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RESEARCH ARTICLE
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Shortening of dioestrus in female dogs using cloprostenol sodium at different stages of dioestrus#

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Abstract

A study was conducted in 18 healthy, fertile, non-pregnant, dioestrous female dogs to evaluate the efficacy of $PGF_{2\alpha}$ analogue (cloprostenol sodium) in shortening the dioestrus. Bitches that were diagnosed non-pregnant by trans-abdominal ultrasonography, on day 30 of breeding and their dioestrual stage confirmed by serum progesterone assay, were randomly allotted to three groups of six bitches each. Group I and II bitches were treated with cloprostenol sodium @ 2.5 µg/kg b. wt., subcutaneously, thrice at 48 h interval, beginning on day 30 and 45 of last breeding, respectively. Group III animals were assigned as control and were left untreated. Fifty days from first injection of cloprostenol, serum progesterone assay was carried out among the animals in Group I and II whereas the same was done among Group III bitches on day 90 after last breeding. Among Group I and II, mean serum progesterone concentrations were 0.93 ± 0.04 and 0.87 ± 0.01ng/mL, respectively whereas the level was 1.72 ± 0.9 ng/mL among control group; highly significant difference ($p<0.01$) existed between control and treatment groups. The present study indicated that administration of $PGF_{2\alpha}$ analogue during dioestrus enhances the pace of luteal regression among non-pregnant bitches.

Keywords: *Dioestrus, $PGF_{2\alpha}$, luteolysis, non-pregnant, bitches*

Running title: Shortening of dioestrus in female dogs using cloprostenol sodium

#Part of MVSc thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

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Canines are non-seasonal, monoestrus, polytocous and spontaneous ovulators (Concannon, 2011). Their prolonged dioestrus and obligatory anoestrus extend the inter-oestrous interval (IEI) up-to 5- 12 months, eventually lowering the profitability of canine breeding industry, due to lesser frequency of puppy harvest. Further, prolonged progestational phase predisposes the bitches to severe systemic diseases like cystic endometrial hyperplasia-pyometra complex (CEH-P) and other less severe conditions like pseudo-pregnancy (Johnston *et al.,* 2001). The present study focused on the efficiency of synthetic $PGF_{2\alpha}$ analogue, cloprostenol sodium, when administered at mid- dioestrus and 15 days later, to shorten the period of prolonged dioestrus in non-pregnant bitches, so as to reduce the IEI as well as risk of pathological conditions.

Materials and methods

Eighteen apparently healthy and fertile bitches, aged 2-8 years and diagnosed non pregnant by trans-abdominal ultrasonography on day 30 from last breeding, were selected for the study. The health status of all the bitches was validated by haematological analysis, peripheral blood smear and wet film examination as well as microscopical examination of faecal sample. Serum progesterone assay was done on the same day, to confirm dioestrus, by chemiluminiscense immune-assay (CLIA) as per manufactures instructions (Roche, cobas e 411 analyzer, USA) and expressed as ng/mL. The selected dogs were randomly allotted into three groups and treated as presented in Table 1.

Progesterone assay was repeated 50 days from first injection of cloprostenol among the animals in Group I and II whereas the same was done on day 90 after last breeding, among Group III bitches. The data obtained were tabulated and analysed statistically (Snedecor and Cochran, 1994) using one-way ANOVA and SPSS version 21.

Results and discussion

Serum progesterone assay during mid-dioestrus, on the day of selection

Mean serum progesterone concentration on day 30 from last breeding was 25.52 ± 2.14, 23.64 ± 2.41 and 22.47 ± 2.46 ng/mL, respectively in group I, II and III. The values did not differ significantly ($p>0.05$) between groups.

Similar values of dioestrual serum progesterone concentration of 15-80 ng/mL were reported by Concannon (2011). Ucar *et al.* (2018) recorded serum progesterone concentration of 32.62 ± 2.42 ng/ml during mid dioestrus. Dioestrus has been referred to as the period of highly active *corpora lutea* (CL) which secretes large amounts of progesterone (Noakes *et al.,* 2019). Observations in the present study indicate the existence of functional CL in the animals under study.

Serum progesterone assay after cloprostenol administration

Mean serum progesterone concentration after 50 days of cloprostenol administration among the bitches of treatment groups and 90 days after last breeding (late dioestrus) in control animals are presented in Table 2.

Table 1. Treatment protocol using cloprostenol sodium in shortening of dioestrus in bitches

Group (n=6)	Day of treatment	Treatment protocol
I	30th day since last breeding date (Day 0)	Cloprostenol sodium* @ 2.5µg/kg b.wt., s/c, three injections at 48 h interval
II	45th day since last breeding date (Day 0)	cloprostenol sodium (Inj. Clostenol – 2mL) @ 2.5µg/kg b.wt., s/c, three injections at 48 h interval
III	Control	Animals were left untreated

*Inj. Clostenol – 2 mL (cloprostenol sodium 250µg/mL): Zydus Animal Heath, Cadila Healthcare Limited, Ahmedabad

Table 2. Serum progesterone profile of the bitches under study after prostaglandin therapy /90th day of last breeding among control animals

Animal no.	Progesterone values (ng/mL)		
	Group I (day 80)	Group II (day 95)	Group III (day 90)
1	0.94	0.82	2.02
2	0.92	0.93	1.78
3	0.9	0.88	1.73
4	0.79	0.86	1.86
5	1.07	0.86	1.54
6	0.86	0.89	1.41
Mean ± SE	0.93 ± 0.04^{b}	0.87 ± 0.01^{b}	1.72 ± 0.9^{a}

Means with different superscripts differ significantly between columns

Mean serum progesterone concentrations of 0.93 ± 0.04 and 0.87 ± 0.01 ng/mL in treatment group I and II, respectively indicates that the bitches have entered in to anoestrus. Okkens and Kooistra (2006) and Kustritz (2012) reported that the bitches were considered to be in anoestrous phase when the serum progesterone concentration reached ≤1 ng/mL. Control animals maintained significantly higher serum progesterone (1.72 ± 0.9ng/mL) than the treated animals, indicating that although serum progesterone concentration has drastically reduced from those recorded on day 30 of cycle (22.47 ± 2.46ng/mL), they had not entered into anoestrum phase. The dioestrous phase in non-pregnant animals may be extended up-to 55 to 100 days and could be associated with varying degree of decline in plasma progesterone levels (Groppetti *et al.,* 2010).

Observation in the present study signifies the efficacy of prostaglandin in inducing luteolysis. Prostaglandins are proven to be more efficient during mid to later stages of dioestrus (Wichtel *et al.,* 1990). Kowalewski *et al.* (2009) reported that an increase in expression of $PGF_{2\alpha}$ receptors during early to late dioestrus enables exogenous $PGF_{2\alpha}$ to act on the CL for induction of luteolysis. Previous study (Ucar *et al*., 2018) reported that multiple low doses of prostaglandins are more effective in inducing luteolysis in bitches. Drop in serum progesterone to basal levels among the treated bitches in the present study corroborates with the findings of Romagnoli (2017) who reported that administration of cloprostenol @ 2.5 µg/kg b wt. subcutaneously, three times at 48 h intervals was 100 per cent effective in inducing luteolysis when used after day 30 of dioestrus.

Slow dip in serum progesterone concentration noticed among untreated bitches in the present study is in accordance with Papa and Kowalewski (2020) who reported that gradual decline in serum progesterone levels occurs from day 35 post-ovulation onwards in non-pregnant animals.

Conclusion

The results of the present study indicate that administration of cloprostenol has shortened diestrum phase and animals have entered into anoestrum at a faster pace than the normal progression to anoestrum in untreated bitches.

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Conflict of interest

The authors declare that they have no conflict of interest.

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RESEARCH ARTICLE
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Occurrence of *Campylobacter* spp. in duck and associated environmental samples in Thrissur district#

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Abstract

Campylobacteriosis caused by Campylobacter spp. is considered as the most common cause of bacterial diarrhoea in humans across the globe. The current research was undertaken to assess the occurrence of Campylobacter spp. in duck and the associated environmental samples. Among 220 samples analysed, 7.73 per cent samples revealed the presence of Campylobacter spp. Majority of the samples contained C. coli (4.55 per cent) and C. jejuni was detected in 3.18 per cent of the samples. The present study revealed a high occurrence of Campylobacter spp. in duck rearing facilities in Thrissur district, Kerala. As the demand for duck products is increasing every year, the risk of contamination by Campylobacter spp. has to be viewed seriously. The study revealed the importance of multifaceted one health approach including human medicine, veterinary medicine, epidemiology, environmental hygiene, public health institutes and epidemiological surveillance agencies to control food-borne diseases and up-gradation of biosecurity measures.

Keywords: *Campylobacter spp. one health*

Running Title: Occurrence of *Campylobacter* spp. in duck of Thrissur district

Campylobacter spp. is considered as an important cause for foodborne gastroenteritis in humans worldwide which is mostly associated with *C. jejuni* and *C. coli* (Wesley *et al*., 2000). Sporadic outbreaks often occur by consumption of raw or undercooked poultry products. In humans, the disease caused by *Campylobacter* spp. is normally mild and self-limiting, but severe systemic

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infection can result in chronic sequelae such as Guillain-Barre syndrome (GBS), irritable bowel syndrome, Miller Fisher syndrome and reactive arthritis. The natural reservoirs of thermophilic campylobacters are free-living birds and commercial poultry. Despite duck being an important reservoir for *Campylobacter* spp. and a major part of modern Asian diet, information regarding the occurrence of the organism in duck is very limited. Therefore, the current investigation was designed with an objective to determine the occurrence of the *Campylobacter* spp. in duck rearing facilities.

Materials and methods

The current research was conducted to assess the occurrence of *C. coli* and *C. jejuni* in duck and environmental samples associated with duck rearing along with molecular confirmation of the positive isolates. The investigation was conducted for a period of 10 months from October 2019 to July 2020. A total of 220 samples from three different duck farms in and around Thrissur district were analysed during the study period. Samples consisted of duck cloacal swabs, soil and drinking water from duck farms. Details of samples are given in Table 1.

