 0.01	0.35 ± 0.02	0.35 ± 0.02	0.13 ± 0.01	0.13 ± 0.00
Pro I (4.5) + Cur (200)	0.50 ± 0.02	5.34 ± 0.39	0.68 ± 0.02	0.72 ± 0.03	0.51 ± 0.12	0.38 ± 0.03	0.39 ± 0.03	0.14 ± 0.00	0.15 ± 0.01
Pro II (9) + Cur (200)	0.45 ± 0.01	4.92 ± 0.11	0.64 ± 0.02	0.66 ± 0.01	0.28 ± 0.00	0.38 ± 0.01	0.40 ± 0.01	0.13 ± 0.00	0.14 ± 0.00

Data are presented as Mean ± SEM (n = 6 mice/group). Pro: Profenofos; Cur: Curcumin. The values were compared using one-way ANOVA followed by Tukey post-hoc test.

Pro II + Curcumin as compared to Pro I and Pro II groups, respectively.

Bone marrow

Histopathological lesions in bone marrow of control and treatment group after subacute exposure are presented in the Fig. 3. Mice of control group revealed normal hematopoietic cellularity of bone marrow tissue. Group treated with Pro I showed congestion and haemorrhages, reduced cellularity with increased fibrosis and slight increase in number of megakaryotic cells. Pro II treated group also revealed same lesions with more severity. Severity of histological alterations was markedly decreased in curcumin treated groups *i.e.* Pro I + Cur and Pro II + Cur as compared to Pro I and Pro II groups, respectively.

Effects of subacute oral exposure of mice to profenofos, curcumin and their combinations on body weight and relative body weight gain

The results of effects of subacute oral exposure of mice to profenofos, curcumin and their combinations on body weight and relative body weight gain are presented in Tables 3 and 4. There was no significant difference in body weight and relative body weight gain in all treatment groups as compared to control.

Effects of subacute oral exposure of mice to profenofos, curcumin and their combinations on organ weight and relative organ weight gain of mice

The results of the effects of single oral exposure of mice to profenofos, curcumin and their combinations on organ weight and relative organ weight gain of mice are presented in Tables 5 and 6. No significant difference was observed in organ weight and relative organ weight gain in all treatment groups as compared to control.

DISCUSSION

Oral administration of profenofos resulted into decrease in Hb and TEC significantly at higher dose in comparison to control. It has also been reported that profenofos exposed animals show decrease in the level of blood cells and haemoglobin in comparison to normal range[12]. The reduction of haemoglobin as well as RBCs counts may be attributed to the toxic effect of profenofos and non-significant decrease of leucocytes due to the inflammation induced as defence mechanism. Reduction in numbers of RBCs probably due to suppressive

and toxic effect on bone marrow and subsequently on haematopoiesis.

It has also been reported that due to profenofos, hematological parameters were decreased substantially in mice[13]. Mice treated with profenofos (25 mg/kg b.wt.) alternately for 30 days showed significant decrease in blood leucocytes, erythrocytes, platelets count and haemoglobin up to 70%, 56%, 15% and 44%, respectively. However, the levels of these blood cells were recovered after treatment with curcumin (120 mg/kg b.wt.) and cumin (150 mg/kg b.wt.). In a comparative study, curcumin was found more effective than cumin as reported earlier[14]. These findings were in accordance to our study.

After oral exposure to profenofos at two doses (4.5 and 9.0 mg/kg b.wt.) and their combinations with curcumin (200 mg/kg b.wt.), no significant change was observed in body weight, relative body weight gain, organ weight and relative organ weight gain. It suggested that profenofos alone or in combination had no direct effect on metabolism at these dose levels. Non-significant changes in body weight and organ weight observed in present studies were comparable with literature where pesticides intoxification did not alter the body weight and organ weight of rats significantly[15,16]. Liver and kidney are the most sensitive organs against pesticide toxicity, as they play key roles in the metabolism and elimination of pesticides[17-19]. Toxic effects of profenofos on female wistar rats at two sub-chronic doses of 1/10th and 1/ 50th of LD_{50}, administered for 45 days each studied by earlier worker[20]. They observed significant decrease in feed intake during the last weeks of treatment, though the body and organs weights increased non-significantly.

Hepatocellular injuries in liver and tubular degeneration of kidney, respectively use on the exposure to profenofos were observed previously[21]. Congested blood vessels and hepatic sinusoids and focal area of coagulative necrosis surrounded with lymphocytic infiltration in hepatic parenchyma, vacuolization, haemorrhage and hyperplasia of Kupffer cells in the liver were reported earlier[22]. In addition, swelling of Bowman's capsules and tubular degeneration in the kidney were reported[23]. All these findings were in favour of our findings.

In support of our findings, it was also reported that profenofos exhibited histopathological changes in liver, kidney, spleen and brain of albino mice treated with sub-lethal concentrations (1/10th, 1/40th and ADI LD_{50}) orally twice a week for 30, 60, and 90 consecutive days. Liver showed hepatic cell damage with degenerative changes. Kidney showed heamorrhages, edema, necrosis and glomeruli shrinkage[24].

Histomorphological studies of ovary and uterus revealed a number of abnormalities with increase in atretic follicles, enhanced percent atresia, degeneration of uterine epithelium and enlarged intracellular spaces in endometrial glands in treated rats. The results infer that the profenofos exposure leads to pathophysiological conditions in the ovary and uterus at dose dependent manner[25] in the profenofos on female wistar rats at two sub-chronic doses of 1/10th and 1/ 50th of LD_{50}, after administration for six week each.

Improvement in the architecture of the liver tissue has been observed in male Wistar albino rats treated with *Curcuma longa* extract as compared to group treated with diethylnitrosamine (DENA) (60 mg/kg b.wt., i/p), which indicates the hepatoprotective ability and antioxidant role of *Curcuma longa* extraction against DENA induced hepatocellular damage[26]. Dexamethasone induced spermatogenesis defects including epithelial vacuolizations, sloughing of germ cells, reduction of seminiferous tubule diameter, reduction in the number of sperm heads and significant maturation arrest ($p < 0.001$) in mice as reported by previous worker[27]. Curcumin (200 mg/kg/day) for 10 days + dexamethasone (7 mg/kg/day) for 7 days treatment significantly prevented these changes ($p < 0.05$). These results were in accordance of our findings that severity of histopathological lesions was decreased in Pro I + Cur and Pro II + Cur-treated groups.

ACKNOWLEDGEMENTS

The work was done in the Department of Veterinary Pharmacology and Toxicology, LUVAS, Hisar.

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Indian J. Vet. Pathol., 48(3) : 244-247, 2024: DOI: 10.5958/0973-970X.2024.00040.1

RESEARCH ARTICLE

Avian cutaneous neoplasms: A retrospective study

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Received: 31.8.2023; Accepted: 4.4.2024

ABSTRACT

A retrospective study was carried out on the biopsy specimens from the avian species received at the Department of Veterinary Pathology, RIVER, Puducherry from the Veterinary Clinical Complex. During the 69 months of study (from January 2017 to September 2022), 15 biopsy samples were collected from different birds. Out of 15 bird samples, 11 (73.33%) were diagnosed as neoplasms. Among the 11 neoplasms, 9 (81.81%) were benign and 2 (18.19%) were malignant. Based on the origin, neoplasms were categorized into epithelial (3 birds, 27.27%) and mesenchymal (8 birds, 72.73%). The epithelial neoplasms consisted of 3 benign (adenoma) and the mesenchymal neoplasms consisted of 6 benign (3 fibroma, 1 osteoclastoma, 1 histiocytoma, 1 lymphoma) and 2 malignant (fibrosarcoma). Grossly, the growths varied from small to large, soft to hard in consistency, solitary to multiple growths found in different sites of the skin. The incidence of cutaneous neoplasms was recorded in different avian species including non-descript, aseel, pigeon, and psittacine birds. The highest occurrence was recorded in 2-3 years of age (6 birds, (54.54%) and the lowest in 0-1 year of age (5 birds, 45.45%). Based on the sex of the birds, out of the 11 neoplasms, 7 occurred in males and 4 in females. With respect to species, pigeon and psittacines were more affected (5 birds, 45.45%), followed by non-descript breeds (4 birds, 36.36%) and aseel (2 birds, 18.18%). Location wise, neoplasm predominantly occurred in wings (2 fibroma, 1 fibrosarcoma, and 1 osteoclastoma), perianal region (1 histiocytoma, 1 adenoma), neck and breast region (1 lymphoma, 1 papillary cystadenoma, 1 fibroma), forehead and eye region (1 fibrosarcoma and 1 adenoma).

Keywords: Avian, biopsy, cutaneous neoplasm, histopathological classification

INTRODUCTION

Birds suffer from a wide variety of neoplastic diseases of both infectious and non-infectious origin. Clinical survey tools can be used to gather data from patients across different institutions have been developed and more evidence-based research to the treatment of avian cancers[1]. Advancements in avian medicine have made the diagnosis of neoplastic disorders increasingly challenging, despite a lack of significant treatment discoveries in the relevant scientific literature[2]. According to clinical investigations, avian neoplasms can affect the skin, mouth, sinuses, kidney, liver, reproductive organs, bones, brain, circulatory system, and connective tissue[3]. Integumentary system neoplasms are prevalent and account for 12 to 70% of all avian neoplasms. In all avian species, the overall expected incidence of neoplasia was 3.8%. There are reports of neoplasia in caged birds as contrasted to free-ranging birds, especially budgerigars, where the rate of neoplasia ranges from 16.8 to 24.2% overall[4].

Of the various neoplasms reported, lipomas and fibromas are observed most frequently[5]. The benign neoplasms known as fibromas are mainly composed of collagenous stroma and fibrocytes[6]. Physical examination could reveal external neoplasms, and needle aspiration, wedge, punch, or surgical biopsies are frequently used for diagnosis. Radiographs, ultrasonography, biopsy, or exploratory surgery are frequently needed for internal neoplasms in order to examine, diagnose, and assess the extent of the neoplastic processes. Cutaneous neoplasm occurs with varying frequencies in birds of all ages[7].

This retrospective analysis aimed to investigate the incidence of cutaneous and visceral neoplasms, along with their signalment (characteristics), and to explore potential correlations between clinical history or histological features and the outcome.

How to cite this article : Abinaya, M., Poobitha, S., Lakkawar, A.W., Uma, S., Kumar, R. and Nair, M.G. 2024. Avian cutaneous neoplasms: A retrospective study. Indian J. Vet. Pathol., 48(3) : 244-247.

MATERIALS AND METHODS

The biopsy specimens from the avian species received during January 2017 to September 2022 at the Department of Pathology, RIVER, Puducherry from the Veterinary Clinical Complex were collected. The neoplasms were categorized based on histological features as benign and malignant. The incidence of cutaneous neoplasms was carried out based on sex, species, age and location. Grossly, the growths varied from small to large, soft to hard in consistency, solitary to multiple growths found in different sites of the skin. The total number of 15 biopsy samples was received during this period. All the samples were fixed in 10%

Table 1. Incidence of cutaneous neoplasms.

Age		Sex		Species		
Below 1 Year	**Above 2 Years**	**Male**	**Female**	**Pigeon/Psittacines**	**Non-descript**	**Aseel**
5 (45.45%)	6 (54.54%)	7 (63.63%)	4 (36.36%)	5 (45.45%)	4 (36.36%)	2 (18.18%)

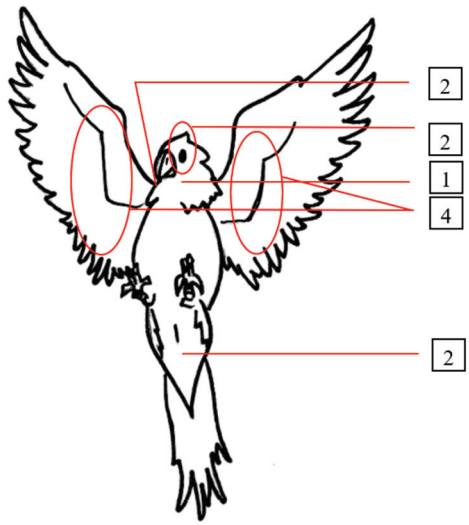

Fig. 1. Location wise classification of Neoplasms.

neutral buffered formalin and processed tissues were sectioned to thickness of 4 - 5 µm and examined with routine Hematoxylin and Eosin staining[8].

RESULTS

The incidence of cutaneous neoplasms (Table 1) was recorded in different avian species including non-descript, aseel, pigeon, and psittacine birds. Out of 15 biopsies, 11 were diagnosed as neoplasms. The occurrence rate was highest among adult birds aged above 2 years, accounting for approximately 54.54% (6 birds), whereas birds below 1 year of age exhibited a lower occurrence of 45.45% (5 birds).

Based on sex, males were predominantly affected, comprising 63.63% (7 birds), in contrast to females, accounted for 36.36% (4 birds). With respect to species, pigeon and psittacines were more affected (5 birds, 45.45%), followed by non-descript breeds (4 birds, 36.36%) and aseel (2 birds, 18.18%). Grossly, the growths varied from small to large, soft to hard mass in consistency, solitary to multiple growths found in different sites of the skin Neoplasms were predominantly based on anatomical location. The wings showed 2 fibromas, 1 fibrosarcoma, and 1 osteoclastoma. The perianal region exhibited 1 histiocytoma and 1 adenoma. In the neck

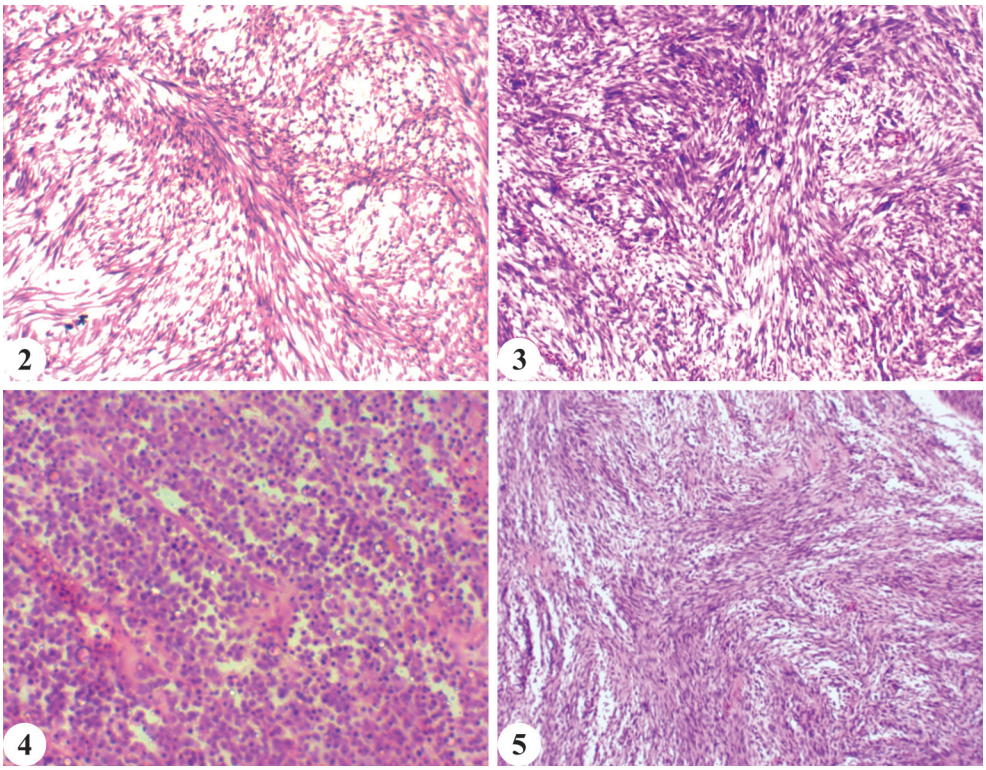

Fig. 2. Fibroma - loosely arranged fibroblasts running in different directions (H&E 400X); **Fig. 3.** Fibrosarcoma - criss-cross pattern of fibroblasts with hyperchromatic nuclei (H&E 200X); **Fig. 4.** Lymphoma - uniform sheet of arrangement of small lymphocyte with scanty cytoplasm (H&E 400X); **Fig. 5.** Neurofibroma - presence of thick and thin collagen strands amidst myxoid material (H&E 200X).

and breast area, occurrences included 1 lymphoma, 1 papillary cystadenoma and 1 fibroma. The forehead and eye region presented 1 fibrosarcoma and 1 adenoma. This distribution is represented in Fig. 1.

Neoplasms were grouped based on the histological examination, of which 9 (81.81%) were benign and 2 (18.19%) were malignant. Further they were categorized into epithelial (3, 27.27%) and mesenchymal (8, 72.73%). Epithelial neoplasms comprised three cases of benign adenoma, and no malignant tumors were detected. In the mesenchymal category, there were six benign neoplasms (3 fibromas, 1 osteoclastoma, 1 histiocytoma, 1 lymphoma) and two malignant mesenchymal neoplasms (2 fibrosarcomas).

In fibroma (Fig. 2), the lesion was characterized by loosely arranged fibroblasts in different directions whereas in fibrosarcoma (Fig. 3), criss-cross pattern of fibroblasts with hyperchromatic nuclei was observed. In lymphoma (Fig. 4), the lesion was characterized by uniform sheet of arrangement of small lymphocyte with scanty cytoplasm whereas in neurofibroma (Fig. 5), presence of thick and thin collagen strands amidst myxoid material was observed. In osteoclastoma, as depicted in Fig. 6, the lesion was distinguished by the existence of multinucleated giant cells, commonly referred to as osteoclasts, along with bone rarefaction, round to polyhedral cells with eosinophilic cytoplasm, was observed in case of histiocytoma (Fig. 7). The proliferation of acinar cells of serous and sebaceous glands in adenoma (Fig. 8) whereas in case of papillary cystadenoma (Fig. 9), papillary projections from the glandular epithelium of serous gland into the lumen were observed.

DISCUSSION

In this study, neoplastic disease affecting different species of birds includes non-descript, aseel, pigeon and psittacine birds. Of which the non-descript freely scavenging birds was the prevalent species. Most neoplasms in avian species affect the integumentary system commonly[4]. A literature survey of neoplasia in pet birds, over a 10-year period, revealed that tumours arising in the integument (31.7%) were more common than from other organ systems[3,4]. The most prevalent tumour in avian species is fibroma. In chickens, connective tissue neoplasms can arise following infection with specific strains of avian leukosis or sarcoma virus. Fibrosarcoma is a malignant neoplasm of fibroblast or mesenchymal cells, which possess the ability to produce collagen fibers[9,10]. Fibrosarcoma occurs commonly in budgerigars, cockatiels, macaws and parrots. Among all neoplasms in budgerigars, fibrosarcomas may account for 3 to 14%. Fibrosarcomas commonly arise from the soft tissues of the wing, leg, head, beak, cere and trunk[11]. The high incidence of neoplasms in these birds may be due to assisted inbreeding, their longer lifespan, potential carcinogenic environmental agents, and the genetic errors or mutations[12,13,17].

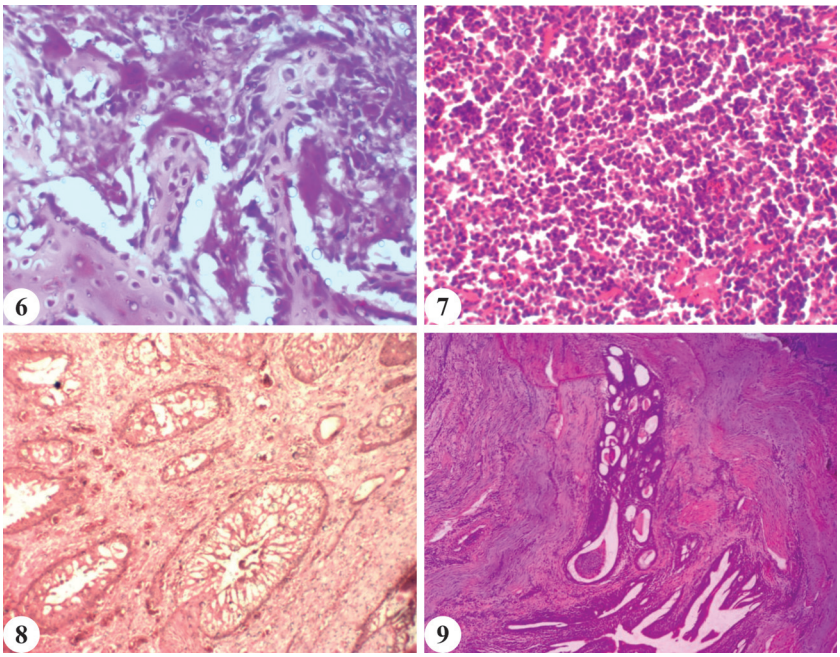

Fig. 6. Osteoclastoma - presence of multinucleated giant cells (osteoclast) and rarefaction of bone (H&E 400X); **Fig. 7.** Histiocytoma - densely populated round to polyhedral cells with eosinophilic cytoplasm (H&E 400X); **Fig. 8.** Adenoma - proliferation of acinar cells giving a glandular pattern (H&E 200X); **Fig. 9.** Papillary cystadenoma - projections arising from the glandular epithelium into the lumen (H&E 100X).

In superficial type adenomas of oesophageal and uropygial gland, the neoplastic crypts varied in size and shape in comparison with the surrounding normal crypts, but the crypt structure itself was plainly visible. On physical examination, the uropygial gland appears enlarged, ulcerated and hemorrhagic.

On histological examination, proliferation of acinar cells giving a glandular pattern and papillary projection arising from glandular epithelium into the lumen was noticed. Neoplasia must be distinguished from adenitis, which usually requires histological examination. Partial or complete removal of the affected gland is usually recommended[1,14,17]. Biopsy sample collected from breast region reveals uniform sheet of small lymphocyte with scanty cytoplasm indicates lymphoma Fig. 4. Lymphoma is the most common lymphoid neoplasia in psittacine and passerine birds. It can involve the spleen, liver, kidneys, GI tract, skin, bone, oviduct, lungs, sinuses, thymus, testes, brain, mesentery, trachea, and pancreas[8]. Numerous reports of exophthalmos in psittacines, particularly young African grey parrots, have been diagnosed as retrobulbar lymphoma. Cutaneous lymphoma often occurs on the head or neck[14,15,16].

Osteoclastoma is known as the giant cell tumour of bone. The neoplasm exhibits profileration of osteoclast (multinucleated bone destroying cells) and rarefaction of bones. Among avian species, it is a rare tumour where the reports are meagre[18]. As the population of birds has increased and as well as due to ageing of many pet birds, neoplasia will undoubtedly be seen more commonly. The etiology of neoplasms in aviary and free-ranging birds are unknown. The studies and reports of neoplasm will be useful in early diagnosis and management of aged birds. Recent advances in oncological studies paved importance in diagnosis of neoplasms in avian species[1,11]. With respect to our retrospective study, the birds which are reported in veterinary clinical complex, RIVER with growth and masses in both cutaneous and visceral regions from 2017 to 2022 were accounted for the study. Of which tumour incidence in non-descript breeds was as similar as pet birds. This is because of increased inbreeding of non-descript birds by small farmers. Based on our findings fibroma and fibrosarcomas has higher incidence.

ACKNOWLEDGEMENT

The authors are thankful to the Dean, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Puducherry for providing facilities to carry put this study.

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Indian J. Vet. Pathol., 48(3) : 248-255, 2024: DOI: 10.5958/0973-970X.2024.00041.8

RESEARCH ARTICLE

Diagnosis of epithelial and round cell tumors by foldscope in comparison with conventional microscope

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Received: 3.11.2023; Accepted: 30.11.2023

ABSTRACT

The present study is a comparative account of images produced by a foldscope and conventional microscope of epithelial and round cell tumors. A total of 100 biopsy samples were analyzed by foldscope and conventional microscope. Out of which, 44 were analyzed as epithelial tumors and eleven as round cell tumors. Epithelial tumors include 17 cases of squamous cell carcinoma, nine cases of mammary carcinoma, eight cases of adenocarcinoma, seven cases of papilloma, two cases each of basal cell carcinoma, hepatoid gland adenoma and one case of sebaceous gland adenocarcinoma. Round cell tumor was included seven cases of mast cell tumor, three cases of transmissible venereal tumor and one case of unclassified round cell tumor. The magnification of the foldscope was equivalent to 200X of conventional microscope. The current study revealed that foldscope is a reliable diagnostic tool for prompt diagnosis of epithelial tumors and round cell tumors.

Keywords: Conventional microscope, epithelial tumors, foldscope, round cell tumors

INTRODUCTION

Foldscope is a useful instrument for quick visualization of a variety of tissue samples. A foldscope is an optical microscope that was developed by Manu Prakash of Stanford University, USA[1]. It is made up of polypropylene paper, a spherical glass lens and a diffuser panel. It is an inexpensive, lightweight device that can be carried to the field and provide 140X magnification[1]. A microscope slide is inserted into the foldscope and assessed by sliding of slide across the folds of paper. The user can magnify pictures by use of a smart phone which gets connected to the foldscope with the help of magnets provided in the kit of foldscope. Neoplasia is an important concern for veterinary diagnosticians and researchers. Histopathology is considered as the gold standard for diagnosis of tumors as it can provide an indication of the 'grade' of a tumor[2]. Tumors of the skin and subcutaneous tissues are the most frequently recognized neoplastic disorders in animals[3]. Researcher diagnosed numerous histopathological slides and clinical samples in animals by using a foldscope. Foldscope can offer high-quality laboratory diagnostic capabilities to resource-constrained areas by bringing diagnostics to the people rather than moving individuals or clinical specimens to distant laboratories[4].

Foldscope, with its innovative design and affordability, has revolutionized microscopy; particularly in resource-constrained settings. According to a study, the low-cost nature of foldscope makes it an accessible tool for students, researchers and healthcare professionals in diverse environments. Its portability, ease of assembly and durability further enhance its utility, allowing for microscopy applications in fieldwork and educational settings. Additionally, foldscope integration with digital technologies, such as smart phone attachments, facilitates image and video capture, promoting data sharing and collaboration in scientific research and education. The customizable nature of foldscope as well as its role in community engagement and healthcare diagnostics, highlights its versatility and impact across various domains[5].

How to cite this article : Sangha, N., Singh, C.K. and Gupta, K. 2024. Diagnosis of epithelial and round cell tumors by foldscope in comparison with conventional microscope. Indian J. Vet. Pathol., 48(3) : 248-255.

MATERIALS AND METHODS

The tissue biopsies (n=100) received from 5th February 2019 to the 2nd November, 2020 in the Department of Veterinary Pathology, GADVASU was incorporated in this study. The biopsy sample were submitted in 10% neutral buffered formalin and processed. Sections were cut at 4-5µ thickness using automatic microtome (Leica Microsystems) after the paraffin blocks were prepared and stained with routine Haematoxylin and Eosin stain[6]. Toludine Blue staining was used to confirm/rule out the presence of chromatin granules in tissues suspected of mast cell tumor[6]. The section was observed to study histopathological changes like hyperchromasia, pleomorphism, anaplastic changes, presence of mitotic figures, cytoplasmic

basophilia and increased nuclear: cytoplasm ratio. Slides were examined under foldscope as well as conventional microscope. The slides were placed in the sample stage of a foldscope and analyzed on a cell phone screen. The magnification was further zoomed in on the screen of cell phone upto 2.1X. Thus, the final magnification of image achieved by foldscope examination was 294X. Therefore, histopathological slides were examined under conventional microscope at 200X magnification. The photography of biopsy slides was done by using Samsung Galaxy S9 Phone attached to foldscope (identity number 0002A7DB323F) and BX61 Olympus, conventional microscope attached to DP25 Olympus camera.

Processing of tissues

The biopsy samples were washed under running tap water and kept overnight in 10% neutral buffered formalin. For dehydration, tissues were added with ethyl alcohol in ascending grades, cleared in two changes of xylene and embedded in melted paraffin wax (Thermoelectron Corporation, Paraplast tissue embedding medium, 60-62^{O}C). The sections of tissues were placed inside paraffin blocks for support while cutting its sections. 4-5 micron thick sections (Leica Microsystems) of the tissue placed in the paraffin block were cut using automatic microtome. To remove the paraffin from the cut tissue section, xylene was used. To rehydrate the tissue it was treated with descending grades of ethyl alcohol followed by pouring running tap water on it. Finally, the tissue section was stained by routine Haematoxylin and Eosin stain.

Hematoxylin and Eosin staining

For staining, first of all, the tissue section was treated with two changes of xylene each for 15 minutes to remove excess paraffin, followed by treatment with series of descending grades of alcohol (Absolute alcohol, 90%, 80% and 70%) each for two minutes and a quick two minute wash in running water.