Isolation and identification of *Campylobacter* spp. from collected samples were carried out by selective enrichment followed by selective plating as recommended by Stern *et al*. (2001) and OIE Terrestrial Manual (2017) with necessary modifications. Cloacal swab was streaked onto modified Charcoal Cefoperazone Deoxycholate Agar plates supplemented with Polymyxin B (P-mCCDA). The plates were then incubated at 42°C for 48 h under microaerophilic conditions. Soil and drinking water samples were subjected to enrichment in mCCD (modified Charcoal Cefoperazone Deoxycholate) broth supplemented with CCDA selective supplement (FD 135). Incubation was done under microaerophilic conditions at 42°C for 48 h followed by selective plating onto mCCDA supplemented with CAT selective supplement (FD 145), Campylobacter supplement V (FD 067) and Polymyxin B selective supplement (FD 003). It was then incubated under microaerophilic conditions at 42°C for 48 h. Greyish, flat, spreading type, shiny, mucoid and moistened colonies with tendency to spread and with or without metallic sheen were considered as *Campylobacter* spp. (Fig. 1).

The confirmation of *Campylobacter* spp. was carried out as per the procedure described by El-Adawy *et al.* (2012) with slight modifications by subjecting all the positive Campylobacter isolates to multiplex polymerase chain reaction. The positive isolates were further analysed for the presence of *16S rRNA* gene specific for *Campylobacter* genus and virulence gene *cad*F. *C. coli* and *C. jejuni* were identified by the detection of *ceu*E gene specific for *C. coli* and *map*A gene specific for *C. jejuni*. The target genes that were detected by mPCR and the primer sequences used in the study are shown in Table 2. A master mix was prepared for each *Campylobacter* spp. before setting up of the PCR reaction by combining the reagents as depicted in Table 3. Cycling conditions for mPCR are depicted in Table 4. The PCR products were stained with SYBR safe dye and detected by submarine gel electrophoresis.

Results and discussion

Cloacal swabs of duck

Per cent occurrence of *Campylobacter* spp. is given in Table 5. The presence of *Campylobacter* spp. in DF2 (22.5 per cent) was significantly higher ($p<0.05$) compared to DF3

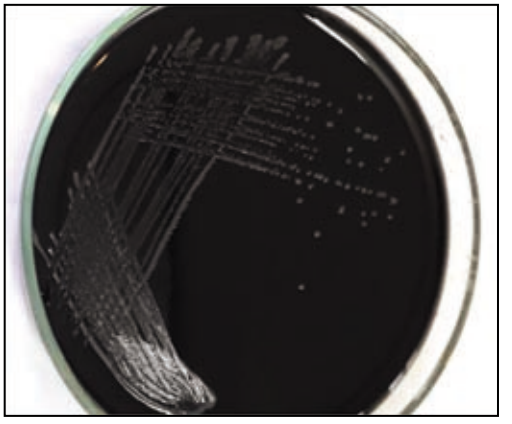

Fig. 1. Greyish, round, spreading type, shiny, moistened colonies of *Campylobacter* spp. on mCCD agar

Table 1. Details of sample collection

Sl. No.	Duck farm	Cloacal swabs	Soil	Water
1	DF1	40	15	15
2	DF2	40	15	15
3	DF3	40	20	20
Total		**120**	**50**	**50**

Table 2. Primers used for the identification of *Campylobacter* spp.

Primer	Primer sequence	Size (bp)	Reference
*16S r*RNA F	5'-GGATGACACTTTTCGGAGC-3'	816	Linton *et al*. (1996)
*16S r*RNA R	5'-CATTGTAGCACGTGTGTC-3'		
*cad*F F	5'-TTGAAGGTAATTTAGATATG-3'	400	Rozynek *et al.* (2005)
*cad*F R	5'-CTAATACCTAAAGTTGAAAC-3'		
*map*A F	5'-CTATTTTATTTTTGAGTGCTTGTG-3'	589	Denis *et al.* (1999)
*map*A R	5'-GCTTTATTTGCCATTTGTTTTATTA-3'		
*ceu*E F	5'-AATTGAAAATTGCTCCAACTATG-3'	462	Denis *et al.* (1999)
*ceu*E R	5'-TGATTTTATTATTTGTAGCAGCG-3'		

Table 3. Components of multiplex PCR mixture

Sl. No.	Name of the reagent	Stock Concentration	Quantity (µL)
1	Template DNA	50 ng/µL	5.00
2	10X PCR buffer	200 m*M*	3.00
3	$MgCl_2$	25 m*M*	2.00
4	*Taq* DNA polymerase	5 Units/µL	0.75
5	dNTP Mix	2 m*M* each	2.50
6	Forward primer of *16S r*RNA gene	10 pmoles/µL	1.00
7	Reverse primer of *16S r*RNA gene	10 pmoles/µL	1.00
8	Forward primer of *cad*F gene	20 pmoles/µL	1.00
9	Reverse primer of *cad*F gene	20 pmoles/µL	1.00
10	Forward primer of *map*A gene	10 pmoles/µL	1.00
11	Reverse primer of *map*A gene	10 pmoles/µL	1.00
12	Forward primer of *ceu*E gene	10 pmoles/µL	1.00
13	Reverse primer of *ceu*E gene	10 pmoles/µL	1.00
14	Nuclease free water		8.75
Total			**30.00**

(2.5 per cent). In the present study, 10 per cent of the total 120 cloacal swabs from ducks were positive for the organism which was in perfect tune with the results of Nor Faiza *et al*. (2013), where the authors reported an occurrence of the organism in 12 per cent cloacal swab samples of duck collected from Malaysia. However, Wei *et al*. (2014) in South Korea isolated *Campylobacter* spp. from 96.6 per cent of duck cloacal swabs. The farm with highest occurrence in the current study had muddy and wet floor compared to the other two farms with rather clean and dry environment.

Soil samples

In the present study, two (13.33 per cent) soil samples each from DF1 and DF2 carried the organism while none of the samples from DF3 harbored the same. There was no significant difference among the three duck farms regarding the occurrence of *Campylobacter* spp. in soil. The lower occurrence in the current research can be correlated with zero prevalence of the organism in soil samples reported by Adzitey *et al*. (2012) which could possibly be due to the poor

Table 4. Cycling conditions used for PCR of *16S rRNA*, *cad*F, *map*A and *ceu*E genes

Sl. No.	Steps	Temperature	Time (min)	No. of Cycles
1	Initial denaturation	95.0°C	10	1
2	Denaturation	94.0°C	1	30
3	Annealing	51.8°C	1	
4	Extension	72.0°C	1	
5	Final extension	72.0°C	10	1
6	Hold	4.0°C	10	

Table 5. Occurrence of *Campylobacter* spp. in duck rearing facilities

Sl. No.	Duck farm	Positive samples							
		Cloacal swab		Soil		Water		Total	
		No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
1	DF1	2	5.00[a, b]	2	13.33[a]	1	6.67[a]	5	7.14[a, b]
2	DF2	9	22.50[b]	2	13.33[a]	0	0.00[a]	11	15.71[b]
3	DF3	1	2.50[a]	0	0.00[a]	0	0.00[a]	1	1.25[a]
Total		**12**	**10.00**	**4**	**8.00**	**1**	**2.00**	**17**	**7.73**

Figures bearing same superscripts do not differ significantly ($p<0.05$)

Table 6. Distribution of genes in *Campylobacter* spp. isolates of duck rearing facilities

Sl. No.	Farm	No of isolates tested	Distribution of genes in the positive isolates (No.)			
			16S rRNA	***ceu*E**	***map*A**	***cad*F**
1	DF1	5	5	2	3	3
2	DF2	11	11	8	3	3
3	DF3	1	1	0	1	1
Total		**17**	**17**	**10**	**7**	**7**

survivability of the organism in soil. The results of the present study are contrary to that of Jensen *et al*. (2006) in Denmark, where the authors attributed the paddock contamination with *Campylobacter* spp. to the higher prevalence (35 per cent) in environmental samples. The occurrence recorded in the current study could be due to spill-over infections to soil from poultry reservoirs.

Water

Only one drinking water sample (collected from DF1) from duck rearing facilities was positive for the organism. There was no significant difference among the three duck farms regarding the occurrence of *Campylobacter* spp. in water. The results can be correlated with that of Adzitey *et al*. (2012), where none of the drinking water samples collected from duck farm harbored the organism. On the other hand, Van-Dyke *et al*. (2010) and Aung *et al*. (2015) recorded higher prevalence of *Campylobacter* spp. in water samples collected from Canada and Malaysia, respectively. The low recovery rate of the organism in the present research might be either due to the absence of the organism in water or the poor survivability of the organism in feed, soil, water and other surfaces exposed to sunlight, high oxygen tension and dry environment.

Overall occurrence in duck rearing facilities

Comparing the three duck farms under study, the occurrence of *Campylobacter* spp. in DF2 (15.71 per cent) was significantly higher ($p<0.05$) than DF3 (1.25 per cent). In farm DF1, 7.14 per cent samples were positive for the organism. The overall occurrence of *Campylobacter* spp. in duck rearing facilities was 7.73 per cent (Table 5). Kafshdouzan *et al*. (2019) reported an occurrence of the organism in 17.33 per cent of duck samples collected from Iran. An incidence of 73 per cent of *C. jejuni* was reported by Pacha *et al*. (1988) from migratory ducks in Pacific North American flyway. The low

recovery of the organism from majority of the samples in the current study could be possibly due to its poor survivability in the environment.

Molecular confirmation of the isolates from duck rearing facilities by mPCR

All the 17 isolates obtained from duck rearing facilities were subjected to mPCR for the simultaneous detection of *Campylobacter* genus specific *16S rRNA* gene, a conserved virulence *cad*F gene, *C. coli* specific *ceu*E gene and *C. jejuni* specific *map*A gene. The result of mPCR is depicted in Table 6.

Among the total 220 samples collected from duck rearing facilities, majority of the samples harbored *C. coli* (4.55 per cent) followed by *C. jejuni* (3.18 per cent). Nor-Faiza *et al*. (2013) also reported a higher occurrence of *C. coli* (88 per cent of total isolates) in duck samples collected from Malaysia compared to *C. jejuni*. On the other hand, according to Wei *et al*. (2014) and Jamali *et al*. (2015), *C. jejuni* was the predominant isolate from duck samples, accounting for more than 80 per cent of the positive isolates. The relative proportion of Campylobacter colonisation in gastrointestinal tract varies with different geographical area and type of host. This could be the reason for differences observed by several authors regarding occurrence of the two *Campylobacter* species.