Next, hematoxylin solution was poured on the sections for about seven minutes and washed under running water for two minutes to remove excess stain. Then, it was differentiated in 1% acid alcohol solution by giving a quick dip in it and it was washed under running water for two minutes to remove excess acid. Next, it was exposed to ammonium solution until sections were observed to be bright blue (just dip) and washed again

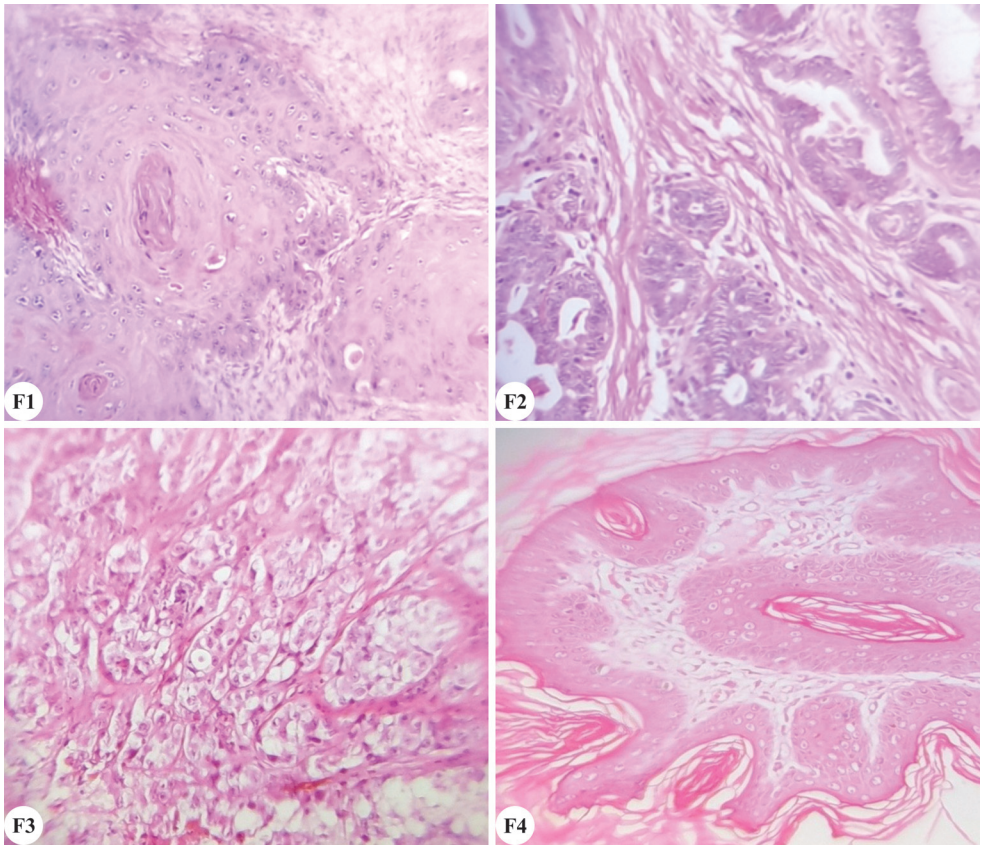

Fig. 1. Epithelial tumor images by H&E x Foldscope 294X. **F1.** Squamous cell carcinoma showing keratin pearl formation, characterized by pleomorphic epithelial cells, large hyperchromatic multiple nuclei with prominent nucleoli. **F2.** Mammary carcinoma showing papillae formation of glandular epithelium that separated by fibrovascular stroma. **F3.** Adenocarcinoma showing pleomorphic cells with hyperchromatic nuclei and vacuolated eosinophilic cytoplasm. **F4.** Papilloma consists of finger-like projections and keratinocytes.

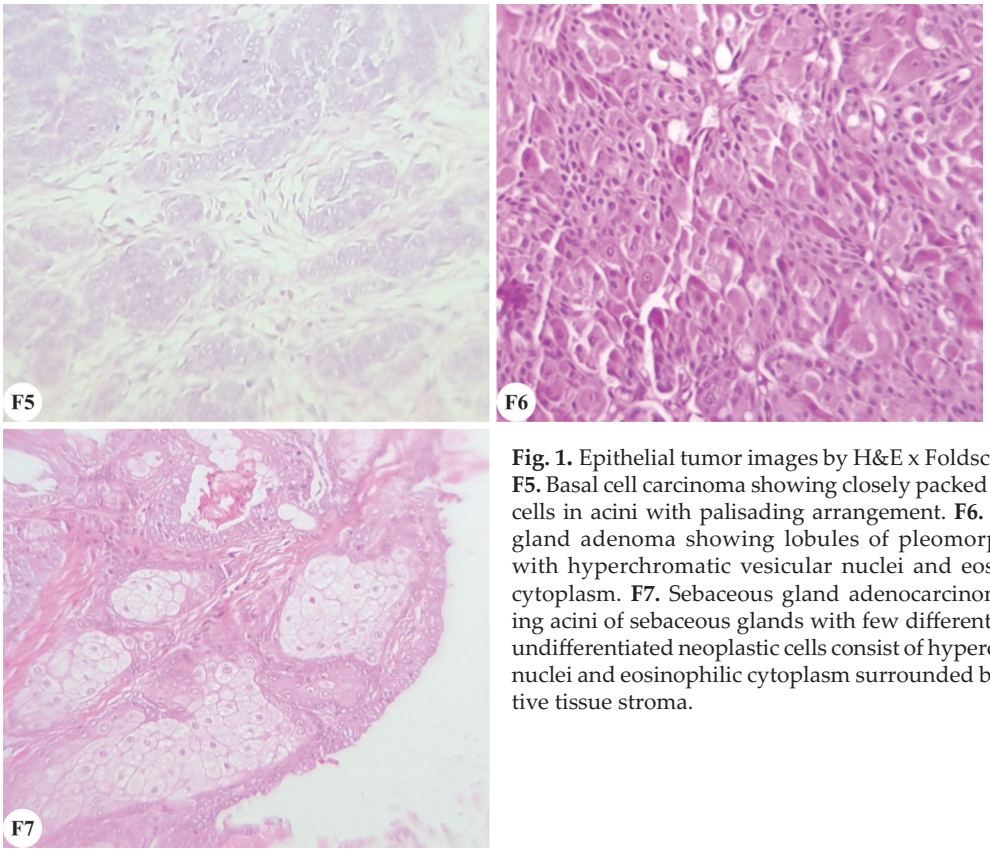

Fig. 1. Epithelial tumor images by H&E x Foldscope 294X. **F5.** Basal cell carcinoma showing closely packed epithelial cells in acini with palisading arrangement. **F6.** Hepatoid gland adenoma showing lobules of pleomorphic cells with hyperchromatic vesicular nuclei and eosinophilic cytoplasm. **F7.** Sebaceous gland adenocarcinoma showing acini of sebaceous glands with few differentiated and undifferentiated neoplastic cells consist of hyperchromatic nuclei and eosinophilic cytoplasm surrounded by connective tissue stroma.

under running water for two minutes to remove excess solution.

Eosin stain was poured on the section and left as such for three minutes. It was washed under running water for two minutes to remove excess stain. For dehydration, ascending grades of alcohol (Absolute alcohol, 70%, 80% and 90%) was used each for two minutes and to remove extra alcohol, it was dipped in two changes of xylene for two minutes each. The sections were mounted using DPX[6].

Toluidine Blue Staining

Toluidine Blue staining was used to confirm or rule out the presence of chromatin granules in tissues in mast cell tumor. Paraffin was removed from the tissues by means of treatment with xylene. Rehydration was achieved by treatment with a sequence of descending grades of alcohol (Absolute alcohol, 90%, 80% and 70%) each for two minutes. Toluidine Blue solution was put on slides for 25 minutes and the excess stain was removed from it by rinsing with running water. Dehydration was achieved by means of treatment with absolute alcohol at ascending grades of concentration. To remove alcohol from the tissues, the section was treated with two changes of pure xylene for two minutes each. The sections were mounted using DPX[6].

RESULTS

The present study is the first attempt to diagnose round cell tumors and second attempt to diagnose epithelial tumor by using foldscope at 294X magnification. Foldscope magnification is 140X and magnification is further zoomed in on the screen of cell phone upto 2.1X. Thus, the final magnification of image achieved by foldscope examination is 294X.

The epithelial tumors (44) and round cell tumors (11) were diagnosed by foldscope in 100 biopsy samples. Epithelial tumors (Fig. 1) included 17 cases of squamous cell carcinoma, nine cases of mammary carcinoma, eight cases of adenocarcinoma, seven cases of papilloma, two cases each of basal cell carcinoma, hepatoid gland adenoma and one case of sebaceous gland adenocarcinoma. Round cell tumor (Fig. 2) included seven cases of mast cell tumor, three cases of transmissible venereal tumor and one case of unclassified round cell tumor. Mast cell tumor was confirmed with Toludine blue staining. The biopsy slides of epithelial tumors (Fig. 3) and round cell tumors (Fig. 4) were re-examined with conventional microscopy at 200X magnification (20X objective x 10X eyepiece). There was complete agreement between the diagnosis of tumors using foldscope and conventional microscope. The one identified round cell tumor which was unclassified with foldscope was diagnosed as lymphoma with conventional microscope.

Foldscope Observations

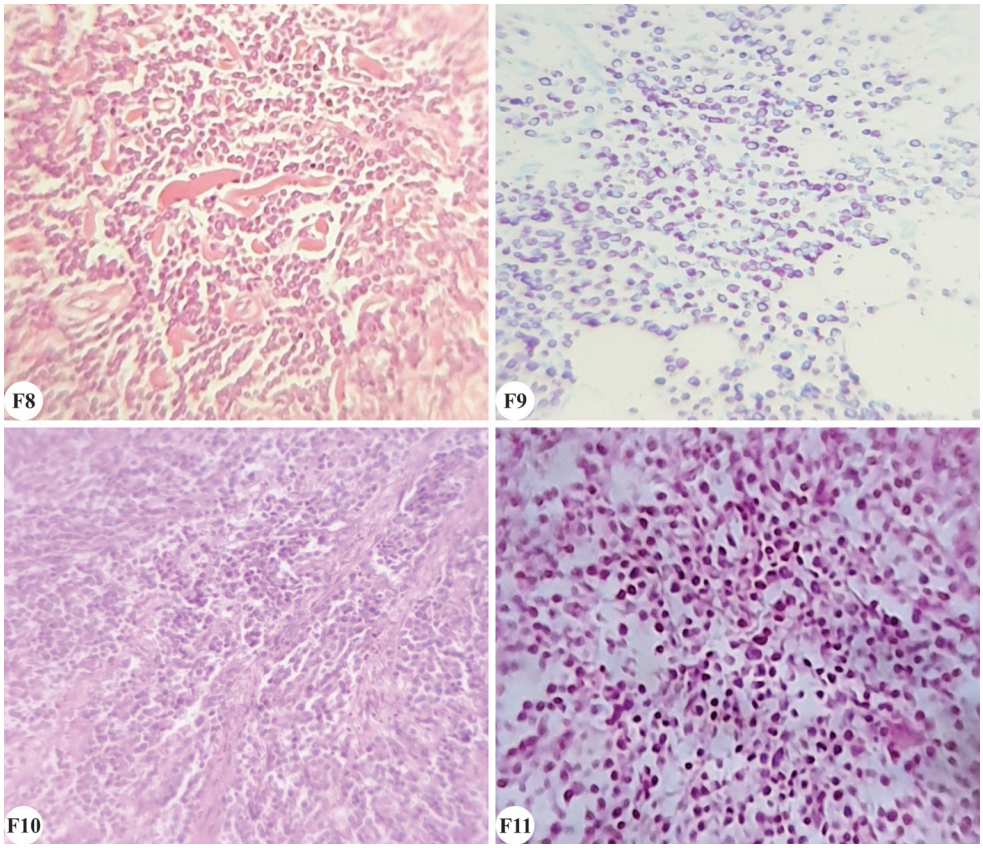

Fig. 2. Round cell tumor images by H&E x Foldscope 294X. **F8.** Mast cell tumor showing round to polygonal shape cells with darkly stained cytoplasm. **F9.** Toludine blue stain showing purple colored metachromatic granules in cytoplasm of mast cells. **F10.** Transmissible venereal tumor showing hypercellularity, uniform round to ovoid cells, hyperchromatic nuclei, high N:C ratio and cytoplasm projections. **F11.** Unclassified round cell tumor showing round to oval shape cells resembling with lymphoid cells and separated by fibrovascular tissue.

Foldscope examination of squamous cell carcinoma revealed pleomorphic epithelial cells, hyperchromatic and multiple nuclei with eosinophilic cytoplasm and keratinous pearl formation (Fig. 1 : F1). In some cases, Individual cell keratinization and proliferation of cords of neoplastic squamous epithelial cells was also visible. The nest of pleomorphic cells had multiple nuclei, anisocytosis, increased N:C ratio with distinct cell border were also observed in few cases. In mammary carcinoma, papillae formation of glandular epithelium separated by fibrovascular stroma and presence of various irregularly sized lobules was observed (Fig. 1 : F2). Flattened pleomorphic cells with prominent hyperchromatic nuclei and vacuolated eosinophilic cytoplasm arranged in tubular pattern was suggestive of adenocarcinoma by foldscope examination (Fig. 1 : F3). In papilloma, finger-like projections (Fig. 1 : F4) lined by stratified squamous epithelium extended to surface of mucosa with pleomorphic cells along with hyperplasia of stratum spinosum layer was seen while in basal cell carcinoma, nests of epithelial cells resembling epidermal basal cells arranged in closely packed acini showing palisading arrangement (Fig. 1 : F5). Acini of cells were separated by connective tissue stroma.

Lobules or cords of pleomorphic, eosinophilic, hepatoid cells having polygonal shape with hyperchromatic and vesicular nuclei were visible by foldscope examination in hepatoid gland adenoma (Fig. 1 : F6). However, variable sized lobules of sebaceous glands separated by connective tissue stroma. Acini of differentiated sebaceous glands and few undifferentiated pleomorphic cells with large, hyperchromatic nuclei and eosinophilic cytoplasm were observed in sebaceous gland adenocarcinoma (Fig. 1 : F7).

In round cell tumors, mild pleomorphic, round to polygonal cells with darkly stained cytoplasm arranged in ribbon like pattern (Fig. 2 : F8) were visible by foldscope examination. Mast cell tumor was confirmed by purple coloration of metachromatic granules in toluidine blue special staining (Fig. 2 : F9). In transmissible venereal tumor, proliferation of large, round to oval shape cells with clear cytoplasmic projections, hyperchromatic nucleus and amphophilic to clear cytoplasm separated by connective tissue fibers (Fig. 2 : F10). Loose sheet of pleomorphic cells with narrow rim of eosinophilic cytoplasm and high N:C ratio was also observed. Foldscope examination revealed round to oval shape

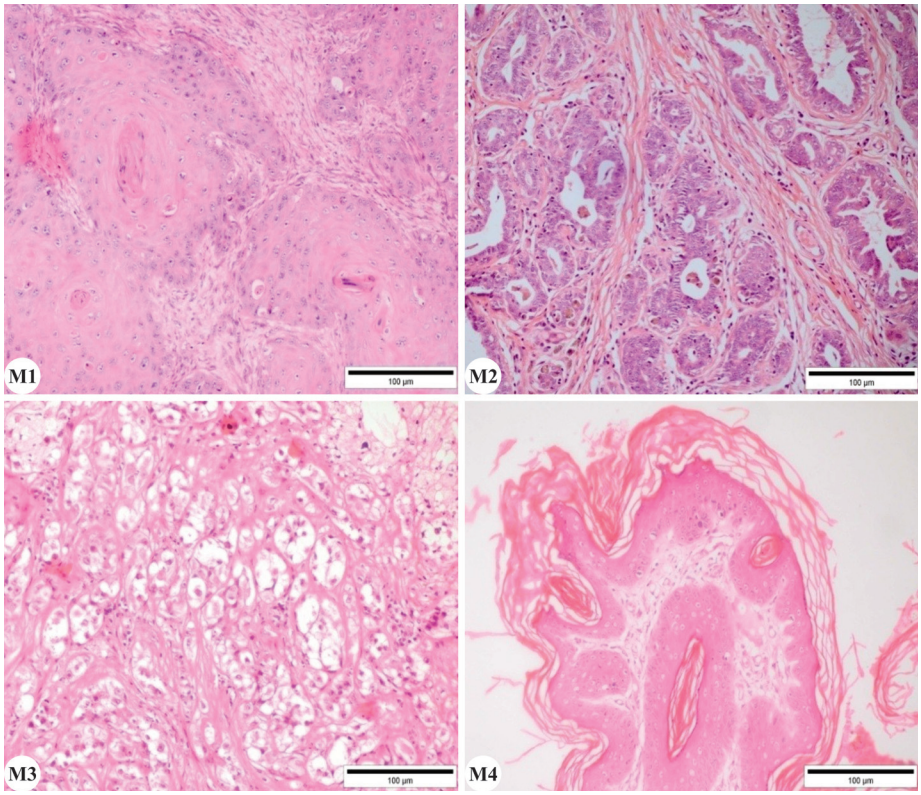

Fig. 3. Epithelial tumor images by H&E x200 X Conventional Microscope. **M1.** Squamous cell carcinoma showing pleomorphic epithelial cells along with hyperchromatic multiple nuclei, eosinophilic cytoplasm and occasionally cell nest. **M2.** Mammary carcinoma showing papillae formation of glandular epithelium that separated by fibrovascular stroma. **M3.** Adenocarcinoma showing pleomorphic cells with hyperchromatic nuclei and vacuolated eosinophilic cytoplasm. **M4.** Papilloma consists of finger like projections along with keratinocytes, pyknotic nuclei and abundant clear cytoplasm.

cells resembling with lymphoid cells, separated by fibrovascular tissue and diagnosed as unclassified round cell tumor (Fig. 2 : F11).

Conventional Microscope Observations

Conventional microscopic examination showed round to oval shape neoplastic squamous epithelial cells with hyperchromatic and multiple nuclei, anisocytosis, eosinophilic cytoplasm and keratinous pearl formation, suggestive of squamous cell carcinoma (Fig. 3 : M1). Individual cell keratinization with prominent intercellular bridges and proliferation of cords of neoplastic squamous epithelial cells arising from epidermis and extending up to the dermis layer was also observed in some cases. The neoplastic cells present in nests along multiple nucleus, prominent nucleoli, anisocytosis, increased N:C ratio with distinct cell border was also seen in few cases of squamous cell carcinoma. Microscopically, there were numerous pleomorphic cells in glandular epithelium and irregularly sized lobules separated by fine fibrovascular stroma suggesting of mammary carcinoma (Fig. 3 : M2). Individually, polygonal to oval shape pleomorphic cells with moderate anisocytosis and hyperchromatic nuclei were also seen. In adenocarcinoma, oval to columnar shape, flattened, pleomorphic cells with hyperchromatic nuclei, vacuolated eosinophilic cytoplasm and distinct cell borders arranged in tubular pattern was noticed (Fig. 3 : M3).

Finger-like projections (Fig. 3 : M4) lined by stratified squamous epithelium extended to the surface of the mucosa with hyperplasia of the stratum spinosum layer, suggesting of papilloma. Microscopic examination revealed hyperchromatic epithelial cells resembling basal cells with of the epidermis arranged in closely packed acini showing palisading arrangement in basal cell carcinoma (Fig. 3 : M5). Acini were separated from each other by connective tissue stroma. In hepatoid gland adenoma, lobules of large, eosinophilic, hepatoid cells having round to polygonal shape with vesicular nuclei, single prominent nucleoli and eosinophilic cytoplasm was seen (Fig. 3 : M6). There were large numbers of undifferentiated neoplastic cells, as well as acini of differentiated sebaceous glandsin sebaceous gland adenocarcinoma (Fig. 3 : M7). Neoplastic cells had mild to moderate pleomorphism, large, hyperchromatic nuclei with prominent nucleoli and eosinophilic cytoplasm was also observed.

In round cell tumor slides, microscope examination revealed hypercellularity, cellular pleomorphism and

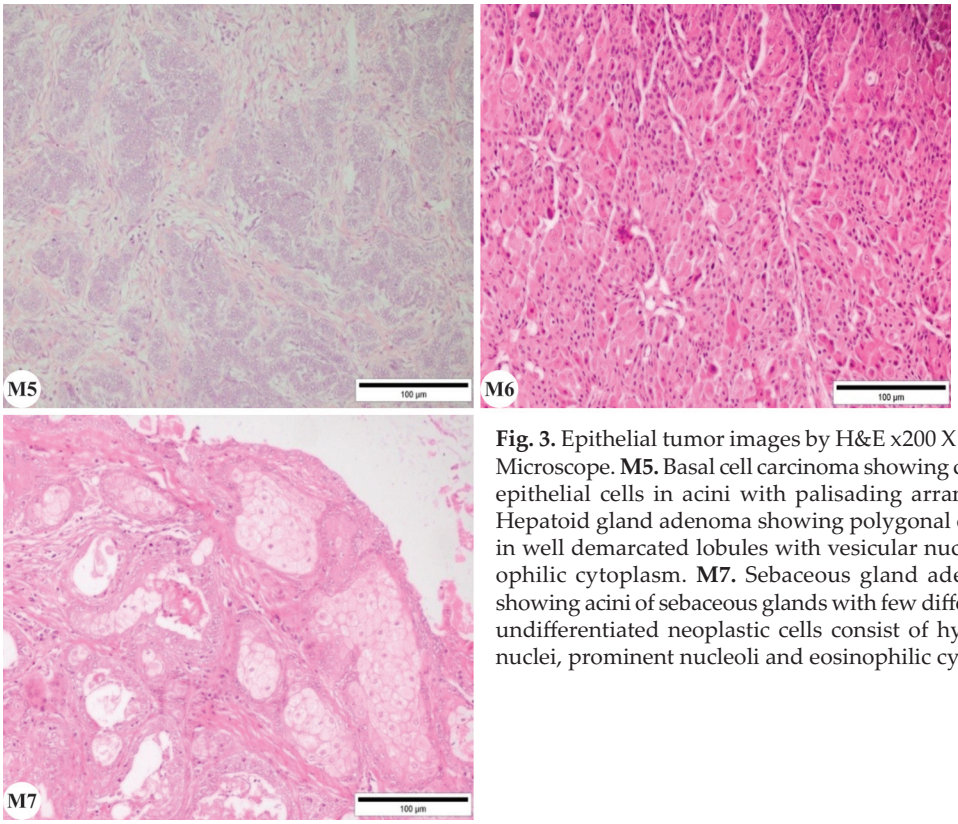

Fig. 3. Epithelial tumor images by H&E x200 X Conventional Microscope. **M5.** Basal cell carcinoma showing closely packed epithelial cells in acini with palisading arrangement. **M6.** Hepatoid gland adenoma showing polygonal cells arranged in well demarcated lobules with vesicular nuclei and eosinophilic cytoplasm. **M7.** Sebaceous gland adenocarcinoma showing acini of sebaceous glands with few differentiated and undifferentiated neoplastic cells consist of hyperchromatic nuclei, prominent nucleoli and eosinophilic cytoplasm.

neoplastic cells arranged in ribbon pattern (Fig. 4 : M8). Round to polygonal cells with centrally placed nuclei, eosinophilic granular cytoplasm and clear cellular borders were also observed in mast cell tumor. Mast cell tumor was confirmed by toluidine blue stain as metachromatic granules were seen in cytoplasm (Fig. 4 : M9). In transmissible venereal tumor, proliferation of uniform round to ovoid shape cells with indistinct outlines and clear cytoplasmic projections and light amphophilic to clear cytoplasm was noticed (Fig. 4 : M10). The nucleus had coarse chromatin and eosinophilic cytoplasm. Neoplastic cells were separated by connective tissue fibers. Loose sheet of pleomorphic cells surrounded by a narrow rim of eosinophilic cytoplasm and high N:C ratio was also observed.

In unclassified round cell tumor slide, proliferation of lymphoblast cells with round to oval shape nuclei, mild to moderate amount of eosinophilic cytoplasm with clear boundaries and high N:C ratio were observed and suggestive of lymphoma (Fig. 4 : M11). Occasionally, binucleated cells were also seen.

DISCUSSION

Only single study was conducted earlier forthe diagnosis of epithelial tumors by using foldscope. No study has been reported, regarding diagnosis of sebaceous gland adenocarcinoma and round cell tumor with foldscope. The present study is therefore, the first attempt for diagnosis of these tumors by using foldscope.

Studies have reported the presence of keratinous pearls along with hyperchromasia, pleomorphism of nuclei and hyperplasia of stratum spinosum layer in squamous cell carcinoma and proliferation of glandular epithelium and mesenchymal cartilaginous growth along with epithelial mesenchymal transition zones in mixed mammary tumor[7]. In adenocarcinoma, neoplastic cells with centrally placed nucleus in sheets were reported[7]. The features of papilloma observations in the present study were in completely agreement with the observation previously mentioned study in which they found finger-like projections with cluster of cells around basement membrane in papilloma and increased thickness of stratum spinosum in acanthomatous papilloma[7]. However, presence glandular, cuboidal cells in palisading appearance along with proliferating nests of basaloid cells in basal cell carcinoma and hepatocytes like cells with proliferative changes in hepatoid gland adenoma was also reported[7].

Conventional microscopic observations of squamous cell carcinoma were completely agreement the study[8]. Whereas, researchers in another study observed proliferation of invasive squamous cells arranged with pseudo-cord appearance[9]. Similar microscopic findings of present study in mammary carcinoma have been reported

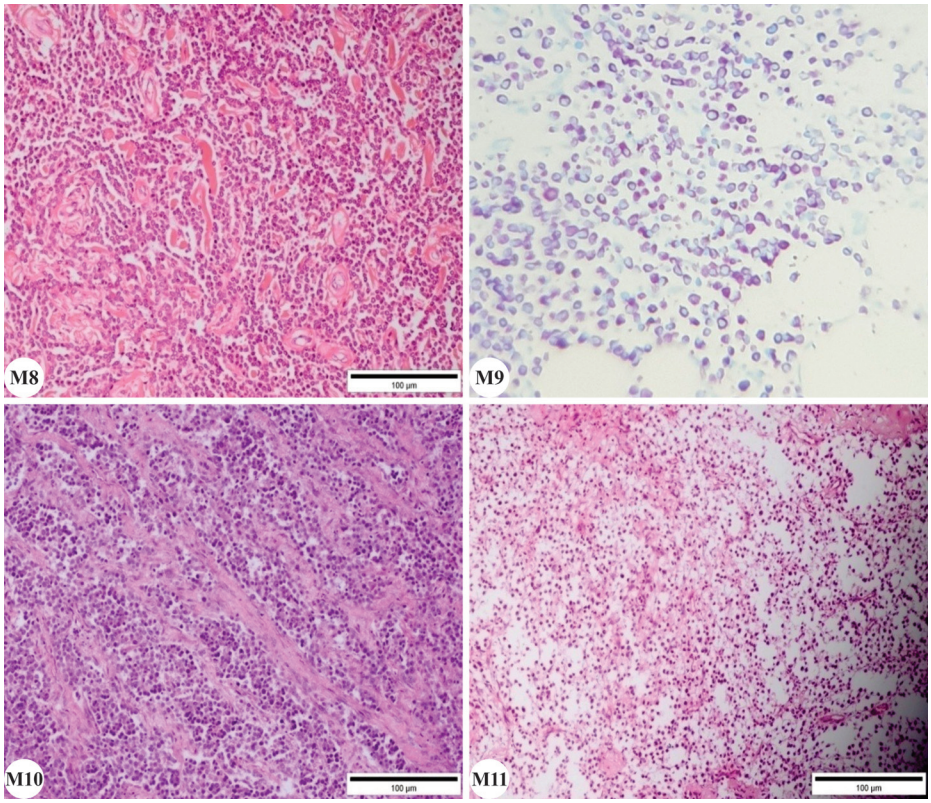

Fig. 4. Round cell tumor images by H&E x200 X Conventional Microscope. **M8.** Mast cell tumor showing round to polygonal shape cells with centrally placed nuclei and darkly stained granular cytoplasm. **M9.** Toludine blue stain showing purple stained metachromatic granules in cytoplasm of mast cells. **M10.** Transmissible venereal tumor showing proliferation of uniform round to ovoid shape cells with indistinct outlines and clear cytoplasmic projections and light amphophilic to clear cytoplasm. **M11.** Unclassified round cell tumor showing proliferation of lymphoblast cells with round nuclei, high N:C ratio and eosinophilic cytoplasm.

by Sassi and colleagues[10]. Similarly, Studies reported that pleomorphic neoplastic epithelial cells, eosinophilic cytoplasm, ovoid hypochromatic nuclei with nucleoli and distinct cell boundaries in case of adenocarcinoma[11] whereas, Baharak and colleagues reported pleomorphic cuboidal to polygonal shape cells arranged in glandular patterns with prominent nucleoli and eosinophilic cytoplasm[12]. Earlier workers[13,14] have similarly reported that the microscopic examination of papilloma slides showed papillary projections of squamous epithelium layer along numerous koilocytes with clear cytoplasm and pyknotic nuclei, acanthosis of stratum spinosum layer in papilloma and proliferation of fibrous connective tissues in fibropapilloma while, Hamad and colleages[15] have reported papillary projections of epithelium, koilocytes with swollen perinuclear halo keratinocytes and inclusion bodies. Similarly, a study also reported proliferation of basal cells with palisading pattern of nuclei[16]. Whereas, Srinivasa and colleagues reported proliferation of basal epithelial cells along oval nuclei, prominent nucleoli and arrangement of rows of palisading cells in finger like projections in adenoid type basal cell carcinoma[17]. Previous studies have similarly reported hepatoid cells with large nucleus, prominent nuclei and vacuolated eosinophilic cytoplasm arranged in lobules and no mitotic figure in adenoma and dissimilar with reports in which cords of neoplastic cells with oval nuclei and eosinophilic cytoplasm arranged in reticular pattern in adenoma were seen[18,19]. Similarly, multilobulated sebaceous glands along with infiltration of pleomorphic epithelial cells separated by proliferative connective tissue in sebaceous gland carcinoma were reported by[20].