Conclusion

All the three duck farms followed semi-intensive system of duck rearing, thus enhancing the chances of horizontal transmission of organism from the environment. Physical barriers that are capable of restricting the access to duck houses and external environment around the farms should be implemented to prevent the introduction of *Campylobacter* spp. via farmers, animals and visitors. Along with that, stringent biosecurity measures are required to prevent the contamination and propagation of organism among different flocks.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Evaluation of therapeutic efficacy of oxytetracycline against caprine respiratory mycoplasmosis using clinical score card method[#]

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Abstract

Mycoplasmosis in goats is one of the challenging and continuous threats to small ruminant farming causing huge economic losses. This study was carried out to evaluate the therapeutic efficacy of oxytetracycline against caprine respiratory mycoplasmosis. Nasal swabs collected from fourteen goats showing clinical signs like cough, nasal discharge and abnormal breath sounds were screened for the presence of *Mycoplasma* spp. by polymerase chain reaction. The severity of the disease as well as the clinical improvement was recorded using a clinical score card. Oxytetracycline was administered intravenously at 15 mg/kg/day for 5 days along with supportive medications. Significant reduction in clinical score was observed after treatment and complete recovery was attained in 62.5 per cent animals.

Keywords: *Mycoplasma, Oxytetracycline, Goat*

Running title: Therapeutic efficacy of oxytetracycline against caprine respiratory mycoplasmosis

Mycoplasma spp. are small, cell-wall less bacteria and they have been implicated to cause various diseases in goats, especially respiratory diseases (Razin, 1992). The practise of rearing goats in groups, their tendency to huddle and stressors like adverse weather conditions predispose them to respiratory mycoplasmosis. The mortality and morbidity associated with

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pneumonia due to *Mycoplasma* spp. have caused huge economic loss to goat-keepers all around the world. The fastidious nature of the organism makes it difficult for *in vitro* isolation and cultivation. For rapid diagnosis of mycoplasmosis, molecular techniques like Polymerase Chain Reaction (PCR) can be used. Treatment of mycoplasmosis mainly involves macrolide antibiotics or tetracyclines.

Materials and methods

Fourteen goats presented to Teaching Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Pookode, with clinical signs like cough, nasal discharge and abnormal breath sounds were selected for the study. All the animals in the study group were subjected to physical and clinical examination after collecting detailed signalment and anamnesis. The assessment of severity of clinical condition associated with caprine respiratory mycoplasmosis was performed using a clinical score card prepared by Love *et al.* (2014) and modified by Gupta (2015). Score card was prepared in such a way that scores from 0 to 3 were assigned to the progressive variants of clinical signs like cough, nasal discharge, respiratory distress and ocular discharge. The scores obtained for each sign were added up to get the final score for a particular animal. The clinical assessment of the final score was carried out as per the interpretations attached which varies from healthy to severe illness (Table 1).

Nasal swabs were collected and kept in 0.5 mL sterile normal saline for DNA extraction. PCR was performed using primers specific for 16S rRNA of *Mycoplasma* spp. (Botes *et al*., 2015). Goats positive for *Mycoplasma* spp. by PCR were treated using intravenous injection of Oxytetracycline @ 15 mg/kg/day (mixed in equal quantity of Normal Saline). Supportive therapy included Flunixine meglumine @ 1.1 mg/kg/day intramuscularly, Chlorpheniramine maleate @ 0.5 mg/kg/day intramuscularly and inhalation with Tr. Benzoin @ 10 mL/L of hot water twice daily. All the treatments were continued for five days. Comparison of clinical scores before and after treatment was done using Wilcoxon Signed Rank Test (Rangaswamy, 1995).

Results and discussion

In the present study, *Mycoplasma* spp. was detected in 71.4 per cent (10 out of 14) of goat nasal swabs tested by PCR. Among the goats with respiratory mycoplasmosis five (50 per cent) had mild illness, three (30 per cent) had moderate illness and two (20 per cent) had severe illness. On the completion of treatment, complete recovery was observed in seven out of ten goats (70 per cent) animals. Of the three animals that did not recover fully, two had mild illness with slight respiratory distress, small amount of watery nasal discharge and single cough when induced and the other had small amount of watery nasal discharge and single cough when induced. On statistical analysis, significant reduction in

Table 1. Clinical score card for assessment of severity of clinical condition associated with caprine respiratory mycoplasmosis.

Clinical condition	Score			
	0	**1**	**2**	**3**
Cough	None	Single induced	Multiple induced	Multiple spontaneous
Nasal discharge	None	Small amount of unilateral/bilateral watery discharge	Unilateral/bilateral cloudy or excessive mucus discharge	Copious bilateral mucopurulent discharge
Respiratory distress	None	Mild	Moderate	Severe
Ocular discharge	None	Small amount	Moderate	Heavy
Interpretations *0 = Healthy, 1-4 = Mild illness, 5-8 = Moderate illness, 9-12 = Severe illness*				

Table 2. Comparison of clinical scores before and after treatment

Sl. No.	Clinical score before treatment	Severity of disease	Clinical score after treatment	Z value (P-value)
1	2	Mild	0	-2.803 (0.0051**)
2	2	Mild	0	
3	3	Mild	0	
4	5	Moderate	0	
5	7	Moderate	0	
6	4	Mild	0	
7	3	Mild	0	
8	9	Severe	3	
9	9	Severe	3	
10	8	Moderate	2	

*** Significant at 0.01 level*

clinical scores were observed after the therapy (Table 2). Complete recovery was observed in 70 per cent goats. Hence, oxytetracycline can be used as the effective treatment regimen for Caprine respiratory mycoplasmosis. Tetracyclines generally act as bacteriostatic antibiotics and inhibit protein synthesis by reversibly binding to 30S ribosomal subunits of susceptible organisms, preventing binding to those ribosomes of aminoacyl transfer-RNA (Plumb, 2018). Recent studies have shown that oxytetracycline have additional mechanisms of action including antioxidant, anti-inflammatory and immunosuppressive activity (Olszewska, 2006). Moreover, the broad spectrum activity of oxytetracycline must have effectively neutralised any concurrent secondary bacterial infection associated with mycoplasmosis. Giadinis *et al.* (2008) reported that oxytetracycline was highly effective in reducing mortality and morbidity associated with caprine respiratory mycoplasmosis. This finding is comparable to the results of the present study. Use of anti-inflammatory, anti-pyretic, analgesic and anti-allergic drugs along with anti-microbials helps in reducing severity of mycoplasmosis and facilitates early recovery (Yatoo *et al*., 2018).

Conclusion

Based on the results of the study, it can be concluded that oxytetracycline can be used to effectively treat caprine respiratory mycoplasmosis. Since recent studies have shown that oxytetracycline has additional mechanisms of action including antioxidant, anti-inflammatory and immunosuppressive activity, they can be used in reducing severity of the disease.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Factors affecting gestation length in small sized breeds of dogs#

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Abstract

A large variation in the gestation length can be expected when the gestation is calculated from the day of mating to birth. The present study was designed to assess the influence of different factors like litter size, breed, parity and age on gestation length in bitches. A total of 89 small sized bitches of different breeds having different age, parity and body weight, were selected for the study. The mean age, body weight and parity of the animals included in the present study were 3.00 ± 0.15 years (1 to 8 years), 9.9 ± 0.25 Kg (6 to 15kg) and 1.14 ± 0.10 (0-4th parity) respectively. The mean gestation lengths observed in different small sized breeds were 60.68 ± 0.55, 59.82 ± 0.54, 60.78 ± 1.74, 58.83 ± 1.35 and 58.17 ± 0.74 in the Pug, Beagle, Dachshund, French Bulldog and the Spitz, respectively. The mean litter size in the study was 4.34 ± 0.13 with a range of 2-8. The mean gestational length in animals having ≤ 3 litter size was 60.83 ± 0.64 days and 59.90 ± 0.43 days in animals having >3 litter size. Age limit of the animals ranged from 1 to 8 years with a mean of 3 ± 0.15 years. The mean gestation length in bitches aged ≤3 years was 60.21 ± 0.23 days (n=63) and this was 60.15 ± 0.3 (n=26) days in bitches in the >3 years group. Mean gestational length observed in nulliparous bitches was 60.86 ± 0.4 days (n=29), while in multiparous bitches it was 59.90 ± 0.5 days (n=60). From the present investigation it could be concluded that no significant difference exists in gestational length between different breeds, litter size, age group and parity.

Keywords: *Gestation length, breed, age, parity, litter size*

Running title: Factors affecting gestation length in small dogs

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Canine gestation length is highly variable when gestation is calculated as the interval from the day of breeding to the day of whelping. Naaktgeboren, 1987 and Concannon *et al*., 1983 reported that gestational length is expressed as the interval from day of mating or from a single mating to the day of whelping. They also reported that gestation length could vary from 57–72 days (14 days), when calculated from the first day of multiple breeding. Gestational length of canines when calculated from mating to parturition varies from 54-77 days (24 days) in various breeds of dogs. This wide variation in gestation length is observed due to the long period of receptivity, long viability of spermatozoa, variations in onset of oestrus and ovulation and litter size (Okkens *et al*., 2001; Kutzler *et al*., 2003). Some authors have reported that litter size, age of the dam, parity and breed have no influence on gestation length, whereas others reported a longer gestation length in smaller litter sizes of either less than 7 or less than 3 puppies (Beccaglia and Luvoni, 2006; Okkens *et al*., 2001). Compared to Rottweilers (65.6 ± 1.6 days), German Shepherds (63.2 ± 1.8 days) had shorter gestation (Mir *et al*., 2011). Perusal of literature revealed conflicting reports about the0 influence of different factors on gestation length. In view of above observations, the present study was designed to assess effect of different factors like litter size, age, parity and breed on gestation length

Materials and methods

A total of 89 small sized bitches of different breeds (Pug, Pomeranian, Spitz, Dachshund, French Bulldog and Beagle) of different ages, parity and body weight, presented to University Veterinary Hospitals, Kokkalai and Mannuthy during the period January 2018 to December 2020 were selected for the study. History regarding age, parity and body weight were recorded. The optimum time for mating was advised based on exfoliative vaginal cytology. Mean age, body weight and parity of the animals included in the present study were 3.00 ± 0.15 years (1 to 8 years), 9.9 ± 0.25 Kg (6 to 15kg) and 1.14 ± 0.10 (0-4th parity) respectively. Pregnancy diagnosis was carried out by day 30 by trans-abdominal ultrasonography and information regarding the day of whelping and litter size were collected. The length of gestation was defined as the interval between the last day of breeding and the day of whelping. The relationship between different factors like litter size, age, parity and breed on gestation length were analysed by Spearman rank correlation coefficient test using SPSS version 24.

Results and discussion

The mean gestation length observed in different small sized breeds were 60.68 ± 0.55, 59.82 ± 0.54, 60.78 ± 1.74, 58.83 ± 1.35 and 58.17 ± 0.74 in the Pug, Beagle, Dachshund, French Bulldog and the Spitz, respectively (Table 1). Even though there was a difference of two days in gestation length for the French Bulldog (58.83 ± 1.35) and the Spitz (58.17 ± 0.74), when compared to the Pug (60.68 ± 0.55) and the Dachshund (60.78 ± 1.74), the difference was non-significant ($p \geq 0.05$). The overall mean gestation length observed in the study was 60.21 ± 0.36 days with a range of 53-71 days and similar findings were observed in the study of Holst and Phemister (1974), Concannon *et al.* (1983) and Okkens (1993). These authors highlighted the inaccuracy of prediction of parturition based on breeding date which is highly variable. The wide variation in the length of gestation may be due to long survivability of canine spermatozoa up to 5-7 days in the female reproductive tract, the long period of receptivity, litter size, variation in the onset of oestrus and ovulation (Concannon *et al*., 1993).

The mean litter size in the study was 4.34 ± 0.13 with a range of 2-8 (Table 1). The mean gestational length in animals having ≤ 3 litter size was 60.83 ± 0.64 days and 59.90 ± 0.43 days in animals having >3 litter size. No significance difference ($p<0.05$) was observed for gestation length in relation to litter size. This is in accordance with Alonge *et al*. (2016) who reported no significant difference in gestation length in relation to litter size. However, Mir *et al*. (2011) reported longer gestation length when the litter size was one or two (65.2 ± 2.6 days) compared to those litters having 3–9 foetuses (62.8 ± 1.9 days) and 10 or more foetus (62.2 ±

Table 1. Influence of different factors like breed, litter size, age and parity on gestation length in different small sized breeds of dogs

Factors		No. of animals	Gestation length (Mean ± SE)
Breed	Pug	37	60.68 ± 0.55^{ns}
	Beagle	28	59.82 ± 0.54 ^{ns}
	Dachshund	9	60.78 ± 1.74 ^{ns}
	French bull dog	6	58.83 ± 1.35 ^{ns}
	Spitz	9	58.17 ± 0.74 ^{ns}
	Overall	89	60.21 ± 0.36
Litter size	≤ 3	30	60.83 ± 0.64
	> 3	59	59.90 ± 0.43
Age	1-3 year	63	60.21 ± 0.23
	> 3 year	26	60.15 ± 0.30
Parity	Nulliparous	29	60.86 ± 0.4
	Multiparous	60	59.90 ± 0.5

Table 2. Correlation between gestation length and factors affecting gestation length

Parity	Breed	Age	Litter Size
-0.084	-0.068	-0.002	-0.168

1.3 days) in large breeds (German Shepherds, Rottweilers and Golden Retrievers) of dogs.

Age of the animals under the study ranged from 1 to 8 years with a mean of 3 ± 0.15 years. Animals of age from one to three years were categorized in Group I and animals that were more than three years of age were categorized in Group II. The mean gestation length in Group I was 60.21 ± 0.23 days (n=63) and 60.15 ± 0.3 (n=26) days in Group II. No significant difference (p>0.05) was observed between the two groups in relation to gestation length (Table 1) and it can be concluded that age of the dam had no influence on gestational length. This is in accordance with Okkens *et al*. (2001) and Mir *et al*. (2011) who studied the relationship between age of the dam and gestation length in large breeds of dogs and reported no significant difference in gestation length of different age grouped bitches.

Parity of animals selected under the study ranged from 0 - 4. Animals which were not delivered even once in life time were considered as nulliparous and animals which have whelped once or more than once were categorized as multiparous. Mean gestational length observed in nulliparous bitches was 60.86±0.4 days (n=29) while in multiparous it was 59.90±0.5 days (n=60). No significant difference was observed in gestational length in relation to parity (Table 1), which was correlated with the findings of Okkens *et al*. (1993) and Eilts *et al*. (2005) who found no influence of parity on gestational length. In recent study Mir *et al*. (2011) found no influence of parity on gestational length in nulliparous (63.1 ± 2.2 days) and multiparous (63.3 ± 2 days) bitches.

The correlation coefficients were not significant (Table 2) which indicate no evidence for the influence of factors like litter size, parity, age and breed on gestation length. Further, assessment was done by making groups based on these factors.

Conclusion

The present study was designed to assess the influence of different factors like litter size, breed, parity and age on gestation length

in bitches. A total of 89 small sized bitches of different breeds having different age, parity and body weight, were selected for the study. The correlation coefficients of different parameters also indicated no evidence for the influence of factors like litter size, parity, age and breed on gestation length. Thus, the study concluded that different factors like breeds, litter size, age and parity did not affect the gestation length in small sized breeds of dogs.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Cyclic adenosine monophosphate modulator supplementation on *in vitro* maturation of bovine oocytes#

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Abstract

Study evaluated the role of cAMP modulator (Forskolin and 3-isobutyl-1- methyl xanthine) supplementation on developmental competence of bovine oocytes. Cumulus oocyte complexes recovered from bovine ovaries of unknown reproductive status were used for the study. Oocytes retrieved by aspiration method were graded based on cumulus cell distribution and culture grade oocytes were selected for the study. A total of 414 culture grade oocytes were taken and divided into two groups. Group I constituted of 201 oocytes in which pre-maturation was carried out for a period of 2 h. In group II, 213 oocytes were selected in which normal maturation was carried out. Maturation was assessed after 24h of culture in CO_2 incubator maintained at 38.5°C in 95 per cent humidified atmosphere of 5 per cent CO_2. Fertilisation was carried out using frozen thawed semen and the presumptive zygotes were then transferred to culture media and cleavage was assessed 48 h after insemination. A significantly higher maturation rate (p ≤0.05) was observed in group I compared to group II (86.85 ± 1.19 vs 79.88 ± 2.67). There was a highly significant increase (p ≤0.01) in cleavage rate in group I (65.92 ± 1.23) compared to group II (59.29 ± 1.50). A higher fertilisation rate was observed in group I (75.35 ± 1.19) than group II (71.88 ± 2.56). It could be concluded that pre-maturation with cAMP modulators improved the developmental competence of bovine oocytes.

Keywords: *Oocyte, pre-maturation, cAMP modulators, forskolin*

Running title: Pre- maturation of bovine oocytes with cAMP modulators

#Part of MVSc thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

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During the last few decades, in vitro embryo production technology (IVEP) in bovines has received greater attention in animal husbandry. The procedure consists of three main steps starting from oocyte recovery, in vitro maturation and fertilisation of retrieved oocytes and in vitro culture of embryos. *In vitro* maturation is an assisted reproductive technology used to produce fully mature oocytes for wide range of applications like embryo production, human infertility treatments, transgenic technologies and cloning etc. It is a major strategic research tool in developmental and reproductive biology (Albuz *et al.*, 2010). It involves gathering of immature oocytes from antral follicles and its subsequent culture under laboratory conditions to generate metaphase II oocytes.

Though IVEP is established, the efficiency is still limited to 30-40 per cent. Various researchers found that one main reason for this reduced efficiency is related to maturation of oocyte. Oocyte maturation involves both nuclear maturation as well as cytoplasmic maturation. Nuclear maturation is initiated from early foetal life onwards and it get arrested at diplotene stage of prophase I. Further maturation happens only after puberty up on LH surge. The oocytes will undergo synthesis and reorganization of cytoplasm during their selection and dominance phase (Luciano *et al*., 2018). Many factors play a role in holding oocytes in arrested stage, among that cyclic adenosine monophosphate is regarded as the major factor responsible for meiotic arrest. Though the major source of cAMP is from surrounding cumulus cells (CC), oocytes also synthesize some amount. There is a chain of exchange between CCs, gap junctions and oocyte. The cAMP produced from CCs is transferred to oocytes via gap junctions meaning gap junctional communication plays a major role in transport. Up on removal of oocytes from follicles, the gap junctional communication will be altered leading to drastic reduction in level of cAMP which will eventually culminate in activation of maturation promoting factor thereby resumption of meiosis (Pan and Li, 2019)

Oocytes exhibit a peculiar property of spontaneous resumption of meiosis when taken out of follicles. This occurs due to rapid drop in levels of cAMP. Oocytes retrieved for *in vitro* studies have not got enough time for synthesis and reorganization because they are taken out of antral follicles at different developmental stages. They won't be competent enough to hold future embryo. So an additional time apart from IVM is essential for cytoplasmic maturation. This can be made possible by incorporating cAMP modulators so that nuclear maturation can be stopped for a while. Forskolin and 3-isobutyl-1- methylxanthine (IBMX) are two cAMP modulators which act synergistically increasing intra oocyte cAMP levels. In view of above observations the present study was designed to investigate the effect of pre-maturation with forskolin and IBMX on developmental competence of oocytes.

Materials and methods

Bovine ovaries of unknown reproductive status were collected from slaughter house in antibiotic supplemented normal saline. Ovaries were washed, extra ovarian ligaments trimmed and after further washing maintained at 36-38 °C till aspiration is completed. All visible surface follicles of 2-8mm size were aspirated and collected in 10 mL test tube and kept undisturbed for 10min in incubator for settling. Later sediment in the bottom was pipetted out and COCs were identified under stereozoom microscope and graded as A, B, C and D.

Experimental design

A total of 414 culture quality oocytes of Grade A and B were selected for the study. Group I consisted of 201 oocytes kept for pre-maturation for a period of two hour before maturation and group II (n=213) maturation was carried out by following standard protocol without pre-maturation with cAMP modulators. In group I, follicles were aspirated in to oocyte collection medium supplemented with 100µM forskolin and 500µM IBMX to prevent fall in cAMP assuming that resumption of meiosis starts up on removal of oocytes from follicles. Pre-maturation medium consisted of TCM-199, supplemented with Earle's salts, foetal bovine serum (FBS), gentamicin, 500µM IBMX and 100µM forskolin. After grading of oocytes,

the same oocytes were transferred to pre-maturation droplets in a ratio of 10-20 oocytes per 100µL of maturation droplet. Oocytes were kept in pre-IVM medium for a period of 2 h. After 2 h, oocytes were washed serially in washing medium and transferred to maturation droplet. In group II, cumulus oocyte complexes were identified using stereozoom microscope and transferred to washing medium. After serial washings culture quality oocytes were transferred to maturation droplet (without pre-maturation) in a ratio of 10-20 oocytes per 100 µL of maturation droplet.