Earlier, similar findings were reported in case of mast cell tumor characterized by centrally located oval nuclei with moderate amounts of granular cytoplasm and distinct cellular boundaries[21] while, pleomorphic round and fusiform cells with centrally placed hyperchromatic nuclei, marked anisokaryosis, anisocytosis and high N:C ratio also reported earlier in MCT[22]. The same study has also reported metachromatic granules observed in cytoplasm of MCT with toluidine blue stain[22].

Microscopic findings in the present study were similar to that described by earlier worker[23] and dissimilar with Paramjit and colleagues, as they reported large round to oval vesicular nuclei with foamy eosinophilic cytoplasm and various mitotic figures arranged in cord-

like fashion in case of TVT[24].

Earlier, it was reported round nuclei with pale cytoplasm and irregularly aggregated chromatin in case of lymphoma[25] while, Guizelini and colleagues reported round neoplastic lymphocytes with distinct boundaries, round nuclei, prominent nucleoli and granular chromatin[26].

CONCLUSION

Foldscope's origami-based design and affordability provide a portable, accessible and customizable microscopy solution, offering advantages in resource-limited settings and promoting widespread scientific exploration compared to traditional microscopes. It is highly likely that foldscope become the approach of choice in future for obtaining expert advice with promptness of internet connectivity and connection with various experts in different fields across the globe. The analysis of related literature has no reported study of round cell tumors and epithelial tumors by foldscope. Hence, the given study is vital as it opens doors for study by foldscope.

ACKNOWLEDGEMENT

The authors are grateful to the Directorate of Research and Centralized Clinical Diagnostic Laboratory, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, for providing facilities to carry out the work.

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Indian J. Vet. Pathol., 48(3) : 256-260, 2024: DOI: 10.5958/0973-970X.2024.00042.4

SHORT COMMUNICATION

Foot and mouth disease outbreak in a pig farm at Nongpyiur, Meghalaya

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Received: 9.2.2024; Accepted: 5.3.2024

ABSTRACT

Foot and mouth disease (FMD) is a highly contagious viral disease affecting cattle, pig, sheep, goat and other cloven-hoofed animals. Its manifestation varies in different species and accordingly, cattle are considered as the indicator host, small ruminants as maintenance hosts, and pigs as the amplifier hosts. This study investigates a suspected FMD outbreak in an organized pig farm at Nongpyiur, Shillong, Meghalaya during early 2020, where the animals exhibited clinical symptoms suggestive of FMD. During the investigation, clinical samples (n=15) along with serum samples (n=42) were collected from affected and in-contact apparently healthy animals. The clinical samples were processed and subjected to serotyping sandwich ELISA and RT-multiplex PCR, where 12 samples were found positive for serotype O. The serum samples were tested using in-house indirect 3AB3 NSP ELISA, where 32 (76.19%) were found positive for 3AB NSP antibodies. The serum samples were also tested in LPB ELISA that could not find any samples to be protective against all three serotypes. However, 28 samples were found to have a 4-fold spiked response against serotype O strain in comparison to other two strains fingering towards an evident circulation of serotype O in the farm. Contaminated feed caried by the vehicles from outside of state was suspected for the source of infection. Few FMD incidences in cattle of nearby areas as admitted by the farmers might also have been another contributing factor. Besides, the commuting of the pig handlers and care-takers from those local areas with a breach in biosecurity measures might have been the precipitating factor behind the disease outbreak in the farm.

Keywords: FMD, multiplex RT-PCR, 3AB3 NSP ELISA, pig, serotype O, typing ELISA

How to cite this article : Rout, M., Bora, K., Pargai, K., Dkhar, L., Kylla, H., Subramaniam, S., Mohapatra, J.K., Singh, R.K. and Singh, R.P. 2024. Foot and mouth disease outbreak in a pig farm at Nongpyiur, Meghalaya. Indian J. Vet. Pathol., 48(3) : 256-260.

Foot and mouth disease (FMD), a notifiable vesicular disease is the first disease for which the World Organization for Animal Health (WOAH) established an official list of disease-free countries. Even being a pathogen discovered 125 years before, the disease is still endemic in about two-thirds of the countries periodically re-emerging in several countries. This is undoubtedly the most serious challenge for livestock health. FMD is caused by seven immunologically distinct serotypes of FMD virus (FMDV) exhibiting indistinguishable clinical signs[1]. In India, serotypes O, A and Asia 1 are prevalent with the first one being responsible for the majority of outbreaks. The disease affects all members of the order Artiodactyla (cattle, swine, sheep, goats and other cloven-hoofed ruminants). Each species varies in its susceptibility to infection and manifestation of the clinical disease along with the ability to transmit the virus. Cattle (indicator host) are usually the most frequently involved in epidemics with maintenance of the FMDV and exhibit frank clinical signs of FMD. Small ruminants (maintenance hosts) are naturally infected with very few or no clinical signs or symptoms in a subdued manner, and persistent infection is less frequent and of shorter duration than in cattle.

Pigs (amplifier hosts), highly susceptible to FMDV are the exhibitors of clear symptoms with production of huge infectious aerosol virus through their respiratory tract. Maximum excretion of aerosol virus in pig coincides with development of clinical disease and lesions on the snout, tongue and feet, and declines over the following 3 to 5 days as the antibody response develops. The role of pigs in FMD episodes was crucial as evident from 38 immediate notifications made to OIE/WOAH from January 2010 to April 2011, where pigs were involved in 7 cases mainly in Eastern Asia, affecting China, Chinese Taipei, North Korea, South Korea and Hong Kong[2]. Meghalaya has the third-highest pig population in India, and pig farming is a crucial component of household economic systems in the state. Meghalaya had a total of 7,06,364 pigs in 2019 (20th Livestock Census, DAHD, GoI). The primary objective of the work was to investigate the suspected FMD outbreak in the organized swine herd at Nongpyiur of Meghalaya state so as to report and create awareness on the

important disease in one of the important species reared in the NE region, so that necessary attention can be drawn and steps towards its prevention can be undertaken by the state animal health authority.

During February 2020, a suspected FMD outbreak was observed in an organized government pig farm at Nongpyiur, Meghalaya. During the time of outbreak, the farm had 32 sows, 15 boars (11 in AI shed at an isolated area and 4 in main shed), 38 piglets, 84 growers thus the total stock was around 169. Out of these animals, 12 sows, 3 boars, 25 piglets, 20 growers were affected. Out of 15 boars, 11 kept in the AI shed were not affected, while 3 out of 4 boars kept in the main shed were affected with FMD. Total 8 pregnant sows were infected with FMD those aborted or delivered still births, but subsequently recovered. The animals were not vaccinated against FMD. During the outbreak, the affected animals were provided with the symptomatic treatment such as Meloxicam injection as analgesic, antipyretic to reduce fever, Himax ointment for local application on the wounds, Amoxycillin antibiotic injection, washing of wounds with Potassium permanganate solution etc. Vitamin and mineral supplements were also given to the affected animals.

The infected animals exhibited clinical symptoms of fever, anorexia, respiratory distress, and lameness. Clinical samples (n=15) (snout epithelium, vesicular fluid, and lesions from the coronary band) were aseptically collected from symptomatic animals in 50% phosphate buffered saline-glycerol transport medium (pH 7.5) on cold chain. The animals during the investigation were bled with all aseptic measures through the ear vein, and serum samples were separated after clotting followed by centrifugation. A total of 42 sera were collected both from affected and apparently healthy in-contact animals.

An indirect ELISA was performed to detect antibodies against FMD virus (FMDV) 3AB NSP[3]. Test sera including the negative and positive controls were diluted @ 1:20 and anti-porcine horse radish peroxidase conjugated antibodies (Komabiotech, South Korea) were dispensed @ 1:12000 dilution. Serum samples producing corrected optical density values ≥55% of that of the positive control were graded as positive.

All the serum samples were tested in liquid phase blocking (LPB) ELISA. Two-fold dilution (from 1:16 to 1:128) of serum samples were tested for determining the serotype-specific FMDV SP-Ab titre to assess the overall status of vaccinal immunity/protective antibody against all three serotypes O, A and Asia 1 in the vaccine using the in-house LPB ELISA kit (ICAR-DFMD, Mukteswar) as per the procedure earlier[4]. The results were expressed as percentage reactivity for each serum dilution as follows:

$$\text{Percentage reactivity} = (OD_{\text{mean of each test serum dilution}} / OD_{\text{mean of antigen control}}) \times 100$$

The antibody titres were expressed as logarithm of reciprocal of serum dilutions giving 50% of the absorbance recorded in the antigen control wells. The samples demonstrating $\log_{10}$ titre of ≥1.8 were graded to have sufficient protective antibody[5].

Clinical samples were tested in a serotype discriminating antigen detection ELISA as described earlier[6]. Samples found negative in serotyping ELISA were subjected to serotype differentiating reverse transcription-multiplex polymerase chain reaction (RT-mPCR) as described earlier[7]. The total RNA was extracted from the samples using RNeasy Mini Kit (Qiagen, Germany). Reverse transcription was performed using M-MLV reverse transcriptase (Promega, USA) and reverse primer NK61[8] followed by mPCR using three serotype-specific positive sense primers e.g., DHP13, DHP15 and DHP9 for serotype O, A and Asia 1, respectively with the reverse primer NK61 using Hotstar Taq DNA polymerase (Qiagen, Germany). The PCR amplicons were analyzed by electrophoresis on 2% agarose gel stained with ethidium bromide and captured through gel documentation system.

During the outbreak in the farm, early signs observed in pigs were fever, inappetence and reluctance to move. Affected pigs were lethargic and stayed huddled together expressing reduced or little interest in feed. Erosive vesicular lesions in upper snout (Fig. 1) and hoof (Fig. 2 and 3) suggestive of FMD were observed in the affected animals. Vesicles in snout and feet after rupture have

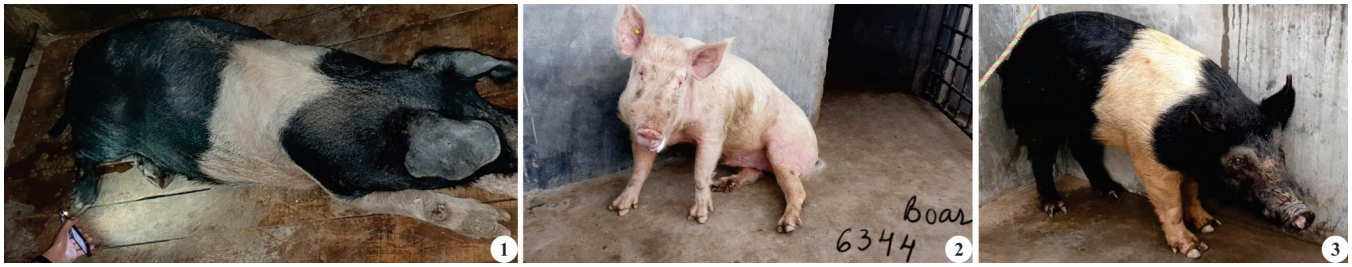

Fig. 1. Erosive carpal lesion due to friction on ground after feet lesion; **Fig. 2.** Frothy saliva and ruptured vesicles on the upper snout of large white Yorkshire boar with evidence of inability to stand; **Fig. 3.** Obvious feet lesions in hoof and coronary band and healed lesions on the upper snout.

caused the superficial epidermis to slough off revealing hyperemia and subsequent hemorrhage on underlying tissue that healed up subsequently. More severe lesions occurred in the feet commencing with lameness and blanching of the skin around the coronary bands. The foot lesions (vesicles between the claws and coronary band) led to inability of the affected animals to stand and they were found struggling and falling down on the floor. As they were housed in pens with rough concrete floors, mechanical injuries led to aggravated erosive lesions on the elbows (carpal region) and hocks (tarsal region). Carpal regions of one or more feet were having erosive lesions due to such mechanical injury on the floor. The vesicles were present on and behind the rim of the snout, in the nares, on the soft tissues of the feet, including the coronary band, heel, the bulbs of the toes and interdigital clefts as reported earlier[9]. Such lesions around the coronary bands have been reported to be the most consistent finding in pigs while legions at other sites may be found less regularly, depending on environmental and other factors[9]. In some affected animals, lesions on the coronary band have led to sloughing of the hoof or the claws. Similar lesions were earlier reported in FMD affected pigs during outbreak in a private pig farm at Kotty in Kollam district of Kerala in 2013 caused by FMDV serotype O[10].

Out of 15 clinical samples tested in serotype differentiating sandwich ELISA, 7 were found positive for serotype O, while in multiplex RT-PCR, 5 more samples were found positive for serotype O (Fig. 4). In NSP serology, 32 out of 42 (76.19%) serum samples were found positive for 3AB NSP antibodies of FMDV. Those

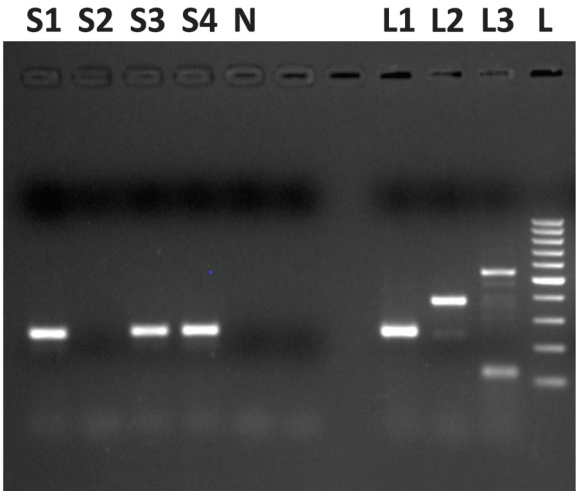

Fig. 4. Figure of RT-multiplex PCR assay showing the amplified product 249 bp of VP1 gene segment corresponding to FMDV serotype O analyzed by 2% electrophoretic agarose gel stained with ethidium bromide. Positive amplification of 3 samples is shown in lanes S1, S3 and S4; Lane N: Negative control; Lane L: 100 bp molecular weight marker; L1, L2, L3 are known products of serotype O, A and Asia 1.

sera found negative were from the animals those could not seroconvert at the time of collection of samples. The paired sera with a gap of 15 days could have been useful in detecting antibodies in those animals, that was not possible. The 42 serum samples tested in LPB ELISA could not find any samples to be protective against all three serotypes. However, 28 samples were found to have a 4-fold spiked response against serotype O strain in comparison to other two strains fingering towards an evident circulation of serotype O in the farm. Anyway, the identification of serotype O in the clinical samples and demonstration of 3AB NSP antibodies in the serum samples were immediately conveyed to the farm authority, after which proper biosecurity measures were enforced with segregation of the affected animals in a separate enclosure. The farm attendants and animal caretakers were instructed to follow sanitary measures with footbath and handwash before and after attending the animals. Such practices could be able to minimize the spread of virus to other healthy pigs in the farm. Notably, early detection and reporting of FMD is crucial for limiting the spread of the virus and minimizing the potential impact of an outbreak. The time to detect, report and confirm FMD is one of the most important measures of the effectiveness of a surveillance system[11]. In this context, it was estimated that if the national movement ban in the 2001 FMD epidemic in the UK had been imposed 2 days earlier, the final size of the epidemic would have been reduced to 48% of its observed size[12]. Similarly, modelling the 2002 FMD epidemic in South Korea could determine that initiating actions 5 days earlier would have produced less than half of the actual observed infected premises[13].

Pigs are more susceptible to FMDV infection through the upper gastrointestinal tract (oropharynx) or oral route than inhalation unlike ruminants[14]. The source of FMD outbreak in South Africa in 2000 was meat scraps fed to pigs and during the 2001 FMD outbreak in the United Kingdom, feeding of unprocessed pig swill was considered the source of the virus introduction. In the present study, during the time of the outbreak in the pig farm, no history of FMD in adjacent areas was recorded. The farm manager was suspecting for contamination in feeds and the vehicles which supplied feed from outside the state. No FMD incidences in cattle in adjacent areas were also reported. However, few farmers have admitted the fact of FMD-like symptoms observed in their cattle but that was never reported by the veterinarian. It might also be a contributing factor being the source of infection. Again, during the time, animal care-takers and handlers were commuting from their residences in the nearby areas, who might have been the probable source of infection and might have mechanically carried the virus into the farm through fomites. Proper biosecurity

measures were also not followed at that time and the breach in such zoo sanitary measures might have allowed the infection to get into. Further, an index positive animal due to huge production of infectious aerosol virus could have been the reason for rapid spread of the virus to other healthy animals in the farm, as infected pigs excrete high concentrations of the virus in aerosol form, being considered a major amplifying host[15]. However, FMDV excretion starts during the incubation period, before the first clinical signs are evident, significantly increasing the risk of virus spreading that is particularly important in pigs[16]. Further, in pig active viraemia and virus excretion start during incubation period[17]; however, the maximum excretion of virus coincides with the development of clinical disease and lesions and declines over the following 3-5 days as the antibody response develops. Such excretion of virus can begin up to 4 days before the onset of clinical signs that is of great epidemiological significance. Pigs excrete massive quantities of airborne virus about 3000 times as much as cattle in their breath[18] thereby constituting an important reservoir for virus amplification. Such epidemiological investigative studies create the foundations for the design and implementation of strategies and policy to prevent or mitigate disease impact, including modeling and risk analysis techniques[19].

Ample evidence supported by many reports demonstrate the importance of porcine species in the epidemiology of FMD. To cite a few, from January 2006 to May 2011, a total 1,795 FMD outbreaks were notified to OIE, affecting 42 countries from 4 continents. Nearly 1100 outbreaks involved pigs, in which more than 25000 pigs were directly affected. The more frequent victim serotype affecting pigs was serotype O that was identified in more than 88% of outbreaks. The role of pigs in the recent FMD epidemics has been significant, mainly in countries from Eastern Asia. Pigs were involved in 7 of 38 immediate notifications made to OIE from January 2010 to May 2011, affecting China, Chinese Taipei, North Korea, South Korea and Hong Kong. In the 2001 FMD epidemic in the UK, pigs also played a significant role, as three of the first five reported outbreaks contained pigs[20].

ACKNOWLEDGEMENTS

The authors would like to express their sincere thanks to the Indian Council of Agricultural Research for providing funds and facilities to carry out this work. We acknowledge the assistance of the farm veterinarians and officials along with their support staff for collecting samples from the infected pigs.

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Indian J. Vet. Pathol., 48(3) : 261-265, 2024: DOI: 10.5958/0973-970X.2024.00043.0

SHORT COMMUNICATION

Pathology of cholangiocellular adenoma: A benign tumour mimicking the malignant neoplasm in a dog

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Received: 4.11.2023; Accepted: 21.11.2023

ABSTRACT

An incidental finding of cholangicellular adenoma in a non-descript dog was described. A seven-year old non-descript dog carcass was presented for necropsy. Liver showed single to multiple aggregated cyst-like structures in the left and right lobes protruding slightly above the capsule. Microscopically, these structures revealed an irregularly circumscribed sub-capsular lesion characterized by hyperplastic bile ducts forming irregular tubules. Hyperplastic bile duct tubules formed inter-anastomosing locules separated by thin non-collagenous stroma. Few locules contained bile increments, cell debris and erythrocytes. Locular content showed positive for neutral mucin with Alcian blue-PAS stain whereas, modified Foucher's stain showed emerald green bile pigments. Tubules were penetrating into the hepatic parenchyma causing isolation and pressure atrophy of hepatic cords. Histochemical profile differentiated CCA from Von Meyenberg complexes as an origin from postnatal life. Margin of the lesion revealed atypical neoplastic cells characterized by anisocytosis, anisokaryosis, few mitotic figures and slightly vacuolated biliary duct epithelium. Although the cause was obscure till date, hepatic injury/disease predispose for CCA as of now. Evidence of splenic infarct, enteritis, interstitial nephritis, pancreatitis in the present case substantiate the possible hepatic injury to be the cause for the CCA. Hence the lesion was diagnosed as CCA with malignancy features in a dog.

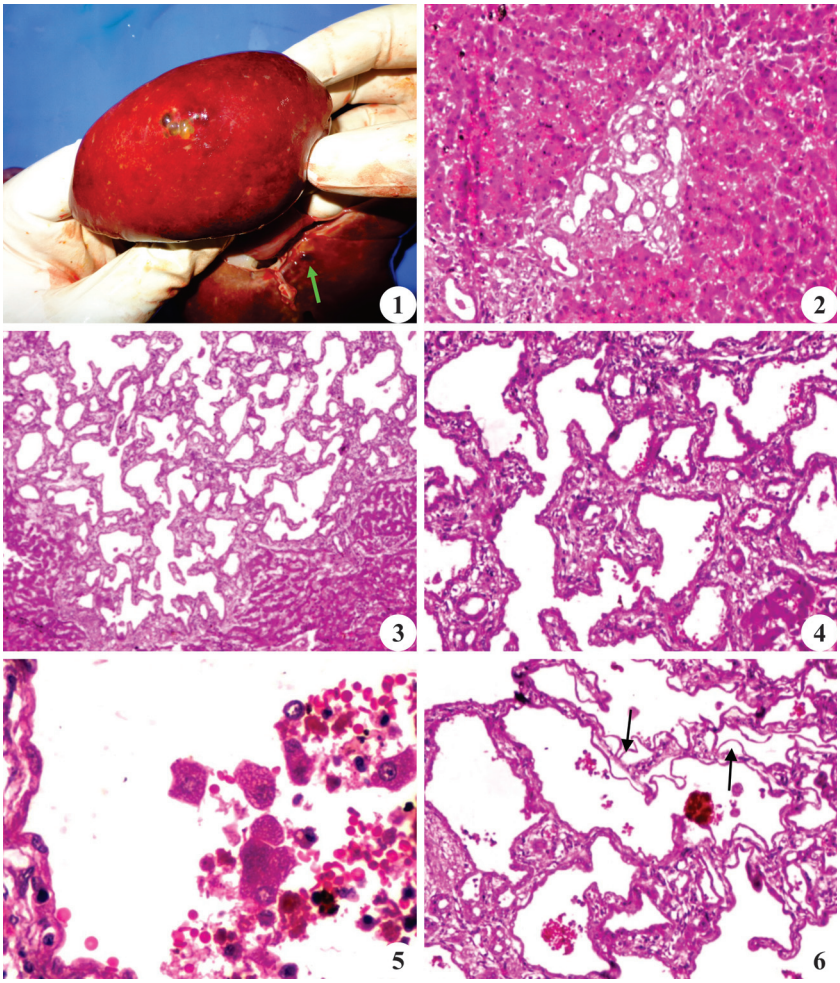
Keywords: Bile duct, carcinomatous transition, cholangiocellular adenoma, dog

Primary epithelial tumours of liver are classified into hepatic, bile duct and of mixed cell origin. Bile duct derived epithelial neoplasm includes cholangicellular adenoma (CCA) and cholangiocellular carcinoma (CCC). CCA is a benign neoplasm composed of well differentiated cholangiocytes[1,2]. It is found to be extremely rare in dogs[3]. CCA is reported less frequently than CCC in dogs[4,5]. CCAs are serene lesion without apparent clinical signs and are mostly found incidentally during imaging techniques and or postmortem examination. CCAs are solitary and well circumscribed benign neoplasm and do not undergo metastasis. They grow by expansion causing pressure atrophy of surrounding hepatic parenchyma[6]. The cause of the CCA is still obscure and thought to be a sequelae of hepatic injury[7,8]. The present case describes the pathology of cholangiocellular adenoma featuring malignancy transition in a non-descript dog.

A seven year old non-descript male dog carcass was submitted for necropsy to the Department of Veterinary Pathology, Veterinary College and Research Institute, Orathanadu, Thanjavur, Tamil Nadu suspected with the history of inappetence, watery diarrhea and sternal recumbency since two days. Systematic postmortem examination was carried out and tissue samples were collected in 10% formalin for histopathology. Tissue samples were processed as per standard paraffin embedding technique. Tissue sections of 4 µm thickness were prepared and stained with haematoxylin and eosin (H&E) as per Bancroft and Layton (2019a)[9]. Duplicate tissue sections were processed for histochemistry using Alcian blue-PAS[10], Masson's trichrome[11] and modified Fouchet's stain as per standard procedure[12].

On gross examination, intestine showed haemorrhagic streaks in the mucosa. The spleen showed multiple irregular raised dark red areas. Kidneys were slightly smaller and capsule peeled off with difficulty. Renal cortex was slightly pale and had irregular surface with tiny pitted areas. Pancreas revealed gelatinization of fat and had scattered chalky white, slightly firm areas throughout the parenchyma. Liver showed scattered areas of greyish white, slightly depressed areas. Diaphragmatic surface of left lateral lobe showed a group of glistening, thin, smooth walled confluent clear cysts of 3-5 mm diameter (Fig. 1) in the middle of the lobe. Few of these lesions were masked under the Glisson's capsule. Ventral surface of the right middle lobe revealed two cysts near the common bile duct

How to cite this article : Prasath, N.B. and Selvaraj, J. 2024. Pathology of Cholangiocellular Adenoma: A benign tumour mimicking the malignant neoplasm in a dog. Indian J. Vet. Pathol., 48(3) : 261-265.



Cholangiocellular adenoma in a dog. **Fig. 1.** Single/multiple, raised, glistening, cystic structures in the left lobe and single cystic lesion in the right lobe (arrow); **Fig. 2.** Hyperplasic and cystic dilatation of bile ducts (H&E x100); **Fig. 3.** Sub-capsular hyperplasic proliferation of bile duct forming tubules (H&E x40); **Fig. 4.** Proliferating bile ducts shows well-differentiated ductular epithelium supported by thin and meagre non-collagenous stroma (H&E x100); **Fig. 5.** Tubular lacule containing bile duct increments, cellular debris (hepatocytes) and erythrocytes (H&E x400); **Fig. 6.** Tubules at certain places shows cystic epithelial lining - arrow (H&E x100).

(Fig. 1 arrow). All cyst-like structures appeared slightly protruding above the capsule. On incision, it contained soupcon of bumblebee yellowish, thin clear fluid with uncollapsed cyst wall. Formalin fixed tissue revealed well defined clear, lobular pattern in the affected portion of tissue.

Histologically, the liver sections revealed sub-capsular bile duct hyperplasia under the capsule forming cystic dilatations (Fig. 2) and irregular bile duct tubules extending deep into the parenchyma. Proliferating tubules formed irregular inter-anastomosing pattern creating locules having resemblance of biliary duct hamartoma or Von Meyenberg complexes (VM complexes) (Fig. 3). Proliferating tubules resemble normal biliary pattern featuring well-differentiated biliary epithelial cells. Tubules were also lined by hypertrophied non-stratified cuboidal or flattened epithelial cells with prominent nucleus, peripherally condensed dense chromatin and indistinct nucleolus (Fig. 4). Tubular lumen was empty and scattered locules contained bile duct increments such as cell debris, erythrocytes and amorphous (granular to homogenous) eosinophilic masses (Fig. 5). At certain places, epithelial lining showed cystic elevations (Fig. 6). Hyperplastic tubules trekked into the hepatic lobules and caused isolation of hepatic cords with atrophy of hepatocytes (Fig. 7). Bile duct tubules were separated with variable stroma consisting of less collagenous, meagre fibroblast tissue and capillaries. Based on the above histological features, the lesion was diagnosed as cholangiocellular adenoma. Periphery of the lesion exhibited hyperplastic bile ducts and biliary epithelial cells with features of cellular dysplasia, anisocytosis, anisokaryosis prominent nucleolus and few abnormal mitotic figures. The area was found to be a transition zone featuring carcinoma (Fig. 10). The spleen revealed red infarcts. The pancreas revealed severe degenerative changes characterized by vacuolar changes in acinar cells,

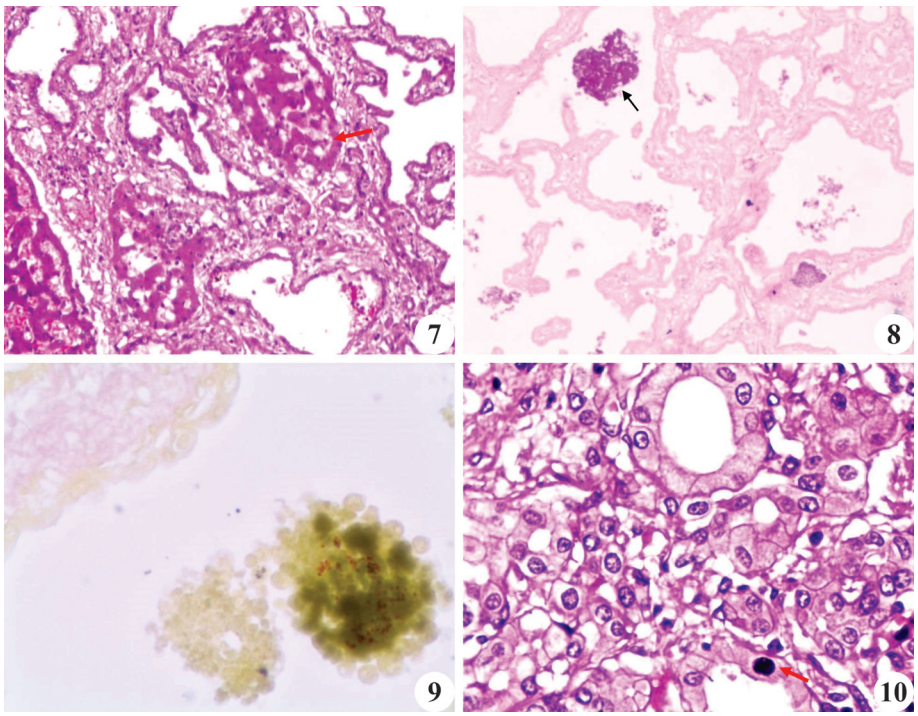


Fig. 7. Proliferating bile duct trekking into the hepatic parenchyma causing pressure atrophy to the surrounding hepatic cords; Isolated hepatic cords (arrow) in-between proliferating bile duct (H&E stain x100); **Fig. 8.** Magenta coloured PAS positive neutral mucin (arrow) - Alcian blue (PAS stain x100); **Fig. 9.** Emerald green bile pigments within the lacule of the hyperplastic tubules with untained cellular debris (Modified Fouchet's stain x400); **Fig. 10.** Malignant transition zone characterised by cellular and nuclear atypia and mitotic figure - arrow (H&E x400).

loss of zymogen granules and scattered mononuclear cell infiltration in the interstitum. The kidney revealed moderate chronic interstitial nephritis with slight atrophy of tubules.

Histochemistry with Alcian blue-PAS stained tissue sections revealed magenta coloured bile acids and found to be positive for neutral mucin (Fig. 8). Modified Fouchet's stained tissue sections revealed emerald green coloured bile pigments within the tubules and differentiated with red coloured erythrocytes within the increments (Fig. 9). Masson's trichrome stained tissue sections revealed thin collagenous stroma separating bile duct tubules.

Liver neoplastic disorders are classified based on morphology and immunohistochemical characterization in humans. Based on these, primary hepatic neoplasms are reclassified more recently based on the origin from the hepatic and its related structures in dogs and cats. These includes primary epithelial tumours (hepatocellular and cholangiocellular neoplasia of adenoma and carcinoma and neuroendocrine carcinoma; mixed hepato and cholangiocellular tumours; hepatoblastoma), hepatic vascular and mesenchymal neoplasia and haematopoietic neoplasia[1,13]. Among these listed neoplasms, cholangiocellular adenoma (CCA) is rare in human and dog. Cholangiocellular adenoma is frequently described as bile duct adenoma, biliary cystadenoma, cystic bile duct adenoma and peribiliary gland hamartomas. It is found to be an uncommon tumour of hepatic origin. The incidence was reported to be 1-2% in human being[8]. Though precise incidence rate is not documented in dog, it is found that 0.6-1.5% of all tumours in dogs are of hepatic origin[1] however, the true incidence of CCA entity is unknown in dog as entire recorded cases were mostly incidental findings during imaging techniques[14] and or during postmortem examinations. The present case is also an incidental finding during necropsy.

Histopathology was found to be an effective technique to diagnose[15] and differentiate CCA from other similar lesions in liver especially VM complexes. Histopathological features observed in this study were in accordance with previous documented evidence in human being and in animals[14,16]. Histological component thought to be a differentiating feature for CCA from VM complexes. VM complex is a congenital developmental malformation characterized by small, well-circumscribed lesion of liver with cystic biliary lesion containing inspissated bile[7]. VM complex exhibits normal biliary anatomy without areas of hepatic parenchyma penetration and cellular atypia. Congenital cystic bile duct was found to contain acid mucin, positive for Alcian blue bile duct epithelium[7]. Evidence of neutral

mucin by histochemistry using Alcian blue-PAS excluded it as VM complex in the present case. Further, the origin of bile duct neoplasm and hepatic neoplasm was from undifferentiated hepatic progenitor cells. The presence of bile pigments with in the well-differentiated hyperplastic bile duct demonstrated by modified Fouchet's stain clarifies the origin of the neoplastic tubules from post-natal cholangiocytes.

The exact cause for CCA is still unclear[16,17]. Though the pathogenesis of CCA was unknown, it was thought to be the localized reactive process of bile duct during hepatic parenchymal injury caused by trauma or chronic liver disease or inflammation[7,8,15]. It was postulated that inflammatory reactions of hepatic parenchyma or toxins carried by affected bile duct prone to develop CCA and hence, the lesion was described as peribiliary gland hamartomas[18]. The cellular debris predominantly hepatocytes and erythrocytes within the locules of tubular bile duct observed in the present study by H&E and modified Fouchet's stain clarifies that the cause of the lesion was probably due to injury to hepatocytes and/or hepatic disease. Interstitial nephritis, pancreatitis, splenic infarct and enteritis forms the supportive evidence for possible toxic injury to hepatic parenchyma in the present study.

The observations of malignant criteria such as anisocytosis, anisokaryosis, mitotic figures in the transition zone in the present case appears to be a lesion progressing to cholangiocellular carcinoma (CCC). This observation was in parallel to the hypothesis put forth by Lee (2016)[19], who reported that these features were insufficient to describe for malignancy[19]. Beside, cystic bile duct adenoma featuring cholangiocellular carcinoma was recorded in a cat[20]. In imaging techniques, CCA appeared to show malignant features with hyper-echoic, rim-like images[15]. Hence, histopathology and precise immunohistochemistry (IHC) profiles are warranted to dissolve the ambiguity. IHC with K19, CD10 and EMA/MUC-1 were found to be an efficient marker for CCA with tubular pattern in dogs. These markers forms basis to differentiate CCA and CCC having similar tubular pattern where CD10 was specific for CCA as per Van Sprundel *et al.* (2013)[1]. We acknowledge the limitation to demonstrate malignancy by immunohistochemistry. Hence, based on the histopatholoigcal features and previous documented evidence, authors concluded that the case was a CCA with carcinomatous transition as an incidental finding during necropsy in a dog.

ACKNOWLEDGMENT

We thank the Dean, Veterinary College and Research Institute, Orathanadu-614 625, Thanjavur and Tamil Nadu Veterinary and Animal Sciences University for the facilities provided.

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Indian J. Vet. Pathol., 48(3) : 266-270, 2024: DOI: 10.5958/0973-970X.2024.00044.7

SHORT COMMUNICATION

Concurrent occurrence of Colisepticaemia and Ulcerative enteritis in Japanese quail breeder flock

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Received: 29.12.2023; Accepted: 24.2.2024

ABSTRACT

Japanese quails have the potential to complement the obvious gap in the protein needs of people, necessitating means to improve their productivity and sustainability. To achieve this, one of the areas that need particular attention is diagnosis of common diseases and its control. Due to intensive production, Japanese quails are susceptible to various infectious and non-infectious diseases. Early diagnosis of these diseases is essential to minimize the economic losses associated with it. The present study was undertaken to elucidate the etiological agents and pathological manifestation in field cases of increased mortality in an intensively maintained Japanese quail breeder flock of 3000 birds of 35 week old during the month of August 2023. Necropsy examination conducted on fifteen dead birds and samples collected and subjected for bacteriological and pathological examination. Affected breeder quail flocks showed cumulative mortality of 21 per cent. Few of the affected birds showed the clinical signs of depression, anorexia, listlessness, partially closed eyes and huddled posture with ruffled feathers. Egg production was dropped gradually from 93 to 68 per cent. At necropsy, dead birds showed good body condition, fibrinous pericarditis, congestion of lungs, marked enlargement of spleen with mottled appearance and moderate dilatation of intestine with multiple greyish white foci. Microbiological examination in specific cultural media liver, spleen and intestinal contents revealed the presence of *Clostridium colinum,* whereas the heart blood and liver swab revealed the presence of *Escherichia coli.* The flock was successfully treated with levofloxacin, amoxicillin and cloxacillin based on the antibiotic sensitivity test. Microscopically, fibrinous pericarditis, myocardial degeneration in heart, vascular changes in lungs, mulifocal necrosis with infiltration of inflammatory cells in liver, lymphoid depletion and reticuloendothelial cell proliferation in spleen and coagulative necrosis with ulceration of intestinal epithelium were noticed.

Keywords: ABST, colisepticaemia, japanese quail, pathology, ulcerative enteritis

The poultry industry is considered as an important sector that meets the great demand for protein sources all over the world. Now, quails are recognized as promising and important alternative species with many advantages over other poultry species. In many countries around the world, quail meat has achieved great popularity as a good source of protein and other important nutrients[1]. In India, Japanese quails (*Cortunix japonica*) have been gained importance as commercial poultry in recent years and have become the third largest commercially reared avian species in number next to chicken and ducks. Being a hardy bird, it can adapt very well to Indian climatic conditions and is resistant to most of the poultry disease conditions[2]. Due to their rapid development rates and/or higher egg output, Japanese quails raised in intensive systems are subjected to the same production stress as chickens. Stress affects these birds' immune systems, predisposing them to various infectious diseases similar to those that plague chicken; however reports on the diseases affecting the quails were scanty[3].

Clostridium colinum and *Escherichia coli* are commensal of the intestinal tract in many poultry species but also appears frequently associated with avian pathology. Colisepticemia is the most severe manifestation of *E. coli* in poultry, which is characterized by the presence of pericarditis, perihepatitis, air sacculitis, and salpingitis[4]. Ulcerative enteritis (UE) is an acute and chronic bacterial infection of quails caused by *Clostridium colinum* occurring most frequently in young quail between 4 and 12 weeks of age, and outbreaks in adult quail has been rarely reported[5]. Diseases associated with *C. colinum* and *E. coli* cause great economic losses in terms of reduction in production, morbidity, mortality and treatment cost. However, there is a paucity of information on the concurrent occurrence of colisepticaemia and ulcerative enteritis in Japanese quails and its therapeutic management. Therefore generating such information helps in planning health care measures and reduces losses in quail industry. The present

How to cite this article : Srinivasan, P., Gopal, K., Murthy, T.R.G., Saravavan, S. and Gowthaman, V. 2024. Concurrent occurrence of Colisepticaemia and Ulcerative enteritis in Japanese quail breeder flock. Indian J. Vet. Pathol., 48(3) : 266-270.

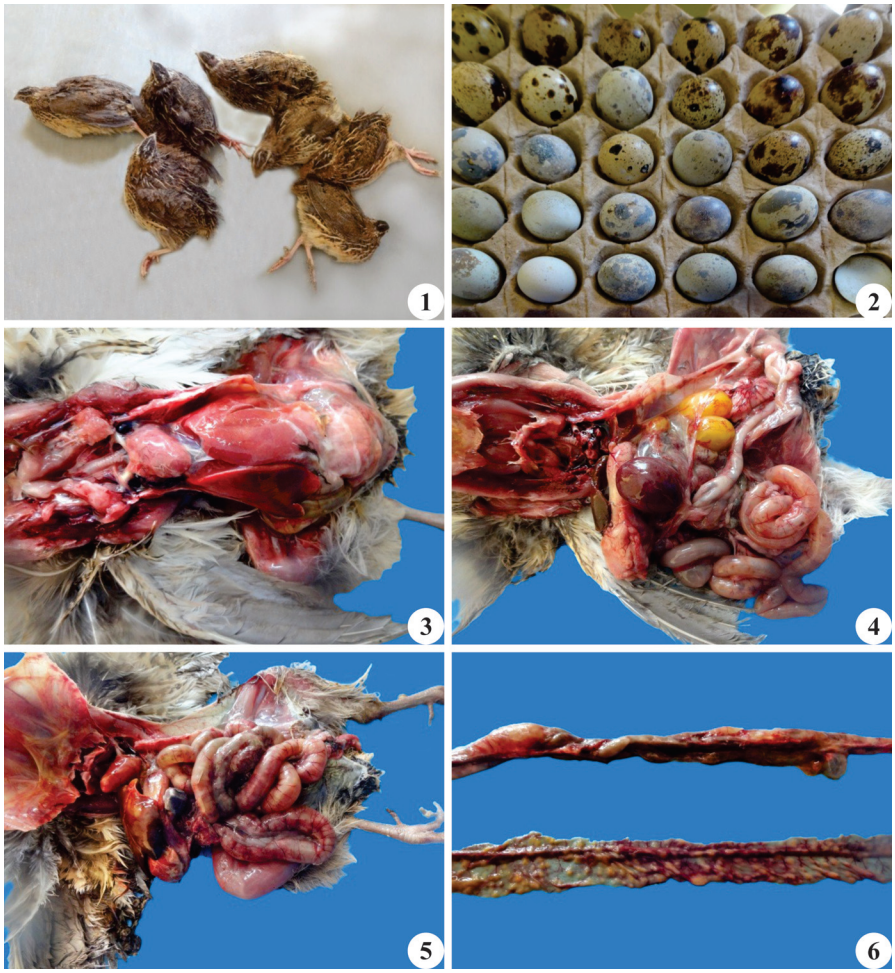

Fig. 1. Japanese Quails showing leg weakness; **Fig. 2.** Eggs in the bottom rows showing pale appearance; **Fig. 3.** Fibrinous pericarditis and perihepatitis; **Fig. 4.** Spleen markedly enlarged with mottled appearance; **Fig. 5.** Intestine was ballooned and serosal blood vessels were congested; **Fig. 6.** Intestinal mucosa showing greyish white ulcers with necrotic debris.

study was undertaken to elucidate the pathological manifestation in field cases of concurrently occurring colisepticaemia and ulcerative enteritis in adult breeder quail.

A Japanese quail flock of 3000 birds was maintained for breeding purpose in an unorganized farm at Coimbatore district of Tamil Nadu. The flock age was 35 week old, fed with commercially prepared compound feed *ad libitum* and maintained in deep litter system of management with paddy husk as a litter material. The flock exhibited leg weakness (Fig. 1) with increased mortality around 25 birds per day hence it was treated with antibiotics (co-trimoxizole) as per the advice of the poultry consultant. In spite of that the mortality was continued and increased to 40 to 50 birds per day with a cumulative mortality of 21 per cent over a period of two weeks. Few birds in the flock exhibited depression, anorexia, listlessness, partially closed eyes and huddled posture with ruffled feathers. Egg production dropped gradually from 93 to 68 percent. Eggs laid by the flock were pale in appearance (Fig. 2) with increased shell breakage.

Fifteen number of dead birds were presented for postmortem examination to poultry disease diagnosis and surveillance laboratory, Veterinary College and Research Institute, Namakkal during the month of August 2023. Necropsies were performed as per approved procedure. After external and internal examination of tissues and organs individually for gross lesions, materials for histopathology were collected from heart, lung, liver, spleen and intestine and fixed in 10 per cent neutral buffered formalin. After fixation, samples were embedded in paraffin, sectioned at 5 μm thickness with a rotary microtome followed by routine staining with hematoxylin and eosin. The slides were mounted with DPX mountant solution and covered with coverslips for histopathological examination.

Heart blood and liver swabs collected from dead birds were placed in Brain Heart Infusion (BHI) broth and incubated at 37^{O}C for 24 hours and subsequently cultured

aerobically in 5 per cent sheep blood agar, MacConkey agar and eosin methylene blue agar (EMBA) for isolation of bacteria. Liver, spleen and intestinal contents were cultured anaerobically in tryptose-phophate agar and incubated at 37^{O}C for 48 hours. Bacterial isolates were identified on the basis of their colony morphology, growth characteristics, sugar fermentation and biochemical characteristics[6]. The bacterial isolates were tested for *in vitro* antimicrobial sensitivity test against commonly used antimicrobial agents by disc diffusion technique on Muller and Hinton agar[7]. Direct microscopic examination of intestinal scrapping was performed to rule out coccidiosis.

The poultry industry is considered an important sector that meets the great demand for protein sources all over the world. Now, quails are recognized as promising and important alternative species with many advantages over other poultry species. In many countries around the world, quail meat has achieved great popularity as a good source of protein and other important nutrients. However, there are some limitations and challenges to quails production among which diseases caused by infectious agents are considered to be a major factors, leads to decrease in growth performance, poor feed conversion, reduction in hatchability, increased mortality and treatment costs[1,8]. Among the infectious agents, diseases caused by clostridia are unusually challenging, because many Clostridial species can be normal inhabitants of the gut, making difficult to determine their pathogenic roles. *E. coli* infection in avian species can cause many clinical manifestations either as primary or secondary pathogen. Colisepticemia is one of the avian disease manifestations of *E. coli* infection and has been reported in chickens, turkeys and quails. The majority of economic losses associated with colisepticaemia results from mortality and decrease in productivity of the affected birds[9,10].

Ulcerative enteritis is a multifactorial and financially devastating bacterial disease caused by *Clostridium colinum* is a normal inhabitant of the intestines of healthy bird[5]. The ulcerative enteritis that results in clinical disease most often occurs either after a change in the intestinal

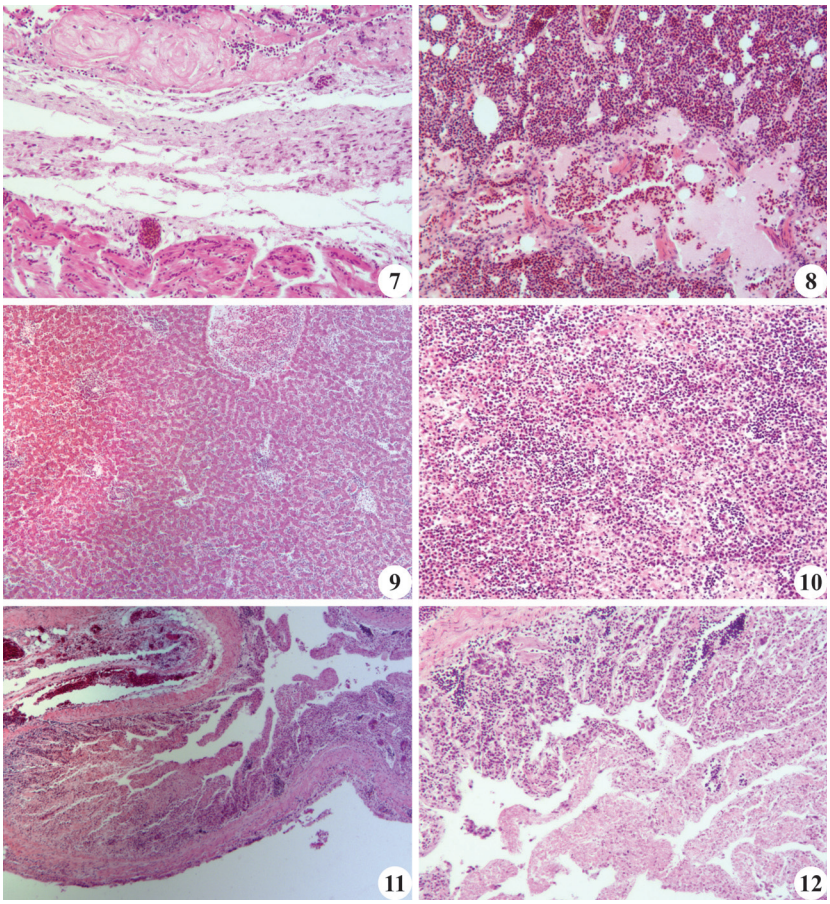

Fig. 7. Heart: Pericardium showing fibrinous exudate with infiltration of heterophils and degeneration of myocardial fibers (H&E x100); **Fig. 8.** Lung showing congestion, haemorrhage and edema in the interlobular septa and parabronchial lumen (H&E x100); **Fig. 9.** Liver showing multifocal necrosis and infiltration mixed population of inflammatory cells (H&E x100); **Fig. 10.** Spleen showing coagulative necrosis and proliferation of reticuloendothelial cells (H&E x100); **Fig. 11.** Intestine showing necrosis and ulceration with inflammatory reaction (H&E x100); **Fig. 12.** Intestine showing desqumation, pseudomembrane formation and shortening of villi (H&E x400).

microflora or from a condition that results in damage to the intestinal mucosa[11-13]. Anything that promotes excessive bacterial growth and toxin production or slows feed passage rate in the small intestine could promote the occurrence of ulcerative enteritis[14]. However, in the present study, there was no evidence of pre-existing or intercurrent disease. Sudden increase in mortality with few premonitory signs such anorexia, depression, partially closed eyes and ruffled feathers indicate acute nature of the disease. More or less similar clinical signs have been reported by earlier[15]. Paleness of egg shell and breakage might be due to disturbances in the digestion and absorption of nutrient from the intestine. Mortality of 38 to 80 per cent was reported in 9 to 12 week old Japanese quail flock[16]. Young quail, from 4 to 12-wk-old, are most susceptible[17]. In the present study, age of the affected flock was 35 week old, moreover predisposing factors were not present which might be cause for low mortality of 21 percent.

Microbiological examination of liver, spleen and intestinal contents of dead birds in tryptose phophate agar revealed circular to slightly irregular greyish white to colourless semitranslucent colonies. Gram's staining of colonies showed gram-positive rods with rounded ends, some have spores which are oval and subterminal. Biochemical reactions revealed that the isolated organism, hydrolyzed esculin, fermented glucose, mannose, sucrose and maltose but not ferment lactose and mannitol. The catalase, urease and indole tests were negative and not liquefied gelatine. Based on the cultural characters of the colonies, cellular morphology of the organism and biochemical reactions, the isolates were identified as *C. colinum*[18]. Direct microscopic examination of intestinal content revealed no evidences of coccidial infection.

Heart blood and liver swabs from dead birds also revealed the presence of *E. coli* organisms, which were identified based on lactose fermenting pink coloured round, smooth and glistening colonies on Mac Conkey's agar, black metallic sheen colonies on EMB agar, indole production at 44^{O}C, gas production in Eijkmann's test and acid and gas production in different sugar fermentation tests[6]. *E. coli* is a normal inhabitant of the chicken intestinal tract with up to a population of 10^6 per gram of intestinal contents. Approximately 10% to 15% of intestinal *E. coli* is considered to be potential pathogens. As long as the intestinal mucosal barrier is intact, the normal microflora of bird is likely to inhibit the translocation of pathogenic *E. coli* from the intestine to the blood stream and organs. *C. colinum* was consistently isolated from the dead birds with intestinal lesions strongly indicates that this bacterium is involved in the aetiology of ulcerative enteritis. When the intestinal barriers are damaged by infection with *C. colinum,* leads to translocation of the APEC which might be cause for colisepticaemia[4].

On necropsy examination most of the birds showed dehydrated dark red appearance of pectoral muscle and presence of feed in the crop. Heart revealed fibrinous pericarditis with petechial haemorrhages on the epicardium (Fig. 3). Liver was moderately enlarged congested and yellowish brown in colour with mild fibrin deposition on its surface (Fig. 3). Lungs were congested. Ovarian follicles were congested and in some birds it showed atresia. Spleen was markedly enlarged, congested with necrotic foci (Fig. 4). Kidney showed mottled appearance. Intestine was ballooned with congestion of serosal blood vessels (Fig. 5). Greyish white multifocal ulcers were prominent and could be seen readily through the serosa of jejunum. On opening, the intestine mucosa showed discrete round, oval or irregular shape ulcers of 2 to 3 mm diameter filled with necrotic debris (Fig. 6). The gross lesions observed in the present investigation were in accordance with the earlier reports on UE in quails and other species of poultry[13,19].

Antibiotic sensitivity test revealed the *Clostridium colinum* was sensitive to amoxicillin and cloxacillin and the *Escherichia coli* was sensitive to levofloxacin. Based on the drug sensitivity pattern the flock was treated with levofloxacin @ 10 mg per kg body weight in drinking water in the morning and amoxicillin and cloxacillin @ 20 mg per kg body weight in drinking water in the evening for five consecutive days resulted in improvement in the health of affected birds in the flock in terms of reduced mortality and regained its production. The absence of other diseases, such as coccidiosis and the immediate response to treatment with sensitive antibiotics, suggests that ulcerative enteritis is the primary disease and not a complication as usually occur. Information on susceptibility obtained in this study may be useful for the selection of antibiotics for the chemotherapeutic prevention or treatment[18].

Histopathological examination of heart revealed thickening of pericardium with fibrinous exudate and infiltration of heterophils and degeneration of myocardial fibers with inflammatory reaction (Fig. 7). Lungs exhibited congestion, haemorrhages and fibrinous exudate with heterophils in the interlobular septa and parabronchial lumen (Fig. 8). Liver showed multifocal coagulative necrosis and infiltration of mixed population of inflammatory cells in portal areas (Fig. 9) and, occasionally, small colonies of gram-positive bacilli. Spleen showed areas of coagulative necrosis around the germinal follicles and reticuloendothelial cells proliferation (Fig. 10). Intestine showed coagulative necrosis of mucosal villi epithelium with ulceration and inflammatory reaction extended into the muscle and serosal layers (Fig. 11). Superficially ulcerated areas contained numerous heterophils, which also infiltrated from these sites to the serosa. In addition,

many mononuclear cells, mainly lymphocytes, infiltrated all layers and formed nodules in the tunica muscularis and periserosal fat. Cellular detritus, many colonies of gram-positive rods, and rapidly proliferating intestinal epithelial cells were associated with the ulcerated areas. Desquamation, denudation, pseudo membrane formation and shortening of villi were also observed (Fig. 12). The histopathological changes in the various organs of affected birds were similar to those observed in typical cases of ulcerative enteritis (Cooper *et al*.,2013), and colisepticaemia[20]. Various changes observed in above organs could be attributed to bacterial exotoxins of *clostridium colinum*, endotoxins and vascular injury induced by *E. coli*.

CONCLUSION

In conclusion, colisepticaemia and ulcerative enteritis not only affects the young birds, but in adult birds also they may cause mortality and create big challenge to quail farming. Ulcerative enteritis in adult Japanese quails has led to necrosis and ulceration in intestinal tract mucosal epithelial cells, breaking the barrier and predisposing systemic colisepticaemia. Systemic necropsy examination is important to make an early diagnosis in the field, to start control measures before laboratory confirmation. Antibiotic sensitivity of the organism and treatment with amoxicillin, cloxacillin and levofloxacin successfully controlled the mortality and regained egg production. This report will familiarize veterinary practitioners with this infrequently reported disease of adult Japanese quails, and to provide information on treatment.

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Indian J. Vet. Pathol., 48(3) : 271-275, 2024: DOI: 10.5958/0973-970X.2024.00051.6

SHORT COMMUNICATION

Pathological diagnosis of Babesiosis in a labrador retriever

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Received: 23.1.2024; Accepted: 12.3.2024

ABSTRACT

A 2-year-old male Labrador retriever dog was presented to veterinary clinical complex, CVSc, Rajendranagar, PVNRTVU, Hyderabad, with the history of inappetence, brownish urine, blood vomiting and diarrhea, and with the past history of tick infestation. Clinical examination revealed rise in the normal body temperature with intermittent relapse of fever, sunken eyeballs with pale conjunctival mucus membrane, severe dehydration, lymphadenopathy, tachycardia and tachypnoea. Faecal sample examination was negative for presence of any parasitic ova. Kidney function tests revealed elevated serum creatinine and BUN levels. Liver Function tests recorded hypoproteinemia, hypoalbuminemia and decreased glucose levels, with elevated serum SGPT values. Haematological examination documented hemolytic anaemia and mild thrombocytopenia. On Peripheral blood smear examination, presence of typical large pyriform, paired Babesia organisms within the erythrocytes were observed. The PCR report confirmed the presence of *Babesia gibsoni* infection. On postmortem examination, grossly blood-tinged frothy exudate was observed in the trachea, while the lungs were emphysematous and hemorrhagic. Hydropericardium was evident in the heart, kidneys were congested with generalized petechial hemorrhages. Hepatic congestion in the liver and engorged blood vessels in gall bladder was documented. Significant splenomegaly and enlarged hemorrhagic lymph nodes were noticed. Histopathologically, emphysema and edema of the lungs, with inter-septal infiltration of the lymphocytes noticed. Focal tubular necrosis, tubular desquamation and interstitial hemorrhages was observed within the kidneys. On further examination, the lymph nodes showed fibrous tissue proliferation, while the spleen revealed lymphocytic depletion and edematous fluid accumulation.

Keywords: *Babesia gibsoni*, blood smear, canine babesiosis, diagnosis, necropsy, RT-PCR

Canine babesiosis, previously known as canine piroplasmosis, is a globally distributed haemotropic intra-erythrocytic tick-borne disease of domestic and wild canines caused by the intra-cellular protozoan parasite belonging to the family Babesiadae[1]. The Babesia species commonly affecting dogs include *Babesia canis, Babesia gibsoni, Babesia rossi* and *Babesia vogeli*[2]. The clinical signs include lethargy, anorexia, pale mucous membranes, vomiting, brownish urine, jaundice, splenomegaly, tachycardia, tachypnea and distinct lymphadenopathy. Vital organs like kidney and liver are affected in chronic cases, resulting in severe inflammatory response syndrome and multiorgan failures[3,4]. The species detection in Babesiosis is commonly based on the morphological appearance of the intra-erythrocytic forms of the parasite in peripheral blood smears[5]. This procedure is simple and inexpensive, but cannot be used to detect atypical cases of babesiosis due to its low sensitivity to parasitemia and is a time-consuming method[6]. Henceforth, various molecular techniques, including conventional polymerase chain reaction (PCR)[7-11], real-time PCR[12] and reverse line blot hybridization (RLB) assays[13-15] have been developed for quick and accurate diagnosis of canine Babesiosis.