Maturation medium consist of TCM-199 (HEPES modified) supplemented with FSH, estradiol-17β, sodium pyruvate, L-glutamine, gentamicin sulphate and FBS. The culture condition set for the study was 38.5 °C in 95 per cent humidified atmosphere of 5 per cent CO_2. Maturation was assessed after 24 h of culture by cumulus cells expansion and first polar body extrusion. Fertilisation was carried out using frozen thawed semen. Sperm oocyte co-incubation was carried out for a period of 18 h. The presumptive zygotes were then transferred to culture droplets after 18 h of incubation. Culture media consisted of SOF supplemented with BSA, Sodium pyruvate, essential and nonessential amino acids and gentamicin sulphate. Cleavage and fertilisation were evaluated 48 h post-insemination under inverted microscope at 40x magnification

Results and discussion

A total of 123 bovine slaughter ovaries of unknown reproductive status retrieved from slaughter house were subjected to follicular aspiration. A total of 554 visible surface follicles having 2- 8mm diameter were aspirated. Mean number of follicles aspirated per ovary in the present study was 4.35 ± 0.24. The result was comparable with that of Rakshitha (2019) who obtained 5.58 ± 0.23 follicles per ovary and was lower than the observations of Manik *et al.* (2003) who observed 6.8 ± 0.7 follicles per ovary. The difference in the number of follicles found in ovaries can be due to the variations in the age of animal, breed, climatic conditions, presence or absence of corpus luteum in the ovary, nutritional, genetic and reproductive status of the animal (Singh *et al.,* 2001)

The recovery rate of oocytes obtained in the present study was 87.88 ± 1.50 per cent. Oocyte recovery rate found in the study was is in accordance with Rakshitha (2019), who obtained recovery rate as 86.44 per cent. However, researchers like Singh *et al.* (2001) and Boonkong *et al.* (2012) obtained lower recovery rates of 67 and 58.6 per cent respectively. The variability in quality of oocytes obtained in the present study compared with other findings might be due to stage of the cycle at the time of slaughter, size and functional status of follicle, age, season, nutritional status and health condition of the animal (Nandi *et al.,* 2002 and Sianturi *et al.,* 2002).

There was a significantly higher maturation rate ($p \leq 0.05$) was observed in group I (Fig. 1) compared to control group (86.85 ± 1.19 vs 79.88 ± 2.67) (Table.1 and Fig. 2) and a highly significant ($p \leq 0.01$) increase ($p \leq 0.01$) in cleavage rate was observed in group I (65.92 ± 1.24) compared to group II (59.29 ± 1.50) (Fig.3) The *in vitro* fertilisation rate of oocytes in experimental groups were 75.35 ± 1.19, and 71.88 ± 2.57 in group I, and II respectively. Even though there is no significant difference in fertilisation rate between experimental groups, a higher fertilisation rate was obtained in group I (75.35 ± 1.19%) than control (71.88 ± 2.57%).

Table. 1. Comparison of *in vitro* maturation rate in group I and II

Treatment group	No of oocytes kept for maturation	Maturation changes observed		Number of matured oocytes	Maturation rate (%)
		Cumulus cell expansion (%)	First polar body extrusion (%)		
Group I	201	79.88 ± 1.69 (161)	6.96 ± 1.31 (14)	175	86.85 ± 1.19[a]
Group II	213	71.88 ± 2.66 (151)	9.65 ± 2.07 (20)	171	79.88 ± 2.67[b]

*** Significant at 0.05 level. Means having different letter as superscript differ significantly*

The intra-oocyte cAMP concentration is regulated by the balance between the activity of two enzymes: adenylyl cyclase (AC) and phosphodiesterases (PDEs), responsible for cAMP synthesis and degradation, respectively. Cell to cell communications between follicular cells and the oocyte via gap junctions is critical for the completion of meiotic and cytoplasmic maturation (Gilchrist *et al*., 2007). It is *via* gap junctions that oocytes get essential nutrients, purines, nucleotides and metabolic support. Our results which indicate increased maturation, fertilisation and cleavage rate showed that the meiotic inhibitory effect was augmented when forskolin was supplemented with IBMX. For this reason, it is assumed that the prolongation of GJC between oocyte and cumulus cells after the treatment of IBMX and forskolin could be attributable to the accumulation of cAMP in cumulus cells and oocytes. The delayed loss of GJC in turn increased the concentration of intra-oocyte cAMP, which finally delayed the progression of GVBD (Luciano *et al*., 2004; Thomas *et al*., 2004).

In the present study, cumulus expansion was found to be greater in cAMP treated group than compared to control group. Also there is a significant difference noticed in IVM rate. It clearly depicts that when oocytes are meiotically arrested by introducing high level of cAMP modulators, the cytoplasmic maturation is getting improved. Oocytes can continue the reconstruction of cytoplasmic machineries by the time. For improving the yield and quality of developing embryos, *in vitro* cessation of oocyte meiosis is necessary. During this resting time, the oocyte will find the opportunity to continue transcription of mRNA, post-translational modifications of proteins, modification of organelles which are essential to sustain normal fertilization and further embryonic development.

It has been clearly understood previously that the chemical mediation by cAMP anologues have positive effect on oocyte maturation (Albuz *et al*., 2010). The increased rate of maturation in cAMP treated group clearly depicts the positive effect of supplementation of cAMP modulators from the initial period of oocyte collection. Richani

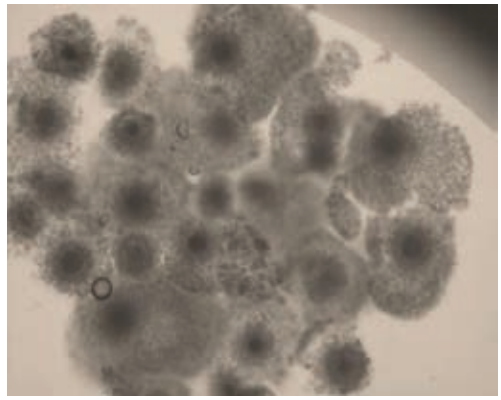

Fig. 1. Cumulus expansion noticed in group I

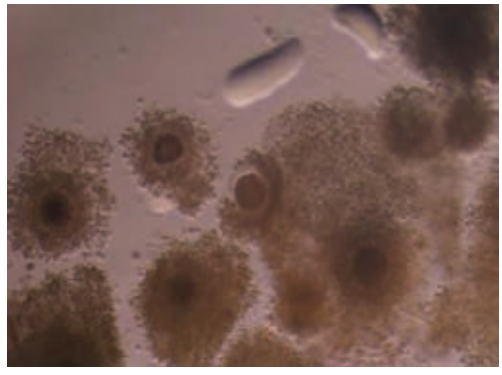

Fig. 2. Cumulus expansion noticed in group II

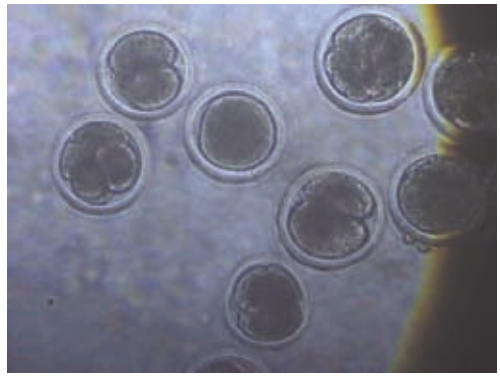

Fig. 3. Cleaved embryos

et al., (2014) found out that the blastocyst yield and quality of embryos was greater when the pre-IVM period was lengthened beyond 1 h suggesting that the duration of cAMP-modulated pre-IVM has important effects on oocyte developmental competence. Extending pre-IVM duration accelerated the time of 2-cell embryo development, which is indicated as a strong predictor of pregnancy success, the same was noticed by (Sugimura *et al*., 2012).

Rose *et al*., (2013) carried out pre-maturation with forskolin and IBMX in ovine oocytes and came up with positive results showing greater developmental competence for pre-maturated oocytes.

Conclusion

Pre-maturation with cAMP modulators is a novel approach in the IVF field with a view to increase the output of IVM by improving cytoplasmic maturation and thereby increasing the developmental competence of oocytes. From the above findings, it is clear that supplementation of cAMP modulators in pre-IVM treatment has a positive effect on developmental competence of bovine oocytes. Hence, it can be concluded from this study that the efficiency of IVEP can be increased by incorporating pre-maturation before maturation so that cytoplasmic maturation can be improved.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Haemato-biochemical changes in tuberculosis infected and healthy Asian elephants (*Elephas maximus*) from South India#

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Abstract

Tuberculosis is known to be a disease of elephants for the past 2000 years. The main causative agent isolated from reported tuberculosis (TB) cases were Mycobacterium tuberculosis. The study focuses on the haematological and serum biochemical changes in the blood of TB infected Asian elephants (Elephas maximus). Twelve apparently healthy elephants and twelve TB infected elephants (confirmed by trunk wash smear positive for acid fast bacilli) were selected for the study. Neonates, pregnant elephants and elephants in musth were not included in the study. The study animals were subjected to haematological and serum biochemical evaluation. The data were analysed statistically. The results showed a significant increase in total leukocyte count, lymphocyte count, monocyte count, thrombocyte count and ESR in TB affected animals compared with apparently healthy animals. Serum creatinine, total bilirubin, direct bilirubin, globulin was significantly high in TB affected animals compared with healthy controls. Assessment of haematological and serum biochemical parameters in TB affected elephants aid in diagnosis and tracking of the infection.

Key words: *Elephant, tuberculosis, haematology, serum biochemistry*

Running title: Haemato-biochemical changes in tuberculosis infected and healthy Asian elephants

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Tuberculosis is one of the major infectious diseases known to the elephant world for the past twenty centuries (Mikota *et al.,* 2001). As the years go by, the number of TB cases are hiking and the elephant population is declining (Lewerin *et al.,* 2005). There can be a plethora of reasons for the decline in the population, however TB can be one of the many reasons. *Mycobacterium tuberculosis* being a devious organism, has complex mechanisms to survive and multiply in the body without being detected by immune system. The course of the disease and its immunobiology are poorly studied in elephants. Although the complex pathogenesis of disease is still under study, the associated immune mediated systemic inflammation has been reported to be significant (Rohini *et al.,* 2016; Ottenhoff *et al.,* 2012) which results in alterations in the blood and serum parameters. If the TB infected animal has an underlying condition or infection which can cause immunosuppression, abnormalities in blood could be much blatant. There are very limited studies regarding the haematological and serum biochemical changes during the course of TB infection in elephants. This study aims to furnish insights into the serum biochemical and haematological changes during TB infection and evaluation of the variability of blood parameters of TB infected elephants when compared to that of apparently healthy animals.