The aim of this study is to investigate the classical signs and post-mortem pathological changes observed in dogs infected with *B. gibsoni* and to establish the probable cause of death based on the haemato-biochemical parameters, molecular diagnosis using conventional PCR, the gross pathological changes and histopathological findings. In the current scenario, researches on gross and histopathological studies in canine babesiosis are scanty, while there is also a growing requirement to develop a rapid, sensitive real-time and conventional PCR assay for the diagnosis of babesiosis to facilitate immediate effective treatment.

How to cite this article : Jayasri, K., Banerjee, R. and Thokala, J.S.K.R. 2024. Pathological diagnosis of Babesiosis in a labrador retriever. Indian J. Vet. Pathol., 48(3) : 271-275.

A 2-year-old male Labrador retriever dog weighing 32 kg was presented to veterinary clinical complex, CVSc, Rajendranagar, PVNRTVU, with the history of inappetence, bloody vomiting, brown discoloration of urine and deteriorating health, with previous history of tick infestation. The dog was subjected to thorough physical and clinical examination, including fecal sample examination to rule out parasitic infections. Haemato-biochemical tests, rapid kit serum agglutination test, peripheral blood smear examination using Giemsa staining technique and

conventional PCR methods were performed to accurately diagnose the case.

Complete blood picture examination was performed by collecting blood in 4 ml 4% EDTA vial and analysed using Mindray BC-5000 Haematology analyzer (Veterinary Clinical Complex, Hyderabad). Liver function tests and kidney function tests were performed by collecting blood in serum vacutainer and segregated serum analysis was performed using serum biochemistry analyzer ERBA DESTINY 180, blood collected from the peripheral ear vein was smeared and stained with wright's - Giemsa staining method[16]. For the confirmatory diagnosis, PCR assay was carried out by conventional PCR method. The sample mixture consisted of 2.5 μl of 10X PCR buffer, 0.5 μl of 10 mMdNTP mix, 1.5 μl of 25 mM $MgCl_2$, 1.0 U of recombinant Taq DNA polymerase, 1 μl each (20 pmol) of the primers and 5 μl of template DNA was isolated from 2 mL of the collected blood sample. The volume was made up to 25 μl with nuclease-free water. The PCR cycling conditions were initial denaturation at 95°C for 5 min, 40 cycles of denaturation at 95°C for 30 sec, annealing at 56°C for 30 sec, and extension at 72°C for 1:30 min, and the final extension was performed at 72°C for 5 min. The PCR products obtained were checked for amplification by electrophoresis on a 1.5% agarose gel and visualized using gel documentation system.

Primers from the 18S rRNA gene was used to amplify *B. gibsoni* to conduct the PCR assay[17]. The sequences of the primers targeted is mentioned below:

Gib599 Forward : 5′CTCGGCTACTTGCCTTGTC3′;

Gib1270 Reverse : 5′GCCGAAACTGAAATAACGGC3′

For further studies, necropsy of the animal was performed with approved consent of the owner. Tissue samples from the lungs, heart, kidney, lymph nodes and spleen were collected and fixed in 10% neutral buffered formalin before processing according to standard histologic procedures[18] in order to observe the histopathological changes under a light microscope at varying magnifications.

The physical examination by visual inspection, auscultation and palpation revealed extreme emaciated condition, abnormal rise in the body temperature with intermittent relapse of fever, sunken eyeballs with pale conjunctival mucus membrane, severe dehydration, tachycardia, dyspnoea, general progressive weakness and lymph node enlargement. Hemoglobinuria and hematemesis was observed. On faecal sample examination, no parasitic ova were noticed. Kidney function tests revealed increased serum creatinine and BUN levels. Liver function tests recorded hypoproteinemia, hypo-albuminemia and hypoglycemia, with elevated serum SGPT values. Heamatological examination recorded hemolytic anaemia and mild thrombocytopenia. On Peripheral blood smear examination, presence of typical large pyriform, paired Babesia organisms within the

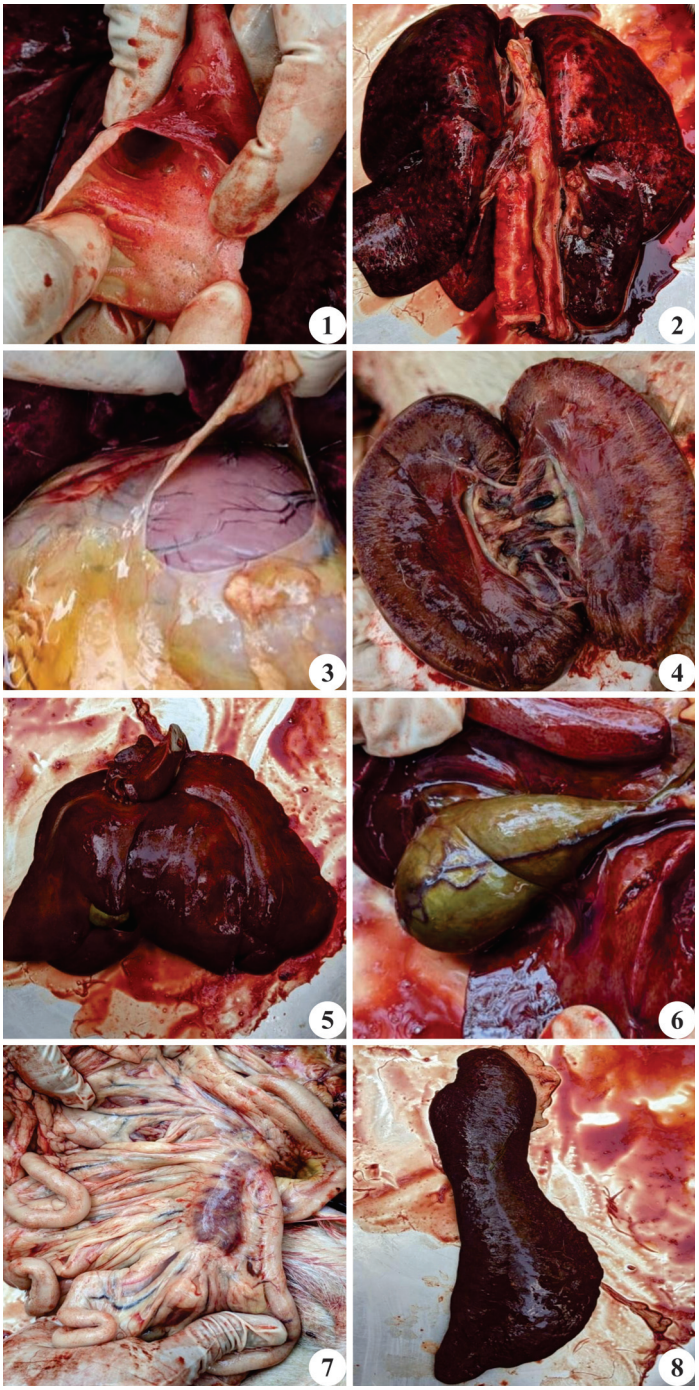

Fig. 1. Trachea showing foamy blood-tinged exudates; **Fig. 2.** Lungs showing emphysema and haemorrhages; **Fig. 3.** Heart showing hydropericardium; **Fig. 4.** Kidneys showing diffuse and petechial haemorrhages; **Fig. 5.** Liver showing congestion; **Fig. 6.** Gall bladder showing engorged blood vessels; **Fig. 7.** Mesentric lymph nodes showing congestion, diffused and petechial haemorrhages; **Fig. 8.** Spleen showing enlargement.

erythrocytes were observed (Fig. 9).

On conventional PCR amplicon of 671 bp was observed that confirmed the presence of *Babesia gibsoni* haemoprotozoan. On the post-mortem examination, the distinct gross lesions observed were blood-tinged frothy exudate in the trachea (Fig. 1) indicative of pulmonary edema, accompanied by emphysematous and haemorrhagic lungs (Fig. 2). Necropsy findings of the heart showed hydropericardium (Fig. 3). Congestion of the kidneys with generalized petechial haemorrhages was observed (Fig. 4), while the liver showed diffused and petechial haemorrhages and congested (Fig. 5). Significant gall bladder enlargement (Fig. 6) with edematous, haemorrhagic mesenteric lymph nodes (Fig. 7) and spleenomegaly (Fig. 8) was also documented. Histopathological examination of the lungs revealed emphysema and edema (Fig. 10), with inter-septal infiltration of the lymphocytes (Fig. 11). Focal tubular necrosis (Fig. 12), tubular desquamation (Fig. 13) and interstitial haemorrhages were reported within the kidneys. The lymph nodes showed fibrous tissue proliferation, while the spleen revealed lymphocytic depletion and edematous fluid accumulation (Fig. 14).

In the present case, the affected dog showed the typical signs of canine babesiosis such as pyrexia, anorexia, depression, pallor of ocular membranes and lymphadenopathy, which corroborates with earlier findings[19]. The Haemato-biochemical evaluations revealed the presence of normocytic and hypochromic anaemia and marked cellular damage in some organs, which corroborate with previous reports[20,21] about the rare complicated canine babesiosis cases. This might be due to intravascular and extravascular haemolysis resulting from direct parasite-induced damage, increased osmotic fragility of infected RBCs, oxidative injury and activity of a secondary immune-mediated process, leading to the possible progressive depression observed in the dog.

The elevated ALT and AST may be due to the haemolysis, as these enzymes are important components of erythrocyte membranes[21]. The increased serum urea level, as observed in the current case report, may have been due to hepatobiliary leakages owing to renal focal tubular necrosis with tubular desquamation, interstitial

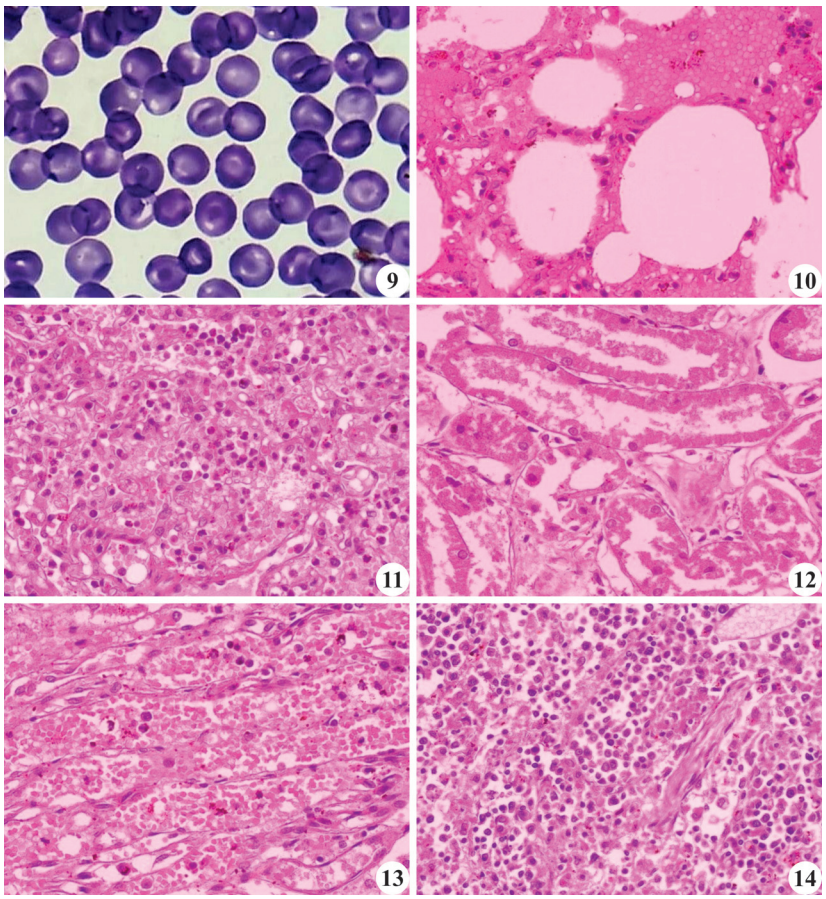

Fig. 9. Peripheral blood smear showing typical pyriform shaped paired *Babesia* organisms within the erythrocytes (Giemsa stain X1000); **Fig. 10.** Section of lungs showing alveolar emphysema and edema (H&E X400); **Fig. 11.** Section of lungs showing inter-septal infiltration of lymphocytes (H&E X400); **Fig. 12.** Section of kidney showing focal tubular necrosis (H&E X400); **Fig. 13.** Section of kidney showing tubular and interstitial haemorrhage (H&E X400); **Fig. 14.** Section of lymphnode showing fibrous tissue proliferation (H&E X400).

haemorrhage, degenerative changes & decreased renal elimination associated with acute kidney injury (AKI) and reduction in glomerular filtration rate. Severe babesiosis infection could be predisposing factor to tissue damage and could account for the recorded decreased serum creatinine level[22]. Similar disproportionally raised urea to creatinine levels in canine babesiosis was reported by earlier worker[23].

Major gross lesions observed were blood-tinged frothy exudate in the trachea, pulmonary edema, hepatomegaly and splenomegaly, corroborating with the lesions documented by earlier worker[24]. Histopathologically, emphysema and pulmonary edema within the lungs were observed, with significant enlargement of the alveolar diameter and packets of diffused edematous fluid accumulation, also accompanied by massive inter-septal cellular infiltration was recorded, which are considered as the typical characteristics of interstitial pneumonia[25]. Interstitial pneumonia can be explained by either increased alveolar capillary permeability or increased hydrostatic pressure, both being the consequence of an acute respiratory distress syndrome (ARDS) documented in the complicated cases of canine babesiosis[26]. The focal haemorrhages and cellular infiltration within the spleen and lymph nodes might be due to the inflammatory response and anaemic hypoxia associated with this haemoprotozoal infection[27]. ARDS & AKI are uncommon but mostly grave complications associated with Canine babesiosis, as were reported in this case.

CONCLUSION

Following thorough examinations of the current case, we infer that the prognosis of an acute and complicated case of canine babesiosis is grave. Early diagnosis using PCR assay and effective therapeutic management are required to help minimize the risk of complications associated with babesiosis and prevent the collapse of the animal. This report is aimed to describe the quick method of early diagnosis of ARDS, AKI & other clinicopathological changes associated with a complicated case of canine babesiosis in dogs, which may aid in the further studies being conducted on this matter.

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Indian J. Vet. Pathol., 48(3) : 276-279, 2024: DOI: 10.5958/0973-970X.2024.00045.3

SHORT COMMUNICATION

Pathology of spontaneous mucinous ovarian adenocarcinoma in a Gramasree hen

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Received: 1.1.2024; Accepted: 28.2.2024

ABSTRACT

Ovarian adenocarcinomas are highly malignant tumours arising from the ovarian surface epithelium, which is characterized by widely varied histomorphology. A dead three-year-old female Gramasree layer bird was brought for postmortem examination. An enlarged nodular ovarian mass with smooth-surfaced coalescing small nodules was present in the coelomic cavity without the involvement of oviduct. Similar individual nodules were seen attached to the serosa of the duodenum.Microscopically, the ovarian nodules were well-encapsulated structures containing pleomorphic neoplastic epithelial cells in diffuse, irregular tubulo-acinar patterns supported by a fibrovascular stroma. Nodules of intestinal serosa also showed neoplastic epithelial cells in the tubulo-acinar pattern. Periodic Acid Schiff stain and intense blue with Alcian blue stain confirmed that a mucinous substance was present inside the tubules of neoplastic growth. Immunohistochemically, tumour cells revealed positive immunoreactivity for pancytokeratin. Based on the gross, cytological, histopathological, and immunohistochemical examinations, the case was diagnosed as mucinous ovarian adenocarcinoma with transcoelomic metastasis.

Keywords: Adenocarcinoma, immunohistochemistry, ovarian neoplasm, pancytokeratin

How to cite this article : Tamilvani, P., Krishna, B.D., Palekkodan, H., Pradeep, M., Arathy, P.S. and George, A.J. 2024. Pathology of spontaneous mucinous ovarian adenocarcinoma in a Gramasree hen. Indian J. Vet. Pathol., 48(3) : 276-279.

The female avian reproductive tract, particularly the ovary, is subjected to several pathological manifestations such as cystic degeneration, yolk peritonitis and neoplasms. Sex cord tumours (granulosa cell tumours, ovarian Sertoli cell tumours), germ cell tumours (dysgerminomas), surface epithelial cell tumours and also virus-induced tumours such as Marek's disease, lymphoid leukosis, myeloid leukosis and reticuloendotheliosis are seen in the ovary[1]. Since ovulation is a continuous physiological process involving the ovaries, it causes oxidative and apoptotic damage to ovarian surface epithelial cells. This persistent damage makes the ovarian surface epithelial cells more susceptible to the development of neoplasms[2]. Thus, the high ovulation rate in hens corresponds with an increased incidence of ovarian adenocarcinomas and their occurrence increases with advancing age[3]. Ovarian adenocarcinomas usually originate from the ovarian surface epithelium, which is a primitive epithelium that loses its stromal characteristics when changing to malignancy[4]. Ovarian adenocarcinomas have varied histological structures, ranging from well-differentiated to moderately or poorly differentiated. Well-differentiated adenocarcinomas are presented as serous, mucinous, endometroid, and clear cell subtypes[5]. The main sites of colonisation by tumour cells are the peritoneum, large and small intestine, liver parenchyma, and lung[6]. This report presents a case of metastatic mucinous ovarian adenocarcinoma in a Gramasree hen.

A three-year-old Gramasree layer hen reared in a deep-litter system was presented for postmortem examination. A detailed postmortem examination of the hen was done systematically. For cytological examination, Leishman stain was used for staining impression smears from tumour tissues. For histopathological examination, the tissues were fixed in 10% neutral buffered formalin, processed by paraffin embedding technique and sectioned in approximately 4-µm thickness for haematoxylin and eosin staining[7]. Special stains like Periodic Acid-Schiff, Alcian Blue, and Masson's Trichrome were used for the histological characterization of tumors[7]. Immunohistochemistry was done with Cytokeratin Pan type I monoclonal antibody (AE1) (Catalogue No. 14-9001-82) from Invitrogen (eBioscience™) to determine the type of tumour.

The Gramasree hen carcass weighed 1.5 kg. External examination revealed an atrophy of pectoral muscles with prominent keel bones. The abdomen was severely distended and pendulous. External parasites such as fleas and ticks were absent. When the carcass was opened, severe ascites was observed with the coelomic cavity containing approximately 250 ml of sanguineous fluid mixed with abundant flakes of blood

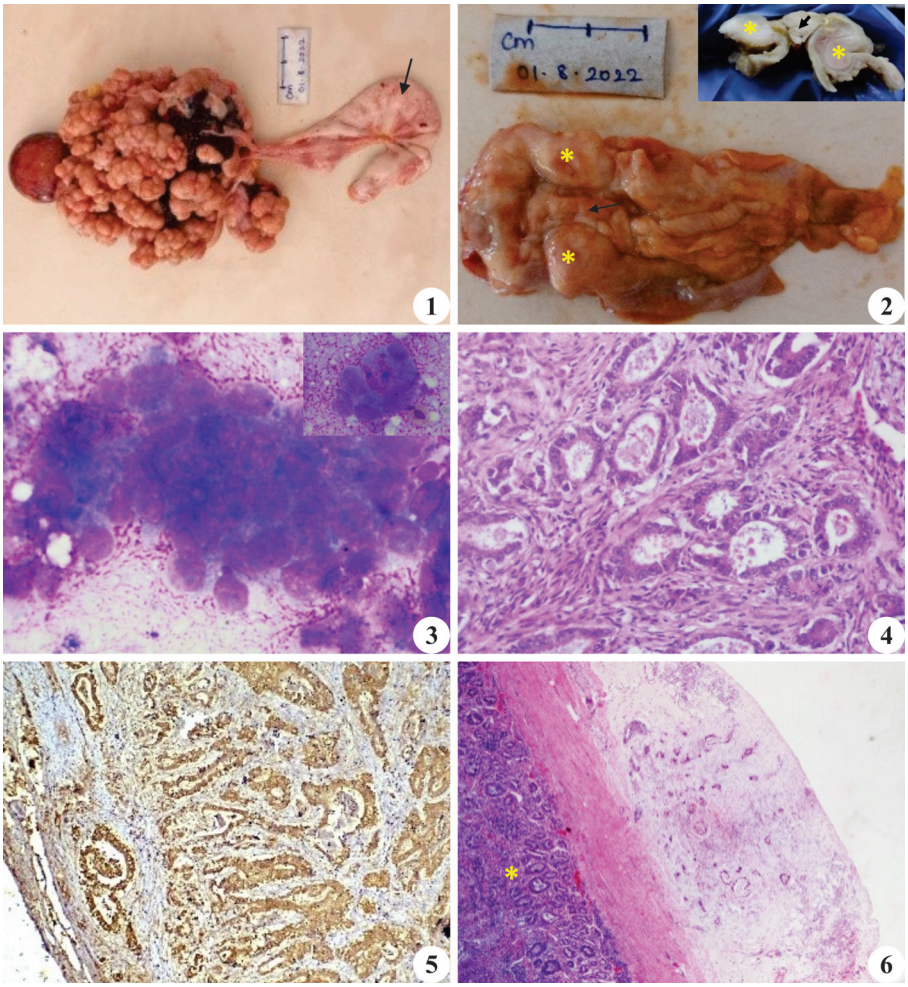

Fig. 1. Ovarian mass with multiple coalescing nodules, a haemorrhagic follicle (asterisk). Oviduct had no nodular structures (arrow); **Fig. 2.** Duodenum revealed individual nodules (asterisk) attaching to the serosa near the pancreas (arrow); Inset: Nodular structure (asterisk) not infiltrating into pancreas (arrow) and limited to serosa only; **Fig. 3.** Impression smear of ovarian nodule revealed clusters of pleomorphic neoplastic epithelial cells. Inset: Acinar pattern formed by neoplastic epithelial cells (Leishman stain x1000); **Fig. 4.** Ovarian nodule showed irregular tubulo-acinar pattern with fibro-vascular stroma (H&E x100); **Fig. 5.** Immunohistochemistry of ovarian nodule for pan-cytokeratin showed diffuse positive immunoreactivity in neoplastic cells (IHC x400); **Fig. 6.** Histomorphology of duodenal nodule.Encapsulated metastatic neoplastic nodule (arrow) attached to the duodenal serosa. Normal mucosal layer of duodenum (asterisk) (H&E x40).

clots. A single multi-lobulated, enlarged ovarian mass completely occupying the coelomic cavity was noticed. Multiple coalescing small-sized nodules (0.5 cm to 3 cm in diameter) with a smooth surface were present, involving 95% of the ovary (Fig. 1). Most of the nodules were well encapsulated, pale pink to beige, and firm, with a solid structure on the cut surface. A few nodules were cystic in appearance and filled with clear fluid. A large haemorrhagic follicle was noticed. Blood clots were randomly present over the nodular surfaces. The mass weighed 200 g and had dimensions of 15 cm x 11 cm x 5 cm. Similar small-sized, solid pink individual nodules (0.5 cm to 1 cm in diameter), 5 to 7 in number, were present attached along the duodenal serosa near the pancreas (Fig. 2). The oviduct and other organs did not show any such nodular outgrowths. The lung was oedematous with diffuse haemorrhagic areas. Coronary blood vessels were engorged. The liver was soft, friable and pale yellow with a distended gall bladder.

The cytological examination of the impression smear from the ovarian mass showed clusters of pleomorphic epithelial cells (Fig. 3), which were round to oval with moderately bluish granular cytoplasm, a high N:C ratio and prominent multiple nucleoli. Marked anisocytosis and anisokaryosis were present. These findings were suggestive of a malignant epithelial tumour. Impression smears from the nodules of the duodenal serosa also had similar characteristics. The normal histology of ovarian follicles was completely evaded by the neoplastic tissue. The nodules were well-encapsulated structures containing diffusely distributed irregular tubulo-acinar

patterns supported by a mild to moderate fibrovascular stroma (Fig. 4). Both ciliated and non-ciliated, cuboidal to columnar epithelial cells forming tubulo-acinar patterns are present in stratified layers in many areas. Neoplastic epithelial cells were highly pleomorphic with intense eosinophilic granular cytoplasm. The nucleus was vesicular, round to oval, and present at the base of the cells with stippled chromatin and prominent multiple nucleoli. Anisokaryosis and a few mitotic figures (0-1 per 5 fields of 400x magnification) were also noticed. The stroma contained the majority of fibroblasts along with loosely arranged connective tissues. The lumen of the larger tubules contained a bluish-pink granular secretory substance that stained faint pink with Periodic Acid Schiff stain and intense blue with Alcian blue stain (pH 2.5). This showed that a mucinous substance was present inside the tubules. The collagen fibres of the fibrovascular stroma were stained blue with Masson's Trichrome stain. Neoplastic cells of ovarian nodules showed positive immunoreactivity for the pancytokeratin (AE1) antibody (Fig. 5). Histopathological findings were characteristic of adenocarcinoma with mucinous differentiation. A similar histomorphology of the neoplastic tubulo-acinar pattern with fibrous tissue stroma was noted in the duodenal serosal nodule (Fig. 6), indicating metastasis from the ovary. Connective tissue predominated in the duodenal nodule, with moderate multifocal infiltration of inflammatory cells such as heterophils, eosinophils, lymphocytes, and plasma cells. In the kidney, the renal tubular epithelium showed degenerative changes along with interstitial haemorrhage. The liver had inflammation and congestion of portal vessels, the central vein, and sinusoids with mild multifocal degeneration of hepatocytes.

In this case, the whole ovarian tissue was involved. However, there was no visible gross nodules or neoplastic cells in the oviduct. This highlights the primary origin of the ovary and eliminates the possibility of an oviductal origin. Except for the duodenum, no other organs or tissues were involved. Most primary intestinal carcinomas have a mucosal origin rather than a serosal incidence[5]. Thus, metastatic foci in the serosa of the intestine ensure transcoelomic spread. In the gross classification of reproductive tumours in hens, this case comes under Class 7, which indicates ovarian tumours with metastasis in other organs without the involvement of the oviduct and is assigned to grade 2 in histological grading as histomorphology is characterised by a moderate number of mitotic figures, poorly differentiated glands and tubules[8].

Moreover, positive immunostaining with cytokeratin (AE1) confirmed the epithelial origin of the neoplasm with primary involvement of the ovary metastasized to the duodenum.

Grossly, primary mucinous carcinoma and metastatic ovarian tumours have a cystic, solid, or mixed appearance. Histologically, mucinous carcinomas in hens were characterised by numerous glandular structures with or without intervening stroma[9]. Glandular structures were composed of columnar epithelium with intercalated ciliated goblet cells with stratification, mitotic figures, and luminal secretion[6]. They can metastasize via transcoelomic, haematogenous, or lymphatic routes, of which transcoelomic is the most common. After initial direct extension into adjacent organs, like the fallopian tubes, uterus, and contralateral adnexa, epithelial ovarian cancer usually disseminates via the transcoelomic route[10]. The development of ascites is a consistent feature in the advanced and metastatic stages of ovarian cancer in both chickens and humans[11]. Hence, ascites, in this case, revealed the severity of the neoplastic condition. Accumulation of ascites fluid is a major consequence, mainly due to reduced lymphatic drainage from the peritoneal cavity due to obstruction of lymphatic vessels by tumour cells. It is also caused by hyperpermeability of microvessels lining the peritoneal cavity, tumour neoangiogenesis, and hypoalbuminaemia secondary to cachexia or dietary deficiency[10]. The age of incidence and gross and microscopic findings were in accordance with earlier case reports in Greater Flamingo[12], Guinea fowl[13], Patridge Plymouth Rock chicken[14] and Native chicken[15].

Ovarian adenocarcinomas should be differentiated from granulosa cell tumours, arrhenoblastoma, mesothelioma, leiomyoma, myelocytomatosis, and metastatic adenocarcinomas[14]. The production potential of most commercial layers declines after 1.5 years, and they are culled at 2 years of age. Hence, ovarian tumours are an economically inconsequential disease condition, as their occurrence increases after the age of two[16]. The age-related spontaneous incidence, similarity in histomorphology, metastatic pathway and ascites fluid accumulation, including their short generation interval, make chicken a more acceptable experimental model for investigating human ovarian neoplasms[17].

CONCLUSION

The present case was diagnosed as a primary mucinous ovarian adenocarcinoma with transcoelomic metastasis based on gross, cytologic, histopathologic, histochemical and immunohistochemical evaluation.

ACKNOWLEDGEMENT

This work is financially supported by Govt. of Kerala through state plan fund 16-17 (RSP/16-17/VI-18 and RSP/16-17/VII-2) and the technical support of Kerala Veterinary and Animal Sciences University is acknowledged.