Materials and methods

Twelve apparently healthy adult captive Asian elephants (*Elephas maximus*) which were negative for acid fast bacilli in trunk wash smear were selected for the study and were included in the healthy group. Twelve animals with trunk wash smear positive for acid fast bacilli (AFB) were selected and included in the TB infected group. Neonates, elephants in gestation, in lactation and in musth were not included in the study. The selected animals were subjected to haematological and serum biochemical evaluation.

Blood collection

Animals were placed in lateral recumbency. Blood was drawn from superficial marginal vein located at the external aspect of the ear using, sterile 16-gauge disposable needle into a vacutainer tube.

Haematology evaluation

Blood samples were collected in 2mL EDTA vacutainer tubes for the evaluation of haematological parameters (Orphee mythic 18vet - analyzer) *viz*. haematocrit (HCT), haemoglobin (Hb), total erythrocyte count (TEC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), total leukocyte count (TLC), erythrocyte sedimentation rate (ESR), platelet count and mean platelet count on the day of presentation.

Serum biochemical evaluation

Blood samples were collected in 4mL serum vacutainer tube for the evaluation of serum biochemical parameters (Master T, Hospitex diagnostics, Italy) viz. blood urea nitrogen (BUN), creatinine, total bilirubin, direct bilirubin, indirect bilirubin, glucose, total protein, albumin, globulin, albumin globulin ratio, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) on the day of presentation.

Statistical analysis

These data were evaluated and analysed by SPSS version 25. In order to compare quantitative variables in TB infected and heathy groups, independent t-test were performed for normally distributed variables. Significance difference were assessed at both 0.01 and 0.05 levels.

Result and discussion

All the animals included in the healthy group were apparently normal and did not show any signs of systemic disorder or infection. Elephants belonging to TB infected group had mild mucus discharges from the trunk. Nonetheless, all the animals were active and alert, with normal behaviour and feed intake.

Blood samples were collected for haematological and serum biochemical

analysis on the day of presentation and were evaluated. The values obtained were statistically analysed. The mean and standard error of mean for the various parameters were tabulated (Table 1, 2). In the present study, total erythrocyte count (TEC), haemoglobin (Hb) and haematocrit (HCT) values had no significant difference between TB infected and healthy group. This is in contrast with the studies done in haematology of TB infected humans (Abdelkareem *et al.* 2015, Rohini *et al.,* 2016, Abay *et al.,* 2018). Nevertheless, individual values for TEC, HCT and Hb showed minor decrease among TB infected group with that of healthy control group. This variability may be due to the dissimilar hydration status of elephants or different stages of infection in the selected animals. The TLC, lymphocytes, MCV, MCH and platelet count were significantly high in TB infected group. This in accordance with Abdelkareem *et al.* (2015) who found that 49 per cent of patients with pulmonary TB (PTB) had leukocytosis. Yaranal *et al.* (2013) and Rohini *et al.* (2016) found that all of patients with PTB had leucocytosis. There was a significant thrombocytosis in TB infected group which is in accordance with the study by Rathod *et al.* (2017) who speculated the potential use of thrombocyte count as a marker for TB and found that 75 per cent of the TB infected patients had thrombocytosis. The ESR values obtained from the study had significant difference with that of healthy controls which is in agreement with the previously published studies (Oliva *et al.,* 2008; Rohini *et al.,* 2016; Kahase *et al.,* 2020). Mandal and Chaven (2016) suggested that the increment in the ESR values were a common observation in active TB cases. The reasons for the increment in ESR values might be due to the increase in fibrinogen, immunoglobulins and C-reactive protein in the blood, which is a common finding in conditions like TB, inflammation and malignant conditions (Cheesbrough, 2005).

Table 1. Comparison of haematological parameters between TB infected and healthy group

Sl. No.	Parameters	Unit	Observation			
			TB infected group	Healthy group	t- value	P- value
1	Total Leukocyte Count	10^3/µL	24.67 ± 0.61	21.57 ± 0.21	4.849**	< 0.001
2	Lymphocytes	10^3/µL	16.29 ± 0.53	11.68 ± 0.16	8.377**	< 0.001
3	Monocytes	10^3/µL	2.48 ± 0.14	3.71 ± 0.11	6.846**	< 0.001
4	Granulocytes	10^3/µL	5.88 ± 0.50	6.05 ± 0.04	0.346^{ns}	0.736
5	Lymphocytes	%	66.31 ± 1.91	54.23 ± 0.50	6.119**	< 0.001
6	Monocytes	%	10.07 ± 0.58	17.51 ± 0.40	10.510**	< 0.001
7	Granulocytes	%	23.63 ± 1.61	28.26 ± 0.30	2.820*	0.016
8	Total Erythrocyte Count	10^6/µL	3.85 ± 0.10	3.76 ± 0.02	0.864^{ns}	0.404
9	Haemoglobin	g/dL	14.11 ± 0.39	13.53 ± 0.02	1.462^{ns}	0.172
10	Haematocrit	%	50.15 ± 1.39	47.95 ± 0.27	1.556^{ns}	0.146
11	Mean Corpuscular Volume	fL	130.33 ± 0.46	127.33 ± 0.09	6.384**	< 0.001
12	Mean Corpuscular Haemoglobin	pg	36.64 ± 0.31	34.13 ± 1.05	2.291*	0.039
13	Mean Corpuscular Haemoglobin Concentration	g/dL	28.10 ± 0.21	28.16 ± 0.15	0.229^{ns}	0.821
14	Red Cell Distribution Width	%	13.82 ± 0.18	14.24 ± 0.05	2.209*	0.046
15	Platelet Count	10^3/µL	1295 ± 149.02	850.58 ± 26.99	2.935*	0.013
16	Mean Platelet Volume	fL	4.62 ± 0.09	5.40 ± 0.06	6.950**	< 0.001
17	Erythrocyte Sedimentation Rate	mm/hr	4.91 ± 0.09	3.88 ± 0.15	5.770**	< 0.001

*** Significant at 0.01 level; * Significant at 0.05 level; ns Non-significant*

Table 2. Comparison of Serum Biochemical Parameters between TB infected and healthy group

Sl. No.	Parameters	Unit	Observation		t-value	P-value
			TB infected group	Healthy group		
1	Blood Urea Nitrogen	mg/dL	12.07 ± 0.16	11.82 ± 0.22	0.922^{ns}	0.367
2	Creatinine	mg/dL	1.96 ± 0.07	1.35 ± 0.05	6.708**	<0.001
3	Total Bilirubin	mg/dL	0.98 ± 0.13	0.66 ± 0.07	2.221*	0.037
4	Direct Bilirubin	mg/dL	0.39 ± 0.04	0.29 ± 0.03	2.123*	0.045
5	Indirect Bilirubin	mg/dL	0.59 ± 0.12	0.37 ± 0.05	1.658^{ns}	0.112
6	Glucose	mg/dL	110.12 ± 6.26	113.47 ± 5.35	0.407^{ns}	0.688
7	Total Protein	g/dL	9.75 ± 0.13	10.03 ± 0.14	1.441^{ns}	0.164
8	Albumin	g/dL	2.85 ± 0.06	3.11 ± 0.09	2.478*	0.021
9	Globulin	g/dL	7.18 ± 0.14	6.61 ± 0.13	2.983**	0.007
10	A/G Ratio	-	0.40 ± 0.01	0.49 ± 0.02	3.334**	0.003
11	Cholesterol	mg/dL	31.41 ± 1.98	32.13 ± 1.79	0.272^{ns}	0.788
12	Aspartate Aminotransferase	IU/L	20.93 ± 2.10	22.89 ± 1.02	0.838^{ns}	0.411
13	Alanine Aminotransferase	IU/L	17.55 ± 2.06	15.34 ± 1.45	0.879^{ns}	0.389
14	Alkaline Phosphatase	IU/L	290.75 ± 23.67	259 ± 30.23	0.827^{ns}	0.417

*** Significant at 0.01 level; * Significant at 0.05 level; ns Non-significant*

The serum biochemistry results obtained in the study projects significant difference in albumin, globulin and albumin globulin ratio. In the diseased group, albumin value attained was lower and globulin value was higher on comparison with the healthy group. Further the albumin globulin ratio attained was lower in TB infected group. This is in agreement with the previous reports (Wong and Saha, 1989; Shingdang *et al*., 2016). The BUN, cholesterol, indirect bilirubin, AST, ALT and ALP values have no significant difference between the groups (Giri *et al.,* 2007). However mean cholesterol level in TB infected group is comparatively less which agrees with Miner *et al.,* (2009) who suggested that cholesterol is crucial for the persistence of the organism. In the present study, the mean ALP value is non-significantly increased in TB infected group. Creatinine value showed significant increase in TB infected group, which is in accordance with Giri *et al.* (2007). Total bilirubin and direct bilirubin values were found to be significantly increased TB group this may be attributable to some underlying hepatic conditions or any intravascular haemolysis (Kerr, 2002).

Conclusion

In the present study, the haematological and serum biochemical parameters differed among TB infected and heathy group. In the context of tuberculosis infection, the changes in the blood chemistry and the failure of these values to return to the normal for a prolonged period can indicate persistent infection. Further blood profiling can aid in correlating the severity of the disease (Kahase *et al.,* 2020). The results of the study demonstrated significant increase in haematological parameters *viz* TLC, lymphocytes, monocytes, MCV, MCH, RDW and platelets. In serum biochemistry there was significant increase in creatinine, total bilirubin, direct bilirubin and globulin. These indices have the potential to form a part of preliminary diagnosis and tracking the response to treatment. Large scale studies are required to substantiate and accurately determine the association of blood chemistry and TB infection in elephants.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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Occurrence of canine mammary and skin/ subcutaneous neoplasms in and around Thrissur district of Kerala during 2017-2020: A review of 265 cases#

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Abstract

The study was conducted in 265 clinical cases of mammary and skin/ subcutaneous neoplasms in dogs presented to University Veterinary Hospitals Mannuthy and Kokkalai during a period of 36 months from October 2017 to September 2020. Mammary neoplasms were found more in females (51.7 per cent) than in males whereas skin and subcutaneous neoplasms were found more in male dogs (48.3 per cent). The maximum occurrence of neoplasms was recorded in the age group of eight to twelve years (38.5 per cent) whereas least occurrence was noticed in the age group up to four years (9 per cent). Labrador and Rottweiler breeds were found more affected with neoplasms (38 per cent each) with highest occurrence of mammary neoplasms in inguinal mammary glands (35.03 per cent) and highest occurrence of skin/ subcutaneous neoplasms on sites involving trunk region (14.06 per cent cases). Eighty-three per cent of the neoplasm cases in the present study were pet dogs with a greater number of dogs maintained in outdoor kennels and 17 per cent of neoplasm was found in free-roaming dogs rescued from streets. Out of total 265 dogs, 37.73 per cent dogs were found to be having commercial dog food as their main feed and 32 per cent dogs were fed with a mixed diet of homemade food and commercial dog food. Among the cases, 14.71 per cent dogs had a previous history of cancer surgery.