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Indian J. Vet. Pathol., 48(3) : 280-284, 2024: DOI: 10.5958/0973-970X.2024.00046.X

SHORT COMMUNICATION

Molecular Detection and Pathology of Cytauxzoonosis in Captive Royal Bengal Tiger

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Received: 18.3.2024; Accepted: 23.4.2024

ABSTRACT

Cytauxzoonosis, a tick-borne, feline hemoprotozoan disease caused by *Cytauxzoon felis*. In the present study, cytauxzoonosis was diagnosed in a 12-year-old male Royal Bengal tiger (*Panthera tigris tigris*). The tiger was presented with a history of anorexia and lethargy. It was severely dehydrated with a temperature of 104.2°F. On the following day, icterus developed and the clinical symptoms progressed to recumbency, coma and the animal subsequently succumbed. Hematologic examination performed shortly before death indicated icteric plasma, severe thrombocytopenia, mild anemia, hematuria and circulating red blood corpuscles demonstrated *Cytauxzoon felis*. Gross necropsy findings comprised of generalized icterus, generalized petechial and ecchymotic hemorrhages along with splenomegaly and large areas of necrosis on lung lobes with congestion. Liver was enlarged with evidence of subcapsular necrosis. Macrophages containing developmental schizont stages of *C. felis* as observed in histopathology partially occluded blood vessels of vital organs of lungs, spleen and liver obstructing the blood flow thereby leading to tissue necrosis that might be one of the pathogenic effects of the *Cytauxzoon* infection in tigers.

Keywords: *Cytauxzoon felis*, cytauxzoonosis, macrophages, PCR, tiger

The wild tiger (*Panthera tigris tigris*) is the largest feline species symbolizing wildlife conservation. The Bengal tiger is a population of the *Panthera tigris tigris* sub-species that ranks as the biggest wild cats alive today. Tigers have been historically found in most of Asia representing the culture of this continent[1]. Cytauxzoonosis caused by *Cytauxzoon felis,* an apicomplexan piroplasmid of the family *Theileridae* is fatal in domestic cats leading to a per acute condition[2,3]. Members of *Theileridae* have erythrocytic and extra-erythrocytic phase, however, *Cytauxzoon* is unique as schizogony in this parasite occurs within mononuclear phagocytes, whereas the schizont stages of *Theileria* and *Gonderia* occur in lymphocyte[4]. The life cycle of *C. felis* involves two stages including non-pathogenic intra-erythrocytic phase (piroplasm) and pathogenic intraleukocytic phase (schizont)[5]. Piroplasms are observed in red blood cells as single signet-shaped, bipolar oval form, tetrads and dots[5] commonly leading to hemolytic anemia. In the leukocyte phase, large macrophages containing schizonts of the parasite lead toluminal obstruction of blood vessels in vital organs such as the liver, lung, lymph node and spleen[6]. The agent is transmitted by tick *Amblyomma americanum*.

Zoonotic diseases though have been largely described in wild carnivores[7], reports on wild felines especially in tigers are scant. The present study reports the first case of clinical and pathological features of fatal cytauxzoonosis in a captive adult white tiger (*Panthera tigris tigris*) in Bannerghatta Biological Park (BBP), Bengaluru, India that is confirmed by PCR. The findings suggest that cytauxzoonosis should be considered as a differential diagnosis in cases of diseases inducing anemia in wild fields.

During the year from 2020-2021, the 12-year-old captive male tiger was exhibiting signs of inappetance, lethargy, weakness, respiratory symptoms indicating distress, dehydration with a temperature of 104.2°F and was under treatment at the veterinary clinic, but was found to be unresponsive. Severe tick infestation was observed in the animal. The condition gradually got worsened and icterus was evident on the following day. It succumbed and necropsy was immediately performed in the post-mortem room (Fig. 1).

The study was conducted during 2020-2021 at Wild Animal Disease Diagnostic Laboratory (WADDL), BBP, Bannerghatta, Bengaluru. The latitude of BBP is 12.85687 and the longitude is

How to cite this article : Manjunatha, V., Rout, M., Srivastava, V., Vijay, N., Umashankar, K.S. and Hegde, R. 2024. Molecular Detection and Pathology of Cytauxzoonosis in Captive Royal Bengal Tiger. Indian J. Vet. Pathol., 48(3) : 280-284.

Fig. 1. Male tiger carcass with icteric body condition.

77.58523 with the GPS coordinates of 12° 51' 24.732" N and 77° 35' 6.828" E.

A volume of 2 ml blood sample collected in EDTA tube was immediately analyzed for hematological parameters using automated human 30s Hematology auto analyzer. Again, 2 ml blood sample collected in clot activator tube, after separation of serum was analyzed for certain serum parameters on the same day such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), total protein (TP), albumin, creatinine (CRT) and blood urea nitrogen (BUN) using TransAsia Chem pro 5 semi-automated analyzer with ERBA Biochemical Reagent kits.

The detailed postmortem examination was conducted and appropriate samples were collected for blood smear examination, tissue samples for PCR and histo-pathological examination. Selected tissues were fixed in 10% buffered neutral formalin, processed in paraffin blocks, sectioned at 5 µm and stained with Hematoxylin and Eosin (H&E).

Blood in EDTA was transported in ice packs. Blood smear examination was performed using Giemsa's staining for suspected hemoprotozoan infection. Thin peripheral blood smears were prepared and were fixed in methanol, then stained with diluted (1:10) Giemsa's stain (Merck Life Science, Mumbai) for 45 min. The blood smears were washed with water and air dried. The stained blood smears were examined under the oil immersion objective (100X) of the light microscope for the presence of parasites. A minimum of 100 fields were examined thoroughly before declaring a sample as negative. The number of parasites per 100 erythrocytes was counted on the smears and recalculated on 1 ml of blood.

Genomic DNA was isolated from blood samples collected in EDTA using DNeasy^{R} blood and tissue kit (Qiagen, Germany) according to the manufacturer's instructions. Extracted DNA was eluted in 100 µl of DNA elution buffer. The DNA concentration was determined using a NanoDrop2000C spectrophotometer (Thermo Scientific, USA) and stored at -20°C till further analysis. PCR assay for *Cytauxzoon felis* targeting the 18S rRNA gene was performed according to the procedure followed earlier[8]. All PCRs were carried out in a final reaction volume of 25µl containing 12.5µl 2X Taq polymerase master mix (Qiagen, Germany), 20 ng (4 µl) of template DNA, 1 µl 10 pmol each of forward and reverse primer and 6.5 µl PCR grade water to make upto 25 µl. The set of primers: Forward primer ITS2F 5′-GTTTCTGAACTCAGATCAAG-3′ and Reverse primer ITS2R 5′-AGACAAGAGTCAATAACTCGATAAC-3′ were used to amplify ITS regions of *Cytauxzoon felis*. The amplification reaction was performed in a total of 25 µl along with positive control and ultra pure water used as negative control. PCR reactions were performed

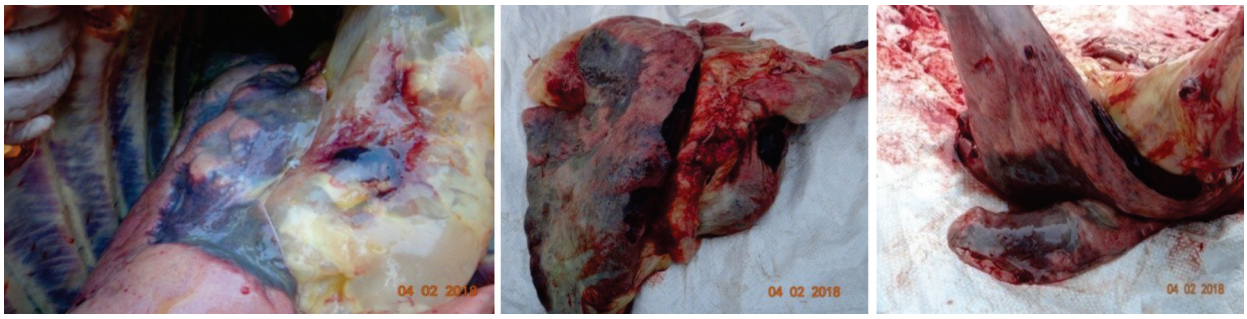

Fig. 2. Edema in mediastinal lobe and large areas of necrosis on dorsal and ventral surface of lungs with patchy consolidation.

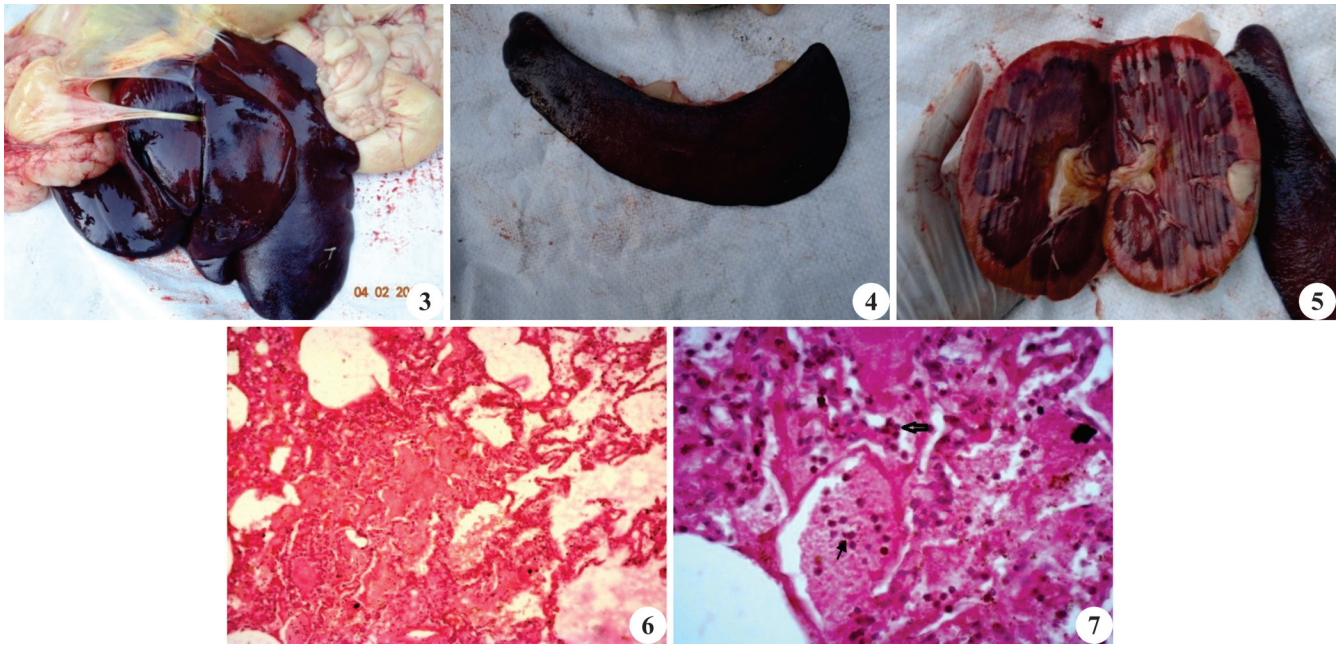

Fig. 3. Liver: Severely congested, enlarged with rounded borders with sub-capsular hemorrhages; **Fig. 4.** Spleen: Dark and moderately enlarged due to severe congestion and diffused sub-capsular hemorrhages; **Fig. 5.** Congested and hemorrhagic kidney; **Fig. 6.** Lung (H&E) microscopic section showing loss of alveoli and alveoli were filled with exudates with necrosis and consolidation along with macrophage infiltration (H&E 10X); **Fig. 7.** Lung (H&E) microscopic section showing loss of lung alveolar architecture, necrosis, edema, conslidation and macrophage containing cytauxzoon schizonts shown with black arrow (H&E 40X).

using the following thermal cycles: 94°C for 5 min of initial denaturation followed by 35 repetitive cycles of 94°C for 30 s, 58°C for 45 s and 72°C for 45 s and final extension at 72°C for 5 minutes. Aliquots of 7 µl of each of the amplified products were analyzed in 1.5% ethidium bromide-stained agarose gel by electrophoresis at 100V for 30 min in TAE buffer and visualized under a UV transil luminator. PCR amplicons were estimated by comparison with known amounts of electrophoretic standards using a 100 bp DNA ladder (Invitrogen, USA).

The study reported here in describes the clinical picture, postmortem gross lesions, histopathogical lesions, laboratory findings and diagnostic procedures for detection of *Cytauxzoon felis* in Royal Bengal Tiger. The molecular confirmation of *Cytauxzoon* sp. infection was established by post-mortem lesions, hematologic findings e.g., icteric plasma, severe thrombocytopenia, mild anemia, hematuria, obtained shortly before death and parasites consistent with *Cytauxzoon felis* observed in circulating erythrocytes. To the best of our knowledge, this case seems to be the first report of molecularly confirmed *Cytauxzoon felis* infection in Royal Bengal Tiger in India and Bannerghatta biome.

The *C. felis* infection in tiger indicates its prevalence in the study site. The incidence of *C. felis* in such felids is related to a higher exposure to ticks in the natural environment and we also observed severe tick infestation in the affected tiger. Although, the exact pathogenic mechanism of the disease is obscure, researchers have hypothesized that schizonts and merozoites present in

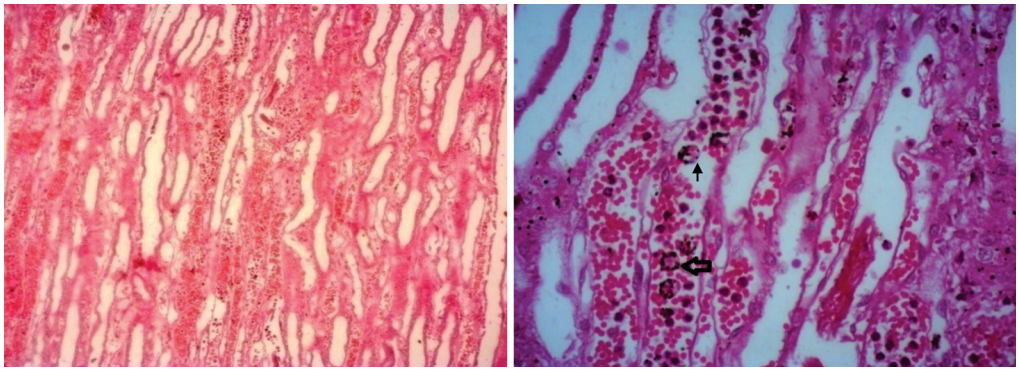

Fig. 8. Kidney (H&E) microscopic section showing tubular cell necrosis and macrophage containing cytauxzoon schizonts in blood vessels (H&E 40X).

the infected phagocytes/macrophages release toxic by-products thereby occluding the minute blood vessels of vital organs leading to cellular necrosis[8,9]. Cytologic blood smear examination revealed morphologically unremarkable leukocytes, while erythrocytes displayed no significant anisocytosis. Intra erythrocytic piroplasms ('dot' shaped organisms inside the erythrocytes) with a lightly basophilic cytoplasm and a basophilic nucleus usually located in an eccentric position. Hematological analysis indicated was a higher WBC count at 19500 cells/µl, RBC count decreased to 3.06 x 10^6/µl, hemoglobin was 8.2 g/dl and platelet count was 1.74 lakhs/µl. Hematology report reveals hemolysis due to multiplying *C. felis* schizonts in the RBCs. Hemorrhage was attributed to thrombocytopenia, anemia and leukocytosis. Serum biochemistry revealed high levels of SGOT at 106 U/L and SGPT 197 U/L and total bilirubin parameter indicates the liver involvement due to huge number of RBC destruction and its metabolites causing the elevated hepatic function parameters. CRT 3.8U/L and BUN 52U/L indicate the involvement of kidneys in the pathogenesis of this infection. Identification of piroplasms in RBCs confirms the infection and compatible history and clinical findings support a diagnosis of *C. felis*.

Gross necropsy findings comprised of generalized icterus, generalized petechiae and ecchymoses, enlarged spleen, liver, and lymph nodes with consolidated lungs. Hemorrhages were diffusely distributed on all the lobes of liver, lungs, kidneys and intestines. Both cranial and caudal lobes of the lungs showed large areas of necrosis especially on the surface and end tissue causing tissue anoxia due to obstruction of blood flow to the end tissues by obstructing macrophages in the minute blood vessels. Edema in mediastinal lobe and large areas of necrosis on dorsal and ventral surface of lungs with patchy consolidation (Fig. 2). All the liver lobes were severely congested with sub-capsular hemorrhages all over the organ appearing very dark (Fig. 3). The spleen was dark and moderately enlarged due to severe congestion and diffused sub-capsular hemorrhages with few areas

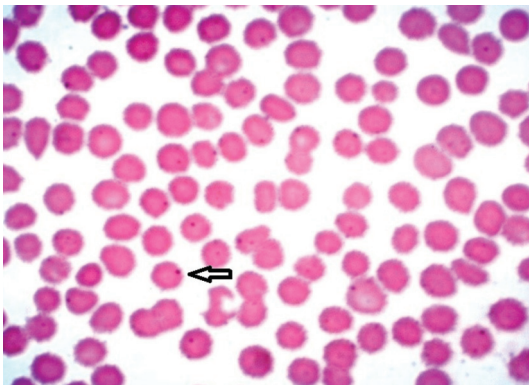

Fig. 9. Blood smear showing the presence of the cytauxzoon in the RBCs.

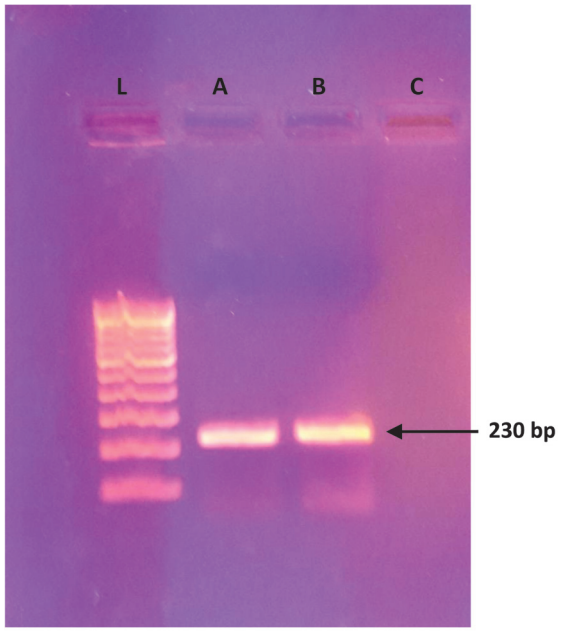

Fig. 10. Result of PCR assay showing amplification of 230 bp specific for *Cytauxzoonfelis* (Lane L : 100 bp DNA ladder, A : Tiger sample, B : Positive control, C : Negative control nuclease-free water).

of necrosis (Fig. 4). Gross appearance of kidney was enlarged, capsule was tightly attached and difficult to peel off, cut surface showed the pale cortex and congested medulla. Kidney showed hemorrhages and congestion as evident from the longitudinal section (Fig. 5). Histologically, intravascular macrophages/mononuclear cells distended by schizonts and merozoites were observed throughout tissues, especially kidney, liver and lungs obstructing vascular structures. Lymph nodes often exhibited number of parasitized mononuclear cells in medullary sinusoids. Lungs showed loss of alveoli and alveoli were filled with exudates with necrosis and consolidation along with macrophage infiltration (Fig. 6). There was loss of lung alveolar architecture, necrosis, edema, conslidation and macrophage containing cytauxzoon schizonts shown with black arrow (Fig. 7). Kidney revealed severe congestion of blood vessels and hemorrhages of arterioles and macrophages contained cytauxzoon schizonts in arterioles causing the anoxia to the succeeding tissue and degeneration of tubular epithelial cells further (Fig. 8). Blood smear showed the presence of cytauxzoon parasites in the RBCs (Fig. 9). The DNA extracted from blood samples was found to be positive for *C. felis* with amplification of 230 bp amplicon using *C. felis* species-specific primers (Fig. 10). The PCR used in the present study revealed high sensitivity and expected specificity with its utility in screening studies. Cytauxzoonosis in tiger has been reported in tigers by several workers. Fatal cytauxzoonosis was reported in a 7-year-old female white tiger[10] as well as in a young Bengal tiger in a German zoo[11].

CONCLUSION

This study describes the clinical picture, postmortem gross lesions, histopathological lesions, laboratory findings and molecular diagnostic procedures for detection of *Cytauxzoon felis* in Royal Bengal Tiger. To the best of our knowledge, it appears to be the first report of molecularly confirmed *Cytauxzoon felis* infection in tiger in India and in Bannerghatta biome. The felines should be protected from tick bites and consistent use of acaricides should be made a routine practice. Even recovered carrier cats should also be protected with acaricides to reduce the risk of transmission of their infection to naive ticks. The occurrence of cytauxzoonosis in tiger has significant implications on management of felids in breeding facilities or biological parks.

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Indian J. Vet. Pathol., 48(3) : 285-287, 2024: DOI: 10.5958/0973-970X.2024.00047.6

CASE REPORT

Melanocytoma: Surgical management and histopathological entity in a cross bred jersey cow

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Received: 19.3.2024; Accepted: 6.4.2024

ABSTRACT

This case report describes the successful surgical removal and histopathology of a melanocytoma in a Jersey cross bred cow. The mass located in the skin over mid ventral abdomen was removed aseptically under general anaesthesia. The mass was 17 cm in diameter and weighed 2.7 kg. Meticulous closure techniques were employed for the surgical site to prevent seroma formation. Standard postoperative care and wound management was followed with antibiotics and analgesics. Histopathological examination revealed cells with pleomorphic nuclei and cytoplasm containing dark brown pigment, suggestive of melanin. Schmorl's reaction showed dark bluish black melanin pigment. Postsurgical examinations on the 12th and 20th day showed proper wound healing. This study reported the occurrence of melanocytoma, a rare cutaneous tumour in a cow and highlights diagnostic and treatment considerations.

Keywords: Cow, cutaneous melanocytoma, histopathology, surgical management

Melanocytoma is a benign tumour that mainly affects white skinned horses, cattle, buffaloes, sheep, and goats[1]. Melanoblast are the cells in the basal layer of the epidermis that contain melanin. Neuroectodermal melanoblasts, which migrate into the hair follicles, dermis and epidermal-dermal junction during development, constitute the melanin secreting cells in skin. Benign neoplasms originating from these cells are usually referred to as melanocytomas, while malignant counter parts are referred to as melanoma or malignant melanoma. Melanomas are firm, hyperpigmented, discrete and single or multiple coalescence growth that appear in the skin or dermo-epidermal region[2]. Cutaneous melanocytomas found to appear in the dermis of adult animals. There have also been reports of melanocytic tumours in juvenile pigs and horses and congenital malignant melanomas in foals[3]. Clinically, melanoma is diagnosed based on the gross appearance of tissues as greyish to black pigmentation within a firm, fleshymass. The majority of melanocytic tumours in cattle are benign, though malignancies can occasionally[1]. This report describes a case of melanocytoma in a Jersey cross bred cow and also elaborates surgical management and histopathology of melanocytoma.

A 3 year and 5 month old female Jersey cross cow was presented to the Large animal referral clinic, Surgery unit of Veterinary College and Research Institute, Orathanadu, Tamil Nadu Veterinary and Animal Sciences University with the history of swelling in the umblical region since 2 months (Fig. 1). On clinical examination, the animal vital signs were completely normal and its overall health was good. Palpation of the superficial lymph nodes revealed no abnormalities. The haematological and biochemical analysis of the blood samples revealed the normal range of blood counts. Physical examination of the tumour showed negative for pain on evincing, with oozing out of blood on manipulation. Under general anaesthetic protocol (Xylazine @ 0.1 mg/kg, Ketamine @ 5 mg/kg and Butorphanol @ 0.2 mg/kg) and local infiltration with 2% lignocaine, a circumscribed incision was made and the mass removed aseptically (Fig. 2).

How to cite this article : Kaarthick, D.T., Prasath, N.B., Vishnugurubaran, D., Jayakumar, K., Vijayarajan, A., Rajesh, C. and Tamileniyan, E. 2024. Melanocytoma: Surgical management and histopathological entity in a cross bred jersey cow. Indian J. Vet. Pathol., 48(3) : 285-287.

The incised surgical wound was closed with cross-mattress suture for muscular layer and horizontal mattress suture for subcutaneous tissue and skin to avoid the dead space to restrain the wound from seroma formation. The removed mass was about 17 cm in diameter and weighed 2.7 kgs. The cut surface of the mass was blackish brown. The representative tissue samples from the mass were collected and preserved in 10% formalin and submitted to the Department of Veterinary Pathology for histopathological analysis. The tissue samples were processed as per standard paraffin embedded technique[4]. The tissue sections of 5 µm thickness were processed

Fig. 1. Cross-bred Jersey cow with ventral mass; **Fig. 2.** Surgically removed mass; **Fig. 3.** Day 12th - Wound healing with granulation; **Fig. 4.** Day 20th - Wound healing; **Fig. 5.** Intracytoplasmic brown pigments in melanocytes around blood vessels, skin (H&E stain x100); **Fig. 6.** Dark blue coloured melanin (Schmorl's reaction x400).

and stained with haematoxylin and eosin (H & E) stain. Duplicate stains were subjected for Schmorl's reaction. Post operatively the animal was treated with Streptopenicillin @ 20,000 IU/kg and Gentamicin @ 4 mg/kg to combat the secondary bacterial infection. In addition, Meloxicam @ 0.5 mg/kg, Chlorpheniramine maleate @ 0.5 mg/kg, Ascorbic acid 10 ml were given as supportive therapy for 5 days with antiseptic wound management.

Post operative examination on 12th (Fig. 3) and 20th day (Fig. 4) showed positive wound healing with good opposition. Hematoxylin and Eosin (H&E) stained sections showed medium-sized polygonal cells with moderately pleomorphic nuclei and cytoplasm containing dark brown coloured pigment suggestive of melanin (Fig. 5). Schmorl's reaction of the tissues demonstrated dark blue coloured melanin pigment in cytoplasm of melanocytes with pink nuclear stain (Fig. 6).

All domestic animals are known for developing melanomas; however, dogs and grey or white horses have a greater chance to be affected by melanoma[5]. Melanoma is a rare kind of skin tumour in cattle, accounting about 5-6% of total tumours[6]. Over a 12-year monitoring period, Nakhleh *et al.* (1990)[7] identified two cases of malignant melanoma among 96 skin and subcutaneous tumours in cattle. The highest occurrence in cattle has been reported in younger animals (less than 18 months old)[8], whereas our study reports the occurrence in a three-year-old animal. Neuroectodermal melanoblasts, which migrate into the epidermal-dermal junction of the skin, follicles, and dermis at the beginning of the development period, usually serve as the source of melanomas. The majority of melanomas are believed to develop from secondary mutations induced due to ultra-violet radiation A (320-400 nm) and UV B (290-320 nm) exposure[9]. In the present case, it originated near the umblical region. According to earlier reports, melanoma appear grossly as a thin,

elastic, black and painless movable mass. Cutaneous melanocytomas show ulcerations and bleeding during the development of tumours[5]. Histopathological observation of the present study was in parallel with earlier reports[5,10].

Surgical management employed in the present case was in accordance with[11]. Electrochemo therapy is an easy, safe and effective local treatment for cases of melanoma. It may also be employed as a substitute for surgery, particularly for smaller nodules where a long-lasting complete response can be obtained with a single treatment session or when the nodule located deeper or inside the body which cannot be removed surgically[12], even though it is less commonly used in cattle. In conclusion, the present report described cutaneous melanocytoma in a cross-bred Jersey cow and its successful surgical management.

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Indian J. Vet. Pathol., 48(3) : 288-291, 2024: DOI: 10.5958/0973-970X.2024.00048.2

CASE REPORT

Colic - Intestine torsion in a horse : Case Report

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Received: 14.3.2024; Accepted: 19.3.2024

ABSTRACT

A seven year old female horse belongs to Sainik School, Kanigiri brought for necropsy to department of veterinary pathology college of veterinary science, Tirupati. At necropsy; subcutaneous tissue was icteric, epicardial and endocardial hemorrhages, consolidated lobes of lungs, nutmeg appearance of liver, difficulty in peeling of capsule of kidney and congestion of cortico-medullary junction were recorded. Twisting of the intestine at greater colon with engorgement of mesenteric blood vessels and severe congestion, hemorrhages on mesenteric mucosa was observed. Thickened mucosa of intestine with necrotic lesions was noted. Microscopically, abundant hemorrhages, severe congestion in sub mucosa, muscularis layer and microvilli were recorded. Villi were ruptured at several places. Mononuclear cell infiltration in the sub mucosa predominantly neutrophils, degeneration of the enterocytes, and desquamation and shortening of the villi were observed and resulted necrotic hemorrhagic enteritis. Finally death occurred due to torsion leads to septic shock.

Keywords: Colic, horse, necrotic hemorrhagic enteritis, septic shock, torsion

Colic refers to abdominal pain caused by disrupted peristaltic action of smooth muscle in the viscera. It affects many animal species, including humans, although equines such as horses, donkeys, ponies, and zebras are especially vulnerable[1]. Colic is distributed worldwide and occurs in different environments. The incidence rate ranges between 3.5 and 10.6 cases per 100 horses per year[2,3]. Colic is a major concern in race horses as there will be severe pain in the stomach and the main etiology supposed to be torsion and result mortality in equines[4] due to stomach rupture, strangulation or enteritis. The etiological agents to this clinical syndrome are several including disease base on system that classifying the cause of colic as obstructive, displacement, gas, parasite, and enteritis[5,6]. Gastrointestinal tract is the most important source for the colic[7]. The prevalence of colic may increase depending on age, breed, managemental factors in equines[8]. The predominant clinical sign observed in colic is pain which may occur due to spasm of digestive system and is manifested as pawing, stamping, kicking or rolling[5,6]. Twisting of intestines on the central axis is called torsion[9]. Torsion of intestine leads to complete blockage distal part which result in arrest of blood supply and further necrosis; which is the most lethal form of Colic. Complete obstruction leads to severe in tolerable pain due to development of inflammatory condition and shock due to intestinal infarction and release of bacterial toxins that may be passed into the blood stream and leads to death.

A seven years old female horse belongs to Sainik School, Kanigiri with history of colic signs *viz;* pain often manifested through behavioral changes, such as pawing, stamping, or kicking at the belly. Restlessness, moving in small circles or repeatedly stands up and lies down, often with exaggerated care since morning died and brought for necropsy to Department of Veterinary Pathology College of Veterinary Science, Tirupati. Detailed postmortem was conducted. Gross lesions were recorded and tissue sections were collected in 10% neutral buffered formalin. Collected tissues were properly fixed and subjected for processing through dehydration in different grades of alcohol, clearing by xylene and embedding by paraffin. The paraffin embedded blocks were subjected for sectioning at 4-6 micron thickness. The cut sections were stained with routine Haematoxylin and Eosin (H&E) stain as per standard protocol[10].

How to cite this article : Harshitha, D., Sarachandra, V., Naik, M.G.T., Reddy, M.V.S.S., Swetha, G., Nakul, P., Nasreen, A., Kumar, A.A., Sujatha, K., Sailaja, N. and Amaravathi, P. 2024. Colic - Intestine torsion in a horse : Case Report. Indian J. Vet. Pathol., 48(3) : 288-291.

The clinical signs as reported in history were kicking of the abdomen and rolling and died suddenly. At necropsy the carcass revealed externally, sunken eyes, pale oral mucous membranes (Figs. 1 & 2). Upon opening the carcass, subcutaneous tissue found to be icteric and around 300 ml of serosangvinous fluid was present in the abdominal cavity (Fig. 3). Heart revealed epicardial and endocardial hemorrhages with left ventricular

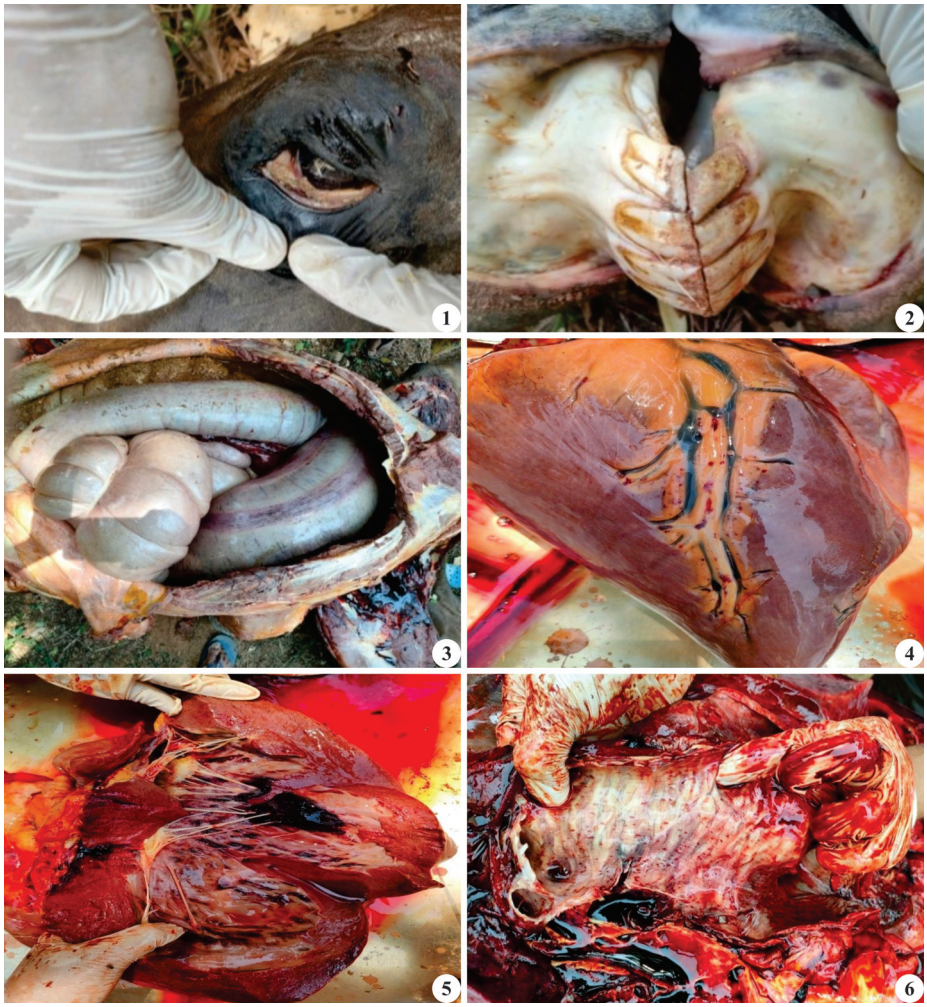

Fig. 1. Carcass of horse showing sunken eyes; **Fig. 2.** Pale oral mucous membrane; **Fig. 3.** Abdominal cavity with presence of serosanguinous fluid; **Fig. 4.** Presence of epicardial hemorrhages; **Fig. 5.** Endocardial hemorrhages with left ventricular hypertrophy and mural thrombus; **Fig. 6.** Tracheal hemorrhages.

hypertrophy and mural thrombosis (Figs. 4 & 5). Consolidation of all the lobes of both lungs, tracheal and bronchial hemorrhages (Fig. 6) was noted. Enlarged liver with moderate congestion and consistency was hard. On sectioning nutmeg appearance was evident (Figs. 7 & 8). The capsule of the kidney could be peeled with difficulty and severe congestion of cortico-medullary junction (Fig. 9) was observed. Intestine was twisted on its axis at greater colon, engorgement of mesenteric blood vessels and severe congestion of mesenteric mucosa, thickened mesenteric mucosa with necrotic lesions, formation of pseudo membrane and hemorrhages were present (Figs. 10 & 11). Histopathologically,

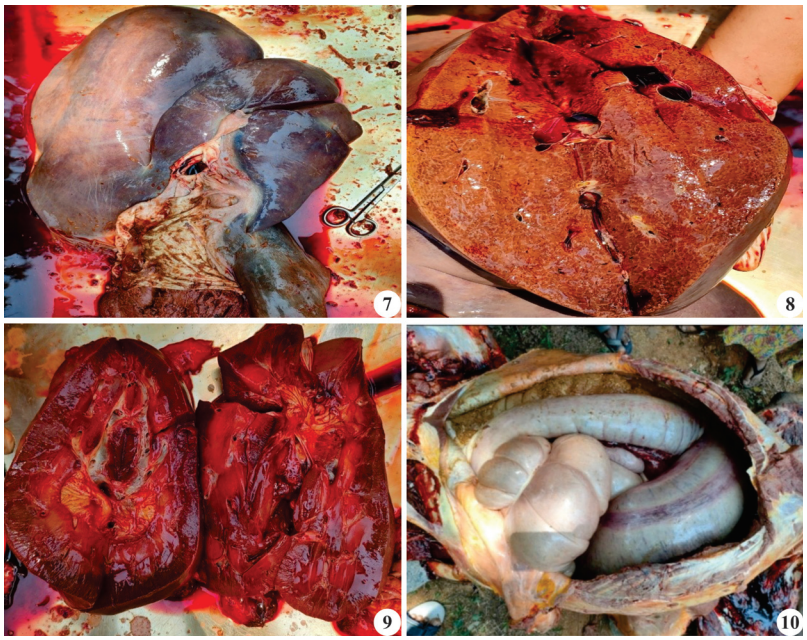

Fig. 7. Carcass of horse showing hepatomegally; **Fig. 8.** Nutmeg appearance of liver; **Fig. 9.** Congestion of cortico medullary junction in kidney; **Fig. 10.** Torsion of intestine at colon.

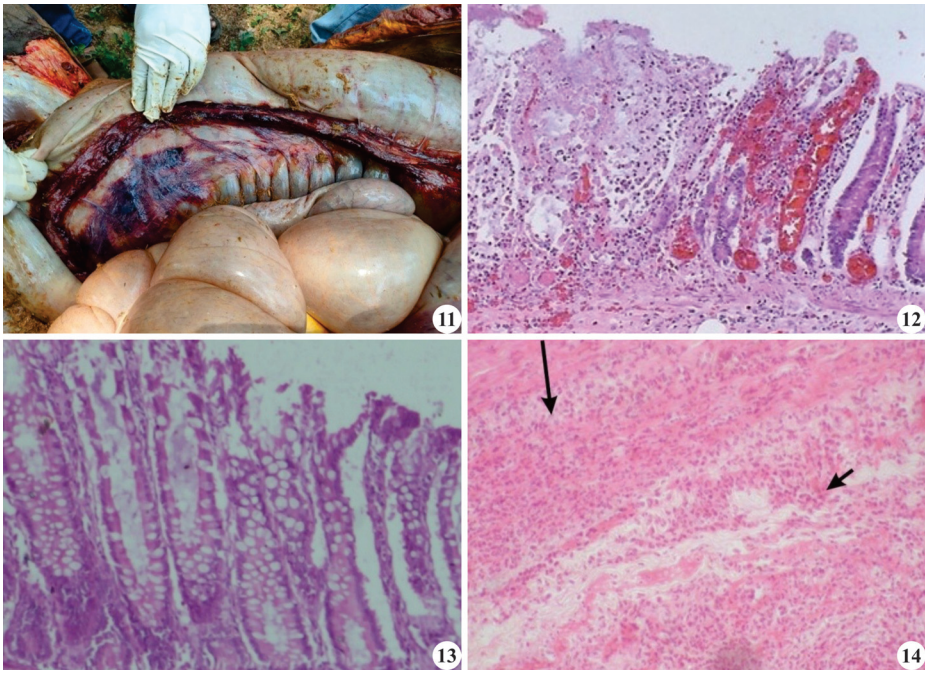

Fig. 11. Carcass of horse showing congested mesenteric blood vessels and hemorrhagic mesenteric mucosa; **Fig. 12.** Section of intestine showing hemorrhages and necrosis in mucosa and villi (H&E X400); **Fig. 13.** Section of intestine showing goblet cell hyperplacia and rupture in villi (H&E X1000); **Fig. 14.** Section of intestine showing presence of mononuclear cells (arrows) predominantly neutrophils in submucosa (H&E X1000).

intestine revealed abundant hemorrhages both in villi and sub mucosa as well. Severe congestion and hemorrhages and thickening in both sub mucosal, muscularis layers and microvilli were recorded. Ruptured villi observed at several places with fibrotic changes. Degeneration of the enterocytes, and desquamation and shortening of the villi mononuclear cell infiltration in the sub mucosa predominantly neutrophils were observed and indicated hemorrhagic necrotic enteritis (Figs. 12 & 14).

The clinical signs observed *viz.* kicking of the abdomen and rolling are common and other symptoms like nibbling, staring at the flank, rolling, and even lying on their back were observed in horses suffering from colic as reported prior to death were in correlation with earlier studies[11,12]. The lesions observed in intestine indicated torsion of intestine. It is the most lethal form of equine colic[13] and leads to complete blockage of intestine and also blood supply to that region resulting in necrosis of the affected part. The blockage of blood supply leads to painful condition causing rapid deterioration. The present findings are similar to the observations made by earlier worker[14,15]. Thickened mucosa of intestine with necrotic lesions, formation of pseudo membrane and hemorrhages might be due to torsion, stagnation of excreta which lead to multiplication of bacteriae and resulted septicemic changes in various visceral organs. The histopathological findings revealed; abundant hemorrhages, severe congestion in submucosal, muscularis layer and microvilli. Degeneration of the enterocytes, and desquamation and shortening of the villi. Mononuclear cell infiltration in the sub mucosa mononuclear cell infiltration indicated necrotic hemorrhagic enteritis. These observations are in line with earlier reports[13,16-18] where complete blockage of intestines lead to stagnation of excreta, which in turn paved way for multiplication of bacteriae and release of toxins into blood stream and further severe intolerable pain and shock due to intestinal infarction and bacterial toxins that passes into blood stream. Hence, the present case can be concluded as death occurred due to torsion lead to septic shock.

ACKOWLEDGEMENTS

The authors are thankful to Sri Venkateswara Veterinary University for providing facilites to carry out this work.

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Indian J. Vet. Pathol., 48(3) : 292-294, 2024: DOI: 10.5958/0973-970X.2024.00049.9

CASE REPORT

Sebaceous gland adenoma in a non descript dog : A case report

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Received: 19.1.2024; Accepted: 6.2.2024

ABSTRACT

A non descript female dog with history of multiple warty growth on lower limbs and flank region persisting from last few months and started increasing in size and number from last two weeks was presented to the Department of Veterinary Pathology. Biopsy sample was collected for histological examination which revealed multiple lobules of bunch of pleomorphic sebocytes along with peripheral reserve cells and trabeculae of connective tissue in between. Dominance of sebocytes along with fewer undifferentiated cells and occasional mitosis was observed at the periphery of the neoplastic lobules suggestive of sebaceous gland adenoma.

Keywords: Adenoma, dog, Sebaceous

Skin tumors are fairly common in canines and felines. Sebaceous glands are found in the skin associated with hair follicle and secrete oily substance which lubricates the skin and hair coat[1] and responsible for the majority of skin's hormone metabolism. Based on histological appearance, these tumours are divided into four basic categories: ductal adenoma, adenocarcinoma, epithelioma, and adenomas. Sebaceous adenoma is benign neoplasm of cutaneous adnexa with sebaceous differentiation. It lies third in incidence among the sebaceous gland tumors with very low prevalence. The head, abdomen, eyelid and thorax are the common sites of sebaceous glands tumors[2]. Prolonged ultraviolet exposure, reduced progesterone and estrogen receptor on sebocytes have been implicated as some of the important determinants of this neoplasm[3]. The aim of this case report is to describe the clinicopathological characteristics of canine sebaceous gland adenoma and to illustrate histological characteristics.

A non descript breed female dog of approximately eight year of age was presented to Department of Veterinary Pathology, College of Veterinary & Animal Sciences, Meerut with history of multiple warty growth ranging from 2 mm to 9 mm, which are mainly concentrated on cutaneous tissue of lower limb and also few on flank, lumber and thigh region (Fig. A, B, C). The bitch was treated with estradiol benzoate at the dose rate 0.01 mg/kg body weight on alternate day for three days for termination of pregnancy due to mis-mating. After 2 days of which the animal was suffered with dermatitis and exhibited signs of erythema, rashes and itching. The animal was treated by private veterinarian with inj. Ivermectin (1 ml S/C) and Amitraz (2 ml in one liter water) topically which lead to mild temporary relief. However the animal was presented to another vet practitioner and the bitch was treated with topical ketoconazole lotion and Zinkovit tablets which also lead to mild temporary relief followed by immergence of few small warty growths (2 to 3 mm size) mainly on the limbs. It remained as it is for long time but they suddenly increased in number and size in last 15 days. The weight of dog reduced from 19.5 kg to 9 kg in one year in addition to appearance of warty growth.

Biopsy sample was collected from warty lesion in 10% neutral buffered formalin and was fixed for next 72 hours, washed overnight in running tap water and dehydrated in increasing grades of alcohol and acetone, cleared in benzene followed by paraffin embedding and prepared paraffin blocks. The tissue sections of 4 μm thickness were prepared by using microtome. The sections were then stained with hematoxylin and eosin as per standard procedure as described by Luna (1968). Haematology and blood biochemistry for liver function test and kidney function test was carried by veterinary specific auto analyzer (Mindray BC-30 Vet, Mindray BS-240).

Microscopic examination of H & E stained tissue section revealed multiple lobules of clustered sebocytes along with marginal reserve cells and trabeculae of connective tissue,

How to cite this article : Chaudhary, S., Jaiswal, V., Katoch, S., Chandra, N., John, J.K., Kumar, A. and Bhatele, A. 2024. Sebaceous gland adenoma in a non descript dog : A case report. Indian J. Vet. Pathol., 48(3) : 292-294.

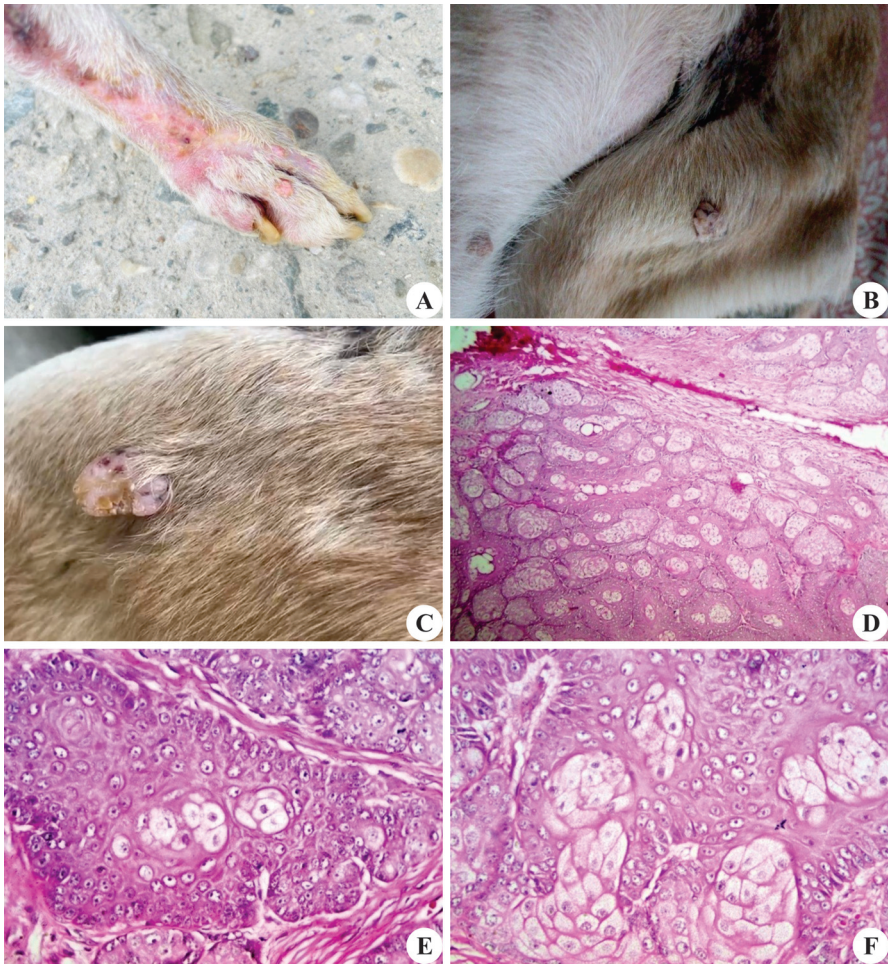

Fig. A, B, C. Gross lesion showing warty cauliflower like growth on the skin of phalanges, thigh and knee respectively. **D.** Microscopic picture of tumor showing multiple lobule of sebocytes separated by connective tissue trabeculae, one to multilayered peripheral reserve cells (Stain H&E, 100X). **E & F.** Microscopic picture of nodule showing mature sebocytes in center with microvesicular (foamy) cytoplasm surrounded by undifferentiated neoplastic cells with prominent nucleoli, coarse chromatin enlarged nucleus and marked anisocytosis (Stain H&E, 400X).

dominance of sebocytes with fewer basaloid reserve cells and ducts. The lobules were divided by trabeculae of connective tissue and pre-existing dermal collagen remnants. Sebocytes were large polyhedral shaped having micro-vacuolated (foamy) cytoplasm, round nuclei, and variability in cell size (Fig. E). Most of the lobules showed well differentiated cells however poorly differentiated cells were also observed at places with interstitial fibrosis (Figs. D, E). At the margin of the neoplastic lobules there is a frame of small, basophilic normal reserve cells, having hyperchromatic nuclei and scant cytoplasm. The thickness of these reserve cells ranged from one to many cell layers showing little to no pleomorphism, however occasional mitoses was observed at places (Figs. C, D). In the present case sebaceous adenomas is extending from the epidermal-dermal interface upto upper some area of dermis. Hematology revealed haemoglobin 5.5 g/dl, RBC 2.82 mill/mm^3, PCV 18.3%, MCV 65.2 fL, MCH 19.5 pg, MCHC 30 g/dl, Platelet 224 thou/mm^3, RDW-CV 0.143, TLC 10.6 thou/mm^3, DLC-Neutrophils 82%, DLC-Lymphocytes 13%, DLC-Eosinophils 2% and DLC-Monocytes 3% while serum biochemistry showed blood urea 123.3 mg/dl, creatinine 2.0 mg/dl, uric acid 1.3 mg/dl, calcium 11.4 mg/dl, phosphorus 10.9 mg/dl, sodium 143.4 mEq/I, potassium 4.10 mEq/I, chloride 99.3 mEq/I while liver function test parameters revealed total billirubin 0.2 mg/dl, direct billirubin 0.1 mg/dl, indirect billirubin 0.1 mg/dl, SGOT (AST) 11.8 U/L, SGPT (ALT) 23.9 U/L, alkaline phosphatase 125.9 U/L, total protein 5.5 g/dl, albumin 2.2 g/dl, globulin 3.3 g/dl and A/G ratio 0.66.

Sebaceous glands are made up of two parts: a glandular portion with undifferentiated cells on the periphery of the gland and mature sebocytes in the centre, and a duct that enters the infundibulum of the hair follicle and is lined by a flattened, undulating, keratinizing epithelium. On the basis of histology four neoplasms arising from sebaceous glands are: sebaceous adenoma, sebaceous gland carcinoma, sebaceous

ductal adenoma and sebaceous epithelioma[5]. Varying prevalence of sebaceous gland adenoma (ranging from 3.3% to 7.9%) had been reported by previous researchers globally[2,6]. Most of the researchers reported its occurrence in head and eyelids of older dogs[2,6]. Sex hormones have been demonstrated as an important driving force for development and differentiation of cutaneous tissue and alteration from normal may lead to neoplastic proliferation of skin and adnexa such as sebaceous glands. Although androgens are considered most important in differentiation of sebaceous glands which is documented both in differentiated as well as basal cells yet estrogens are also important and frequently detected in normal basal cells[7]. The current case has been treated with Estradiol benzoate, amitraz and ketoconazole with history of dermatitis. Amitraz acts as alpha$_2$-adrenergic receptor agonist which inhibits GnRH (gonadotropin releasing hormone) release. Ketoconazole is known to alter the steroid synthesis resulting in alteration of both sex hormones[8]. Ketoconazole have been reported to persistently increase the estradiol-testosterone ratio[9]. Other important factor was concurrent dermatitis causing continuous irritation leading to moderate alopecia exposing cutaneous tissue to ultraviolet light of sun having additive effect. So in nut shell dermatitis along with treatment which might have altered the hormonal status resulted in neoplastic proliferation and adenoma formation in present case. More than fifty percent of sebaceous gland growths are simply hyperplasia characterized by crusty cauliflower like growth, histologically confined up to level of hair bulb while sebaceous adenomas extend from epidermal-dermal interface into dermis, may go deeper involving subcutis and characterized by multilobulation. Other than this sebaceous hyperplasia are composed of hyperplastic lobules of mature sebocytes clustered around a large sebaceous duct, which frequently connects to the follicular infundibulum[5]. Hematology revealed normocytic hypochromic anaemia might be due to toxic effect of amitraz, relative neurophillia due to secondary bacterial infection of cutaneous lesions while serum biochemistry showed increased blood urea and creatinine due to renal damage caused by amitraz[10] and mild reduction in albumin level due exudation of protein rich fluid from erythematous cutaneous lesions.

CONCLUSION

In general tumors are classified on the basis of differentiation of different cell type, their orientation, mitosis and supporting stromal tissue and identified on the basis of most differentiated cells in histological examination of biopsy samples. The current case was having preponderance of pleomorphic sebocytes with less than 50% basaloid reserve cells and ducts leading to final diagnosis of presented case as sebaceous gland adenoma.

ACKNOWLEDGMENTS

The authors are thankful to the Dean, College of Veterinary and Animal Sciences and the Vice Chancellor, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut for providing the facilities for carrying out this work.

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Indian J. Vet. Pathol., 48(3) : 295-298, 2024: DOI: 10.5958/0973-970X.2024.00050.X

CASE REPORT

Pathology of ceruminous gland adenoma associated with *Staphylococcus epidermidis* otitis externa in a cat

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Received: 14.2.2024; Accepted: 23.3.2024

ABSTRACT

A two-year old intact domestic male cat (*Felis catus*) was presented in the Veterinary Clinical Complex, Dr G.C. Negi College of Veterinary and Animal Sciences, Palampur, Himachal Pradesh with a history of continuous unilateral purulent discharge from the right ear canal along with the signs of otalgia and hyperemia of the infected area. There was continuous head shaking and scratching of the infected ear. Otoscopic examination revealed the presence of three different growths of irregular shapes partially obstructing the right ear canal. Biopsy sample from the growths present in the ear canal of a cat was submitted to the Department of Veterinary Pathology. Gross examination of the growth revealed pigmented, friable and pale growths which were smooth in appearance. Cytological examination was suggestive of fibrinopurulent inflammation associated with bacterial infection in addition to the presence of clusters of columnar to round cells showing moderate pleomorphism, hyperchromasia, increased nuclear to cytoplasmic ratio and prominent nucleoli. Masson's trichrome staining revealed the presence of fibrous tissue capsule around the tumor. Histopathological examination revealed columnar epithelial cells lining the glandular lumen showing moderate pleomorphism, hyperchromasia, increased nuclear to cytoplasmic ratio, prominent nucleoli and loss of polarity along with the presence of very few mitotic figures. Cytological, gross and histopathological findings confirmed the case of ceruminous gland adenoma associated with external ear infection of *Staphylococcus epidermidis* in a cat.

Keywords: Adenoma, cat, ceruminous gland, otitis externa, pathology, *Staphylococcus epidermidis*

Although neoplasms associated with skin and subcutaneous tissues are easily and early recognized by the owners, tumors developing in the ear canal may not be visualised until clinical signs appear. Of all the tumors diagnosed, skin neoplasms constitute around 30% in dogs and 35-45% in cats[1]. These are the most commonly occurring tumors in dogs and second most common tumors of cats[2]. Aural tumors comprise 1-2% of all the tumors occurring in cats[3] and 85% of these occurring in feline species have been observed to be malignant while in dogs it is around 15%. Ceruminous gland adenocarcinomas when compared to adenomas are reported more often in cats[4]. Ceruminous glands are the specialized tubular apocrine sweat glands present in the cartilaginous part of external acoustic meatus with their ducts opening either into the regional hair follicles or onto the epidermal surface. This gland's secretion along with sebum and desquamated stratified squamous epithelium constitute cerumen or the ear wax[5,6]. Continuous irritation to the epithelium of ear canal caused during chronic otitis externa may subsequently be followed by hyperplastic changes in the associated structures which may ultimately develop into dysplatic and neoplastic ones[7].

A two-year old intact domestic male cat (*Felis catus*) was presented in the Veterinary Clinical Complex, Dr G.C. Negi College of Veterinary and Animal Sciences, CSK HPKV, Palampur, Himachal Pradesh with a history of continuous unilateral purulent discharge from the right ear canal along with the signs of otalgia and hyperemia of the infected area. There was continuous head shaking and scratching of the infected ear. Otoscopic examination revealed the presence of three different growths of irregular shapes partially obstructing the ear canal. Sterile swab taken from the infected right ear was inoculated on Nutrient agar followed by sub-culturing on selective media i.e. Staphylococcal Agar No. 110 (Himedia). The pure and isolated bacterial colonies were subjected to Gram staining and the species was further confirmed by carbohydrate fermentation tests. Later, these masses were surgically excised using laser technique and submitted in the Department of Veterinary Pathology. Touch smears were prepared from the cut surface of the tumor growth and were air-dried, methanol-fixed and stained with Giemsa stain. The biopsy sample was preserved and

How to cite this article : Bisht, E., Patil, R.D., Verma, A., Kumar, R. and Asrani, R.K. 2024. Pathology of ceruminous gland adenoma associated with *Staphylococcus epidermidis* otitis externa in a cat. Indian J. Vet. Pathol., 48(3) : 295-298.