Keywords: *Mammary, skin and subcutaneous neoplasms, dogs.*

Running title: Occurrence of canine mammary and skin/ subcutaneous neoplasms

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In the past few decades, an uprise in the cancer incidence in the companion animals were recorded. More than 50 per cent older dog population develops cancer with one-fourth of the reported populations eventually dying of it. (Lewis *et al*., 2018). The leading cause of death in dogs is still identified as cancer and proves to be a constant focus of research in veterinary medicine. University Veterinary Hospitals of Mannuthy and Kokkalai of Kerala Veterinary and Animal Sciences University serves as referral hospitals of the state and a large number of cases of neoplasms were reported in the past decade. The present study was envisaged to document trends in occurrence of neoplasms in dogs over the past three years in and around Thrissur District of Kerala, India.

Materials and methods

Occurrence of mammary and skin/ subcutaneous neoplasms presented to University Veterinary Hospitals Mannuthy and Kokkalai during a period of 36 months from October 2017 to September 2020 were studied. Signalment and anamnesis including the information on age, breed, sex, body weight, general body condition of the animals, general information on lifestyle/ history of illness, home and environment factors, nutritional information and previous history of concurrent illness were recorded. The data collected were analysed to find out the trends in the occurrence of neoplasms.

Results and discussion

A total of 265 cases of superficial neoplasms were reported among the 14140 clinical cases screened during a period of three years at University Veterinary Hospitals Mannuthy and Kokkalai. Out of 265 cases, 137 cases were canine mammary neoplasms (51.7 per cent) and 128 cases were skin/ subcutaneous neoplasms (48.3 per cent). The increased number of mammary neoplasms than skin/subcutaneous neoplasms were in accordance with Aleksić-Kovačević *et al.* (2005) and Choi *et al*. 2016 who reported mammary neoplasms as the main type of neoplasms in dogs. However, these observations were not in agreement with the findings of Gupta *et al*. (2012) and Karnik *et al.* (2020) who reported cutaneous tumours as the most commonly occurring neoplasms in dogs followed by canine mammary neoplasms.

Age

The maximum occurrence of neoplasms were recorded in the age group of eight to twelve years (38.5 per cent) followed by four to eight years (29.1 per cent) and more than twelve years (20.38 per cent), whereas least occurrence (12.08 per cent) were reported in the age group up to four years. The trend of minimum incidence below four years scaling to an increase in four to eight years followed by peak incidence at 11 years and decrease thereafter at age of 12 years was recorded by Dhami *et al*. (2010) and Vascellari *et al*. (2016).

Breed

Breed wise occurrence of neoplasm was seen slightly more in Labrador retrievers (38/265, 14.34 per cent) and Rottweilers (38/265, 14.34 per cent) followed by German shepherds 36 / 265, (13.58 per cent), non-descript /crossbreed (34 / 265, 12.83 per cent), Dachshund (33/265,12.45 per cent), Spitz (28/265, 10.57 per cent), Doberman pinschers (23/265 ,8.68 per cent), Chinese Pug (22/265, 8.30), Great Dane (2/265, 0.75 per cent), Pitbull terriers (2/265, 0.75 per cent) Lhasa Apso (2/265, 0.75 per cent) Golden Retrievers (2/265, 0.75 per cent) ,Neapolitan mastiff (1/ 265, 0.38 per cent), Basset hound (1/ 265, 0.38 per cent), Weimaraner (1/ 265, 0.38 per cent), Jack Russel Terrier (1/ 265, 0.38 per cent) and Beagle (1/ 265, 0.38 per cent) (Fig.1).

Pure breeds were more affected (87.16 per cent) than non-descript breeds (12.83 per cent) in the current study and this was accordance with Vascellari *et al*. (2016) and Hemanth *et al.* (2015). These findings are contradictory to the observation of Dayananda *et al*. (2009). It could be concluded that even though specific breed predisposition to neoplasms was not established in the majority of cancer types, the increased breed wise occurrence in Labrador retriever, Rottweiler and German shepherd dog breeds could be attributed to breed preference among local populations.

Gender

Neoplasms were seen more in females (168/ 265, 63.4 per cent) than in males (97/ 265, 36.6 per cent). This was in accordance with Karnik *et al.* (2020). Even though canine mammary neoplasms were seen more in female dogs (130/137 cases, 94.89 per cent), male dogs were also reported with mammary neoplasm (7/137 cases, 5.11 per cent). Similar findings were documented by Dhami *et al.* (2010) and Patel *et al.* (2019). The occurrence of skin and subcutaneous neoplasms was seen more in males in the present study. This result was found in agreement with the observations recorded by Aleksić-Kovačević *et al.* (2005) and Dayananda *et al.* (2009).

Gland wise occurrence of mammary neoplasm

Gland -wise occurrence of mammary neoplasms in dogs are depicted in Fig. 2. Out of 137 mammary neoplasm cases, inguinal mammary glands accounted for a maximum of total mammary neoplasm (35.03 per cent) followed by cranial abdominal glands (23.36 per cent), caudal abdominal glands (16.79 per cent), and caudo-thoracic glands (10.22 per cent) cranio-thoracic (8.03 per cent), respectively. This was in accordance with Ginn *et al*. (2007) and Panchkhande *et al.* (2019). A similar pattern of ascending involvement from cranial thoracic to inguinal glands was reported by Dhami *et al*. (2010).

Region wise occurrence of skin/ subcutaneous neoplasm

Region wise distribution of skin/ subcutaneous neoplasms in dogs are depicted in Fig.3. Out of 128 cases of skin/ subcutaneous neoplasms, 18 cases were found to be involving trunk region, 13 croup and loin region of the body, 13 cases in the forehead, 12 cases in the hindlimb, 10 cases in the forelimb manus, 11 cases on tail base, nine cases in the thigh region, eight cases each on the perianal and digit and neck regions, seven cases in the axilla and four cases each on the lip region, shoulder region and para preputial /penis. In the present study, trunk region was found to be having a maximum number of skin and subcutaneous neoplasms which was in accordance with Mukaratirwa *et al.* (2005) who observed larger incidence of skin and subcutaneous tumours located on the trunk which were more likely diagnosed as epithelial tumour than non-epithelial tumours and attributed increased incidence of neoplasms in the trunk region due to the larger exposure area for carcinogens.

Bodyweight

Out of 265 dogs, 105 dogs (39.62 per cent) were found emaciated whereas 62 cases were found to be obese (23.4 per cent) and 98 dogs (36.98 per cent) were found to be in the normal body condition. This was in accordance with Philibert *et al*. (2003) who documented that fat content influences the development and biological behaviour of canine mammary gland neoplasms. Neoplasms were seen more (39.62 per cent) in dogs which were emaciated than the dogs with normal body weight (36.98 per cent of animals) in the present study. The reason for emaciation in the majority of animals with neoplasm may be due to cancer cachexia of malignant neoplasms as documented by Arnold *et al.* (2001).

Rearing pattern

Out of 265 cases of neoplasms, 220 (83 per cent) cases were owned by people and 45/265 dogs (17 per cent) were dogs with free-roaming lifestyle rescued from streets by animal welfare organizations. Out of 220 owned dogs, 150 dogs (68.19 per cent) were maintained in outdoor kennels / chained with an average 6-8 hours free-roaming time inside the compound and 56 dogs (25.45 per cent) were strictly maintained indoors and 14 dogs (6.36 per cent) in the study were community dogs with free roaming lifestyle.

Environment

In the present study 137/220 dogs (17 per cent) of the owners reported that they reside near a farm, or any other agricultural/ horticultural /industrial area Among the owned dogs, 45 out of 220 dog owners (20.45 per cent) reported that they regularly used rodenticides, herbicides or pesticides in their house/premises. The increased occurrence of neoplasms in the

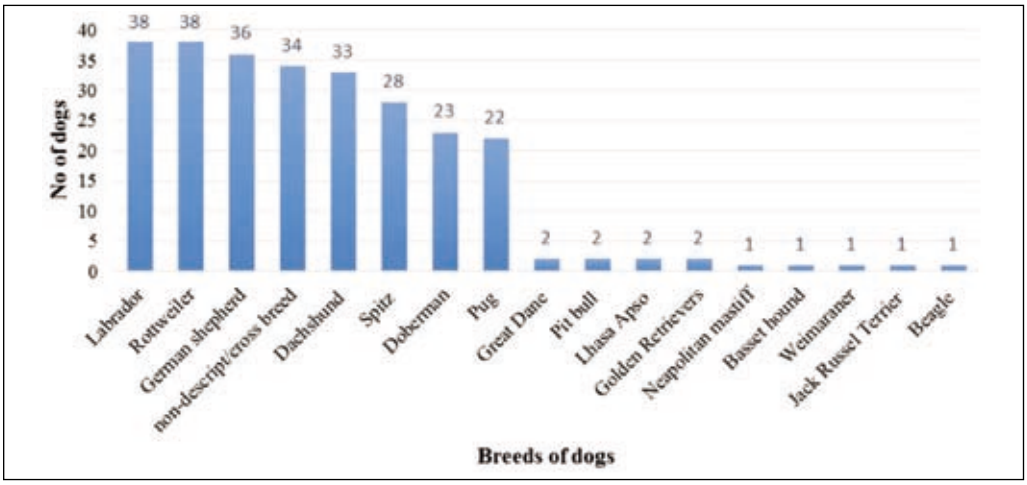

Fig. 1. Graph showing breed-wise occurrence of neoplasms in dogs (n=265)

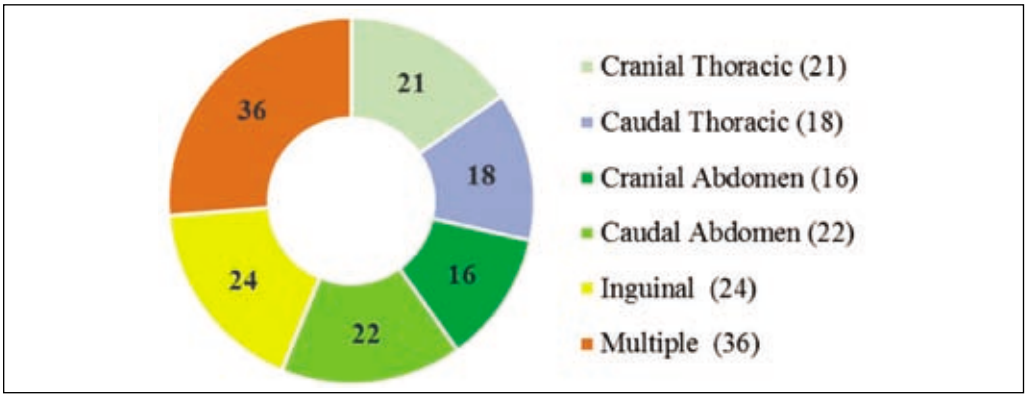

Fig. 2. Gland -wise occurrence of mammary neoplasms in dogs (n=137)