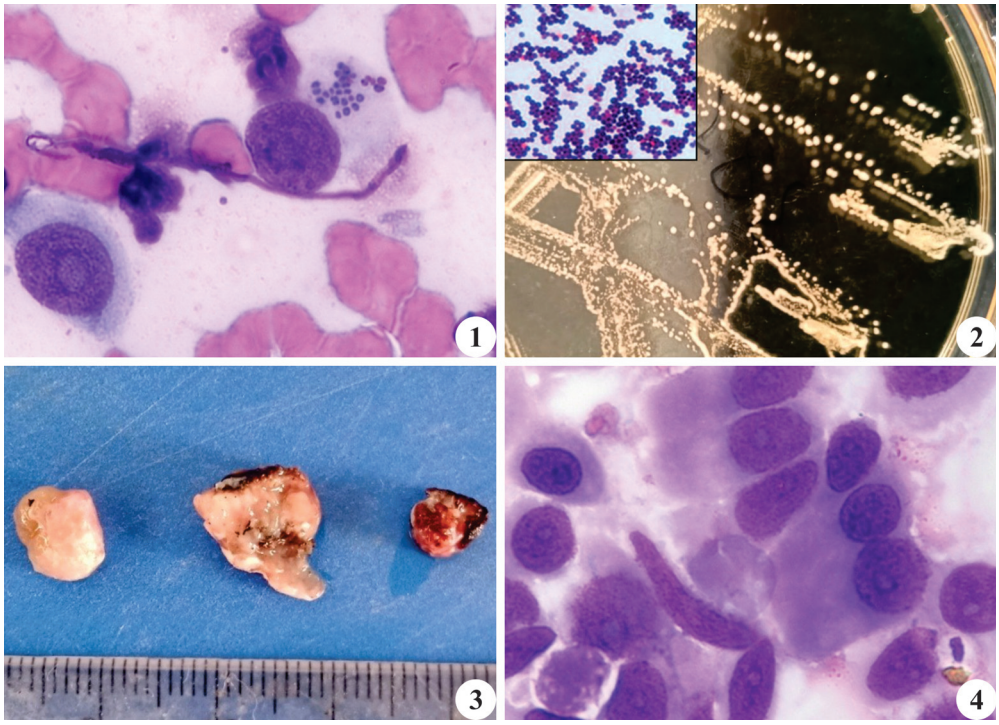

Fig. 1. Presence of neutrophils admixed with fibrin and bacterial colony comprising cocci (Giemsa 1000x oil immersion); **Fig. 2.** Round, convex, cream colored *S. epidermidis* colonies without pigment production on Staphylococcal Agar No. 110. Inset: Gram-positive *S. epidermidis* cocci in bunches (Gram stain 1000x oil immersion); **Fig. 3.** Gross morphology of tumor masses revealed pigmented, friable growth with irregular border, round to oval shape and smooth appearance; **Fig. 4.** Cytosmear revealed cluster of columnar to round cells with pleomorphism, hyperchromasia and prominent nucleoli (Giemsa 1000x oil immersion).

fixed in 10% neutral buffered formalin. After 72 hours of fixation, the tissue sample was processed as per the standard protocol followed by paraffin embedding and block making. Histologic sections of 3-4 microns were cut and then stained with haematoxylin and eosin and Masson's trichrome stain as per the standard protocol[8].

Microscopic examination of the smear prepared from ear swab revealed the presence of abundant neutrophils admixed with fibrin and bacterial colonies comprising cocci in addition to the presence of neoplastic epithelial cells (Fig. 1). Microbiological examination revealed small, round, convex, white colored bacterial colonies, which revealed gram positive cocci on Gram staining. The bacterial growth on Staphylococcal Agar No. 110 revealed round, convex, cream colored colonies without pigment production (Fig. 2). The carbohydrate fermentation profile of the isolate was found to be positive for glucose, maltose, sucrose and lactose while it was negative for mannitol, trehalose, raffinose and salicin which led to its identification as *Staphylococcus epidermidis*.

Gross examination of the biopsy revealed three 5 to 10 mm in diameter round to oval masses attached to ear canal. The growth was found to be pigmented, friable, pale and smooth in appearance (Fig. 3). Impression smear revealed the presence of clusters of cuboidal to columnar epithelial cells showing moderate pleomorphism, hyperchromasia, increased nuclear to cytoplasmic ratio, prominent nucleoli and rare occurrence of mitotic figure (Fig. 4).

On histopathological evaluation, the neoplastic cells were mainly arranged in tubulo-papillary pattern giving cystic appearance at places (Fig. 5). Round to columnar epithelial cells lining the glandular lumen were evident exhibiting moderate pleomorphism, hyperchromasia, increased nuclear to cytoplasmic ratio, prominent nucleoli and loss of polarity along with occasional mitotic figures (Fig. 6). The cytoplasm of these neoplastic cells was distinct and eosinophilic while the nuclei were round to oval in shape. At places, the necrotic cellular debris with or without desquamated epithelium and apoptotic cells were seen inside the glandular lumen (Fig. 7). Masson's Trichrome staining showed the tumor masses were well encapsulated by fibrous connective tissue admixed with collagenous stroma (Fig. 8).

Tumors of external ear canal in domestic animals are histologically classified into ceruminous tumors, plasmacytoma, feline nasopharyngeal polyp, hyperplastic inflammatory polyp and cystic apocrine hyperplasia or ceruminous cyst[9]. Ceruminous gland tumors are further classified as adenomas, adenocarcinomas, and mixed or complex ceruminous gland tumors[10]. Although ceruminous gland tumors are more common in dogs

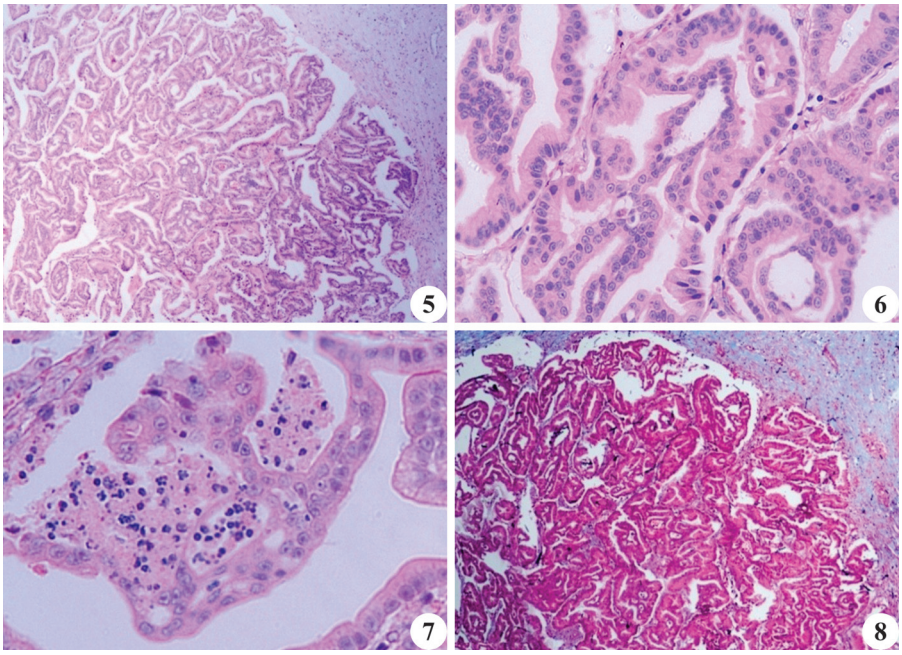

Fig. 5. The neoplastic glandular epithelium were arranged in tubulo-papillary pattern and encapsulated with fibrous connective tissue (H&E 40x); **Fig. 6.** The glandular epithelial cells exhibited loss of polarity, hyperchromasia, pleomorphism and prominent nucleoli (H&E 200x); **Fig. 7.** Necrotic cellular debris admixed with desquamated epithelium seen in the glandular lumen (H&E 400x); **Fig. 8.** Presence of fibrous connective tissue capsule around the tumor (Masson's Trichrome stain 40x).

and cats but occurrence in other species have also been reported including horses[11,12], foxes[13] and ferrets[14]. Unlike canines and felines, very rare instances of these neoplasms are evident in humans[15,16].

It becomes important to diagnose these tumors as it acts as a guide to clinicians for further treatment plan in addition to detection of malignancy. Surgical treatment options for ceruminous gland tumors in dogs and cats include total ear canal ablation and bulla osteotomy (TECABO) and CO_2 laser ablation. The former however, results in partial or complete hearing loss and is costly too. Chances of recurrence in all kinds of ceruminous gland tumors have also been reported with two out of twenty six cases treated showing recurrence (8%)[17].

As stated earlier, prolong irritation to the ear canal epithelium is one of the important predisposing factors for the development of ceruminous gland neoplasms. In the present case, *Staphylococcus epidermidis* was found to be the major etiological agent involved in causing fibrinopurulent otitis externa which might have subsequently led to the formation of tumor masses in the ear canal. Hence, cytological, gross and histopathological findings confirmed the presence of ceruminous gland adenoma associated with otitis externa due to *Staphylococcus epidermidis* infection in the ear canal of a domestic cat which were in accordance with the earlier reported studies[18].

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Indian J. Vet. Pathol., 48(3) : 299-301, 2024: DOI: 10.5958/0973-970X.2024.00052.2

CASE REPORT

Hepatocellular and bronchogenic carcinoma in a rabbit *(Oryctolagus cuniculus)*: A case report

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Received: 23.8.2023; Accepted: 2.11.2023

ABSTRACT

An adult male rabbit was presented for necropsy in the Department of Veterinary Pathology, DGCN COVAS, Palampur. The post-mortem examination of the rabbit revealed the presence of multiple nodular growths on the liver along with multiple pea-sized nodular and raised dirty fluid-filled cystic structures on the lung parenchyma. Fine needle aspiration cytology (FNAC) examination has shown the presence of pleomorphic cells with increased nuclear-cytoplasmic ratio and profound cellular atypia. The histopathological evaluation revealed the presence of pleomorphic hyperchromic neoplastic cells with prominent changes in nuclear components (anisokaryosis, prominent nucleoli) in hepatocytes and air spaces in lungs were filled with eosinophilic fluid as well as pleomorphic neoplastic cells. The present case was diagnosed to have died of hepatocellular and bronchogenic carcinoma based on macroscopic, cytological and histological examinations.

Keywords: Bronchogenic carcinoma, hepatocellular, necropsy, rabbit

How to cite this article : Jamwal, S., Kumar, R., Verma, A., A., Choudhary, S. and Asrani, R.K. 2024. Hepatocellular and bronchogenic carcinoma in a rabbit *(Oryctolagus cuniculus)*: A case report. Indian J. Vet. Pathol., 48 (3) : 299-301.

Rabbits (*Oryctolagus cuniculus*) are one of the most commonly used laboratory animals to conduct biomedical research. The incidence of cancers is less in rabbits in comparison to other mammalian species[1]. Uterine adenocarcinoma is the most common neoplastic disease of rabbits followed by lymphoma/lymphoid leukemia[1,2]. Pulmonary and hepatic neoplasms are not common in rabbits. Among the hepatic tumors, lymphoma, bile duct adenoma and carcinoma are the most common types of tumours. Tumors in other parts of the body such as the uterus show metastasis to the liver in rabbits[3]. Pulmonary carcinomas can be classified into bronchial, alveolar or broncho-alveolar carcinomas among which pulmonary adenocarcinoma is main type of pulmonary tumors[4].

The present case was observed in an adult male rabbit which was brought to the Department of Veterinary Clinical Complex (VCC), Palampur, Himachal Pradesh, India. The animal died during treatment and was presented for necropsy to the Department of Veterinary Pathology, DGCN COVAS, CSKHPKV, Palampur, Himachal Pradesh, India. Cytological smears were prepared from the growths and were fixed with methanol followed by staining with Giemsa stain. On necropsy examination, approximately 0.5 cm thick tissue sections were collected in 10% neutral buffered formalin (NBF) for histopathological evaluations. The fixed tissue sections were dehydrated in ascending grades of alcohol, cleared in benzene, and impregnated in molten paraffin. The paraffin blocks containing tissue sections were cut into the 2-4µ thickness and were stained with Haematoxylin and Eosin (H&E) stain as per the standard protocol[5]. The stained tissue sections were evaluated and micro-photographed (Olympus BX40).

Post-mortem findings revealed multifocal, raised, variable-sized, creamish, white colored, nodular lesions on the liver parenchyma (Fig. 1). The lungs exhibited the presence of nodular, raised, dirty fluid-filled cystic structures along with adhesions involving the thoracic wall (Fig. 2) and the abdominal cavity was filled with serosanguinous fluid along with fibrin threads (Fig. 3). Cytological examination of impression smears prepared from the liver has shown pleomorphic cells with increased nuclear-cytoplasmic ratio and prominent nucleoli along with profound cellular atypia (Fig. 4). The results of cytosmear in the present investigation were quite similar to the findings of hepatocellular carcinoma in dogs[6]. The histopathological evaluation was found to show the presence of pleomorphic hyperchromic neoplastic cells with prominent changes in nuclear components (anisokaryosis, prominent nucleoli) with frequent mitotic figures in hepatocytes (Fig. 5). The gross and histopathological findings of the liver in rabbit in this study has a parallel correlation

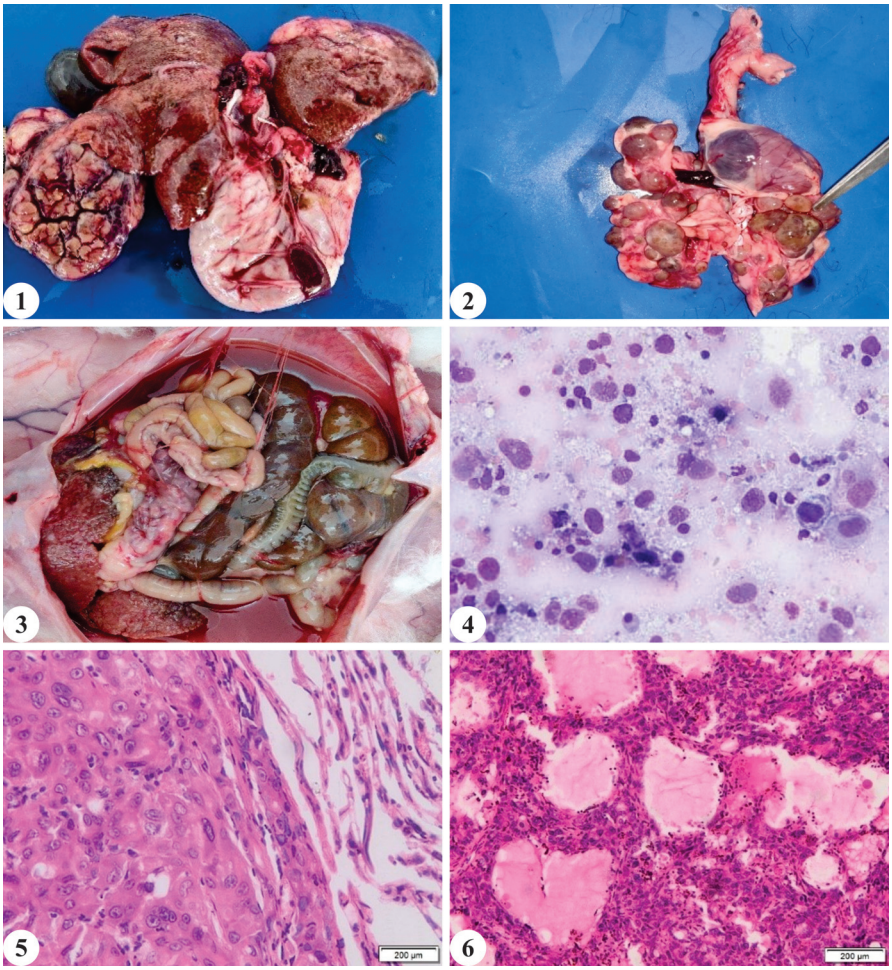

Fig. 1. Liver parenchyma showing the presence of multifocal, raised, variable-sized and creamish nodular lesions; **Fig. 2.** Lung surface showing the presence of nodular and raised dirty fluid-filled cystic structures; **Fig. 3.** Abdominal cavity filled with serosanguinous fluid along with fibrin threads; **Fig. 4.** Pleomorphic cells with increased nuclear-cytoplasmic ratio and prominent nucleoli (Giemsa stain, 400x); **Fig. 5.** Liver showing tumour cells with hyper chromatic nuclei, frequent mitotic figures and nuclear pleomorphism (H&E x200); **Fig. 6.** Lung tissue showed the air spaces filled with neoplastic cell populations of varying shape and size with eosinophilic fluid accumulation (H&E x100).

with the documentation of hepatic cell carcinoma in rabbits[7-9] and dog[10,11] done earlier. Microscopic examination of lung tissue has shown a neoplastic cell population of varying shape and size along with eosinophilic fluid accumulation in air spaces (Fig. 6), which were in accordance with earlier reports in rabbit[10,12] and dog[13-15].

The incidence of hepatic and pulmonary tumours in rabbits is rare or less reported. In this case report we have examined the death of a rabbit due to the occurrence of hepatocellular and bronchogenic carcinoma. This case shows the importance of through diagnostic investigations in cases of suspected rabbit diseases. A multidisciplinary approach, combining a clinical examination, advanced imaging methods and histological analysis can be used for an accurate diagnosis and for providing appropriate treatment. The most preferred treatment for many tumours in rabbits is surgical resection, however metastatic cancers frequently have a poor prognosis, thus failure to treat it by surgical excision in the early stages often results in the death of the animals.

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SUPERANNUATION

Dr B.N. Tripathi

Dr B.N. Tripathi, *FRC Path,* Vice-Chancellor, Sher-e-Kashmir University of Agricultural Sciences & Technology, Jammu born in UP on 20 July, 1962. He Graduated from Veterinary College, Mathura, CSA University of Agriculture & Technology, Kanpur (UP), (now DUVASU), 1984 and MVSc and PhD from Indian Veterinary Research Institute, Izatnagar (UP) in 1987 and 1990, respectively. He joined ICAR services on 9th October, 1990 as Scientist (ARS) to Principal Scientist at IVRI and ICAR-NRCE, Hisartill 2008. Further elevated as Head of Animal Health Division, Central Sheep & Wool Research Institute, Avikanagar from 2009-2012, Director, CCS-National Institute of Animal Health, Govt. of India, Ministry of Agriculture & Family Welfare, Department of Animal Husbandry, Dairying and Fisheries, Baghpat from 2012-2014. Director, ICAR-National Research Centre on Equines, August 2014 to January 2020 and Deputy Director General (Animal at IVRI Science), ICAR, New Delhi, 30th January 2020 to 19 June, 2023. During 1998-99, he got opportunity to serve as Postdoctoral Scientist, Institute of Animal Health, Compton (UK) and Visiting Scientist, Moredun Research Institute, Edinburgh, 2003-04; International Wellcome Trust Travel Fellowship, 2003-04. He has awarded International Travel Grants from Indian National Science Academy (INSA), 2005, Department of Science and Technology, Govt. of India 2005, 2012; Asian African Society of Mycobacteriology, 2018.

Dr Tripathi has vast professional experience of over 34 years (including 15 years of administration). He has published 200 original research papers in high impact journals, authored 5 books, prepared 4 manuals, contributed 5 technical bulletins, 3 policy papers, two National Action Plan (Disease control), 20 technologies (diagnostics & vaccines), 10 patents (granted/applied), 20 diagnostic and 4 vaccine technologies including Ancovax and Lumpi-ProVac vaccines, 9 patents, over 200 NCBI Accessions etc. He has guided 16 PG and PhD Students at IVRI Izatnagar.

Dr Tripathi is recipient of more than dozen prestigious awards viz; Sardar Patel Outstanding ICAR Institute Award, 2015 for ICAR-NRCE, Hisar. University Best Teacher Award, Drs Jain and Vegad outstanding pathologist award, 2006; International Wellcome Trust Fellowship (London) 2003, Best Research Paper Awards of IJVP 2003, 2005; Prof. Rama Rao Best PhD Thesis Award for three students, 2006, 2013; Dr Talpatra Oration Award 2023, Dr CM Singh Samman, Dr CM Singh Oration Lecture-2020, Dr NN Dastur Oration Award etc. He has visited large number of countries for academic, research and as Govt. of India representative. He acted as member of Indian delegation for OIE, WHO, SAARC, ASEHN, BIMSTEC meetings in India & abroad. He acted as National coordinator of Animal Genetic Resources in India and Vice-Chair (Asian Region) & Rapporteur for 12th Inter-Governmental Technical Working Group (12th ITWC), FAO, Rome. His distinguished contributions rewarded him with Royal College of Pathologists (FRC Path), London UK; National Academy of Agricultural Sciences (FNAAS), National Academy of Veterinary Sciences (FNAVS), National Academy of Dairy Sciences (NADSi), Indian Association of Veterinary Pathologists (FIAVP), Society for Immunology and Immunopathology (FSIIP), Indian Society for Veterinary Immunology & Biotechnology (FISVIB); FIAAVR, Diplomat & Charter member Indian College of Veterinary Pathologists (ICVP).

His professional excellence made him to be Chief Editor of Indian Journal of Animal Science, Chief Editor of Indian Journal of Veterinary Pathology and IAVP Newsletter, 2006-2011; Secretary General, IAVP 2013-2016, Secretary, ICVP 2012-14; Member Task Force DBT, 2017-18; Member of Board of Management (BOM), LUVAS, Hisar (2014-17), GADVASU (2015-18, 2019-22); SKUAST-Kashmir (March, 2021-continues); Member of International delegation from ICAR for various meetings of BRICS, BIMSTEC, SAARC, ASEAN, FAO, WHO and OIE and at present holding the post of President, Indian Association of Veterinary Pathologists (2019-2025). He has successfully discharged his professional duties and superannuated from the ICAR services on 31st July, 2024. The Indian Association of Veterinary Pathologists wish him a healthy, happy and peaceful family life.

SUPERANNUATION

Dr K.P. Singh

Dr K.P. Singh, Joint Director, CADRAD and Principal Scientist, ICAR-IVRI, Izatnagar, obtained his graduation degree in Veterinary Science from Veterinary College Mathura in 1984, Master and Doctorate degree in Veterinary Pathology from Deemed University, ICAR-IVRI, Izatnagar in 1987 and 1990 respectively. After PhD, joined Agricultural Research Services as Scientist on 9th October, 1990 at ICAR-IVRI Izatnagar. Became Scientist (Sr. Scale) in 1995, Senior Scientist in 1999 and Principal Scientist in 2007. Served as Head, Division of Pathology, during Jan, 2019 - Feb, 2021. Availed many foreign assignments on deputation like visiting Scientist at Institute for Animal Health, Compton, UK during 1996; Wellcome Trust Fellow at Institute for Animal Health, Pirbright, UK during 2002-2004; Expert Pathologist at Veterinary Research Centre, Muscat, Oman during 2008-2009. Beside these, presented research papers in International Conferences and visited countries like United Kingdom, Italy, France, Netherland, Muscut Oman. Extensively worked on immunopathology and molecular epidemiology of rabies and bluetongue. Submitted more than 150 gene sequences to International Gene Databank. Received and honoured by various awards like Young Scientist Award of Indian Association of Veterinary Pathologists (1993, 2007, 2016, 2019, 2022, 2023), Fellow-National Academy of Veterinary Sciences (FNAVS), Fellow-Indian Association of Veterinary Pathologists (FIAVP), Fellow-Society for Immunology and Immunopathology (FSIIP), Diplomate Indian College of Veterinary Pathologists (DipICVP). Published 278 research papers in various journals of national and international repute, presented more than 150 research papers in various conferences/symposium, delivered more than 100 invited lectures in various scientific forums/trainings. Having life membership of various scientific societies including IAVP and SIIP. Actively involved in research, diagnosis, postgraduate teaching and guided 10 MVSc and 13 PhD students. Teaching of Veterinary Pathology Courses for PG students from 1996-2023 and UG since 2016. Attended more than 125 disease outbreaks in the field with suitable advice. Contributed in various Institutional building/Professional activities viz: Hostel Warden (2012-2014), Chief Hostel Warden (2013-2021), Co-ordinator Human Hospital, (2020-2024), Convener, Broad Subject Matter Area (BSMA) for PG uniform syllabus of Veterinary Paraclinical Subjects (2018-2019), Convener, Revision of ICAR Agricultural Research Services (ARS) entrance examination syllabus (2022), Organizing Secretary-Veterinary Pathology Congress-2023 and National Symposium on "Advances in Veterinary Pathology for Diagnosis and Control of Emerging Diseases of Livestock and Poultry" at ICAR-IVRI, Izatnagar, Course Director - 20 days, ICAR sponsored Winter School entitled "Advances in livestock disease diagnosis using Clinico-pathological and molecular techniques" during October, 2019 at Division of Pathology, ICAR-IVRI, Izatnagar, Organizing Secretary-National Congress on Wildlife Health and Annual Convention of Association of India Zoo and Wildlife Veterinarians (AIZWV) during January, 2017 at ICAR-IVRI, Izatnagar, Vice President, Indian Association of Veterinary Pathologists - 2022-2025, Chief Editor (2012-2014), Managing Editor (2014-2017), Editor (2010-11) of Indian Journal of Veterinary Pathology, Secretary General, Indian Association of Veterinary Pathologists (IAVP) - 2017-2022, General Secretary, Association of Indian Zoo and Wildlife Veterinarians (AIZW) 2008-2017, Treasurer, Indian Association of Veterinary Pathologists (IAVP) during 2006-2008. Handled 28 research projects as PI and Co-PI, the important one were Indo-UK, DBT-BBSRC project on "Development of Diagnostic Systems, Reference Collections and Molecular Epidemiology Studies for Important Arboviral Pathogens of Livestock in India" and ICAR-All India Network Programme on Bluetongue (AINP-BT). ICAR-All India Network Project on "Challenging and Emerging Diseases of Animals, All India Network Programme on "One Health approach to Zoonotic Diseases", NCDC - "National One Health Program for Prevention and Control of Zoonoses (NOHPPCZ)" DBT - "Establishment of the consortium for One Health to address Zoonotic and Transboundary Diseases in India, including the Northeast region". Indian Association of Veterinary Pathologists (IAVP) wishes Dr KP Singh, a very happy, healthy and peaceful post retirement life ahead.

SUPERANNUATION

Dr Syed S.Y.H. Quadri

Dr Syed Quadri born on 13.7.1962 at Hyderabad and did his graduation and post graduation between 1979-1987 from APAU Hyderabad and further acquired FELASA "C" in 2002 and DICVP in 2008. He started his career with the poultry industry working as a Veterinary Pathologist with M/s Venkateshwara Group at their Poultry Diagnostic and Research Center, Pune and later joined the National Institute of Nutrition, Indian Council of Medical Research at the Laboratory Animal Science Center in the division of Pathology. He is involved in work related to designing of animal facilities, running the laboratory animal breeding and health monitoring program. We bred laboratory animals such as mice, rats, guinea pigs, rabbits, dogs, sheep, monkeys of several strains for research purposes and also helped in designing and set up and further obtaining AAALAC accreditation in more than a dozen CRO's with animal facilities. He was a part of the team to develop the National Animal Resource Facility for Biomedical Research (NARFBR) ICMR at Turkapally, Hyderabad. He has 36 years of experience in animal breeding, supply, health monitoring, animal experimentation, animal welfare and ethics. Established and set up facilities to breed transgenic animals such as db/db mice, ApoE, RR rats, Fabry mice. The Institute has been supplying laboratory animals and feed to over 350 research institutions both in government and private. During the Covid-19 pandemic we had the privilege to supply over 2500 Golden Syrian Hamsters to various organizations such as Bharat Biotech, Hyderabad; THISTI, Faridabad; NIV, Pune and Indian Immunologicals, Hyderabad for Corona Vaccine testing. He has privileged to serve in more than two dozen institutions as nominee of the CPCSEA in implementing the animal welfare rules. As Veterinary Pathologist and Toxicologist was Coordinator of the Advanced Centre for Preclinical Toxicology at ICMR-NIN, contributed in safety and efficacy data generation for submission to the Drug Controller General of India. Products tested were Japanese Encephalitis Vaccine, Human Papilloma virus vaccine, recombinant DNA antirabies vaccine, genetically modified cotton, tomato, Brinjal, okra, several drugs, ayurvedic products and involved in more than 100 studies generating more than 20 crores for the Institute.

Dr Quadri is credited with number of awards from university and also from different national and international professional societies and agencies. He acted as visiting scientist for National Jewish Hospitals, Denver, USA and Okhlahoma Medical Research Foundation, Okhlahoma, USA in 2012 and 2013 respectively. He has attended number of workshops, trainings, symposiums, conferences and presented papers both nationally and internationally. He has vast teaching experience for the past three decades at various institutes viz TANUVAS, Chennai, ICMR-NIN, Hyderabad, MANAGE, National Institute of Agricultural Extension Management, Ministry of Agriculture and Farmers Welfare, GoI., nominees of AWBI and CPCSEA. He is also reviewer of external project reports of various private and government laboratories like BIOCON Limited, Bangalore, Metahelix Life Sciences Private Limited, Bangalore, Dr Reddy's Laboratories Ltd. Hyderabad and IIT, Pune. He has published more than 45 research papers in both national and international journals of repute and contributed a half a dozen of book chapters. He has vast experience in writing technical reports.

Dr Quadri is a member of several national committees viz FSSAI, Bureau of Indian Standards, Export Promotion Council, BIRAC, Dept of Biotechnology and internationally, member of Asian Federation of Lab Animal Sciences, Strategic Planning Committee of the AAALAC, Bethesda, an International accreditation body, Member of guidelines committee at FASEB, USA.

Dr G.V. Sudhakar Rao, Professor & Head, Madras Veterinary College, TANUVAS, Chennai and **Dr Sarojini Tamuli**, Professor & Head, Assam Veterinary College, AAU, Guwahati have successfully discharged their professional duties and superannuated. The Indian Association of Veterinary Pathologists wish them a healthy, happy and peaceful family life.