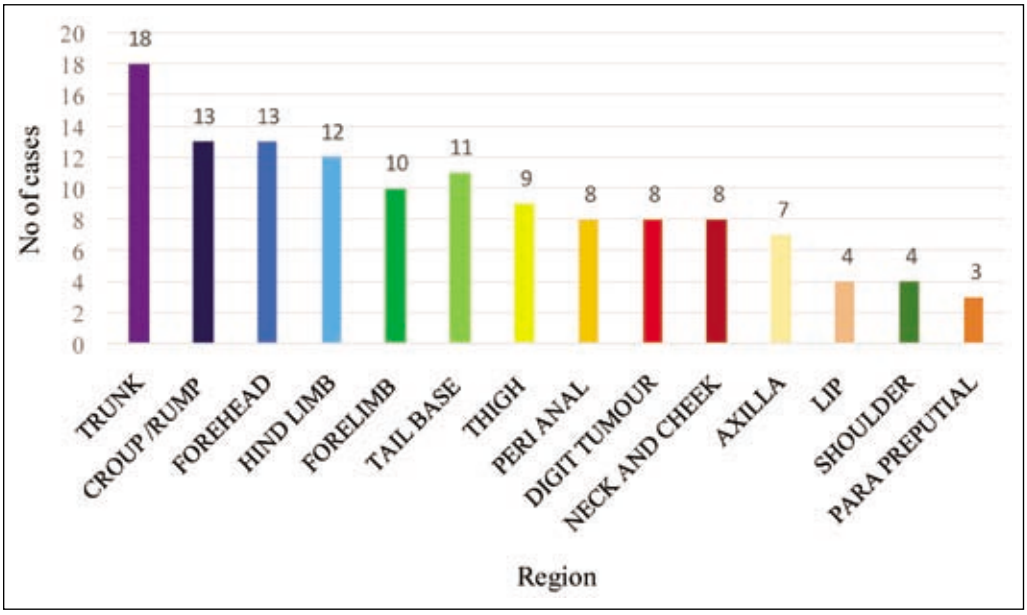

Fig. 3. Region-wise distribution of skin/ subcutaneous neoplasms in dogs (n=128)

dogs could be attributed to increased exposure to environmental carcinogens as documented by Reif *et al*. (1998) and ultraviolet rays as reported by Guzman et al. (2003). The increased incidence of neoplasms in the outdoor dogs may also be attributed to the illegally burned household waste and automobile exhausts as reported by Comba *et al.* (2006). In the present study 7.72 per cent, owners reported cigarette smoking or the presence of smokers at home. This was in accordance with Reif *et al*. (1992) who reported a weak relationship between passive smoking and the risk of developing lung cancer in dogs.

Diet

Out of total 265 dogs, 100 (37.73 per cent) dogs were found to be having commercial dog food as their main feed, 66 dogs (24.9 per cent) were reported to be maintained on exclusively home food, 85 (32 per cent) dogs were fed with a mixed diet of homemade food and commercial dog food and 14 dogs (5.28 per cent) were free-roaming dogs with scavenging eating habits.

Feeding practices finds itself a vast area contributing to cancer. Even though the current study cannot rule out the potential effect of commercial pet foods on cancer, an increased occurrence of neoplasms among the dogs solely fed with commercial in the present study. Dobson *et al.* (2008) detected carcinogenic property of simple triazine compounds mainly melamine and cyanuric acid in commercial pet foods. Further studies are needed to emphasise the findings of an increased incidence of neoplasms in dogs maintained with commercial pet foods. In the present study least occurrence was reported in the animals fed with homemade food alone. Neoplasms were also detected on dogs with scavenging eating habits in the present study. This could be attributed to the ingestion of carcinogenic contaminants as reported by Gavazza *et al.* (2001).

Previous history of neoplasms

Among the cases, 39 dogs had a previous history of cancer surgery, of which 20 cases were mammary neoplasms and 19 cases were skin and subcutaneous neoplasms. These observations were in accordance with Neeman and Ben-Eliyahu (2013) who reported that surgical trauma could initiate specific factors that can influence the cancer recurrence and surgery-induced vascular endothelial growth factor (VEGF) which could potentiate cancer stem cells (Ceelen *et al*., 2014).

Conclusion

The present study recorded the occurrence of 265 cases of mammary tissue and skin / subcutaneous neoplasms in and around Thrissur district of Kerala from October 2017 to September 2020. *).* Most of the of the neoplasm cases (eighty-three per cent) in the present study were pet dogs with a greater number of dogs maintained in outdoor kennels and the remaining cases of neoplasm was found in free-roaming dogs rescued from streets.

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Conflicts of interest

There were no conflicts of interest reported by the authors.

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Chorioptes texanus causing mange in goats: comparison of two therapeutic protocols#

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Abstract

Out of 34 caprine dermatological cases examined, 12 were detected positive for Chorioptes texanus. Pruritus, alopecia, crusts, thickening, wrinkling, cracks and fissures on legs, axillae, inguinal region and perineal region were the symptoms noticed in caprine chorioptic mange. In affected animals, reduction in haemoglobin concentration and per cent lymphocytes were noticed, along with elevation in the values of the total leukocyte count, per cent neutrophils and per cent monocytes. Values of serum glucose, zinc and copper were normal. All the 12 cases were treated with ivermectin at 10 days interval till two consecutive skin scrapings were negative for mites. In addition, all goats were treated with vitamin A supplements throughout the period. Six goats were treated topically with permethrin spray while other six animals were treated topically with lime sulphur spray. Both treatment protocols caused recovery of the animals from clinical symptoms, however, a skin scraping after 1 year revealed the presence of mites.

Keywords: *Chorioptes spp., permethrin, lime sulphur*

Running title: *Chorioptes texanus* mange: comparison of two protocols

Chorioptes spp. of mites infest cattle, horses, sheep and goats (Constable *et al*., 2017). They are transmitted by direct contact and contaminated fomites. They have a life span of 60 days and complete their life cycle on the body of host in three weeks (Smith and Sherman, 2009).

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Two valid species of *Chorioptes* were reported previously *viz*., *C. bovis* and *C. texanus* (Lusat *et al*., 2011). The clinical signs of animals with chorioptic mange include alopecia, pruritus, thickening and wrinkling of the skin with scabs, crust and fissures on the feet and tail (Reddy *et al*., 2013). Severe infestation leads to decreased meat and milk production as well as poor hide quality, which may result in heavy economic loss in leather industry (Asmare *et al*., 2016).

Materials and methods

Goats presented at Teaching Veterinary Clinical Complex, Kerala Veterinary and Animal Sciences University, Pookode, with a history of skin lesions were selected for the study. Skin scrapings and scab materials from these animals were examined microscopically for the presence of mites. Complete blood count of infested goats was estimated by three part fully automated haematological analyzer (Mindray BC-2800Vet). Serum glucose was estimated by semiautomatic biochemical analyzer (Master T, Hospitex Diagnostics, Italy) with commercially available diagnostic kits (Biosystem diagnostics Pvt. Ltd), while serum zinc and copper were estimated using atomic absorption spectrophotometry (Perkin-Elmer, USA).

The infested goats were divided into Group I and Group II, consisting of six animals each. All animals were given inj. Ivermectin (Neomec 10 mL) @ 200 µg/kg BW S/C at 10 days interval until two consecutive skin scrapings became negative for mites. They were also given vitamin A supplements as Lavitone H injection @ 1 mL/ 25 kg BW I/M on the first day of treatment and continued the supplementation orally (Lvitone H liquid 5mL daily) throughout the treatment period. The group I goats were treated topically with permethrin 0.05 per cent as spray (Durvet Permethrin 10 per cent) over the lesions once. Group II goats were treated with lime sulphur two per cent (Demoscanil 250 mL) as spray over the lesions once. Response to treatment was evaluated clinically and treatment groups were compared based on the mite count per field in each review. Samples collected from six apparently healthy goats served as the control group. The data collected were statistically analyzed using SPSS version 24.0.

Results and discussion

Microscopic examination of skin scrapings revealed the presence of *Chorioptes* spp. in 12 out of 34 cases with skin lesions like hair loss and pruritus. The mites were small round, having cup shaped suckers on the short unjointed pedicel and distinctively round mouth parts as seen in Fig. 1 and Fig. 2. These characteristics were previously reported by Taylor *et al*. (2007). The length of the opisthosomal setae 1 and 2 of adult male were 49.57± 2.14 µm and 147.91± 3.95 µm respectively. Therefore, they were identified as *C. texanus.* Similar observations were previously recorded by Lusat *et al*. (2011). *Chorioptes texanus* was reported earlier in countries like Canada (domestic goats and raindeer), Israel (dairy cattle), Germany (cattle) and Korea (cattle). Caprine chorioptic mange was reported in India, by Reddy *et al*. (2013) and Dharanesha *et al*. (2015) in the states of Andhra Pradesh and Karnataka respectively. However, Reddy *et al*. (2013) did not identify the species of mite involved in the causation of the disease. Dharanesha *et al*. (2015) identified the mites as *C. caprae* in goats from Karnataka, based on the characters described by Soulsby (1982) and did not consider the length of opisthosomal setae for the species differentiation.

In the present study, the skin lesions were mainly observed on the legs (11 cases) and the axillae, inguinal region, perineal region, tail and ear (eight cases). The lesions of chorioptic mange in goats, most commonly appeared at the lower limbs (Smith and Sherman, 2009). In the present study, lesions at lower limbs were observed in 10 cases. Five out of 10 cases showed feet lesions. Dharanesha *et al*. (2015) observed the lesions of chorioptic mange in goats of Karnataka mainly on lips, muzzle, ears and periorbital areas and rarely around hoof, accessary claws and interdigital space. In the present study, main lesions observed were alopecia with crusting, erythema, thickening, wrinkling and erosions on the skin (Fig. 3). These lesions were previously reported by Reddy *et al*. (2013) and Jesse *et al*. (2016).

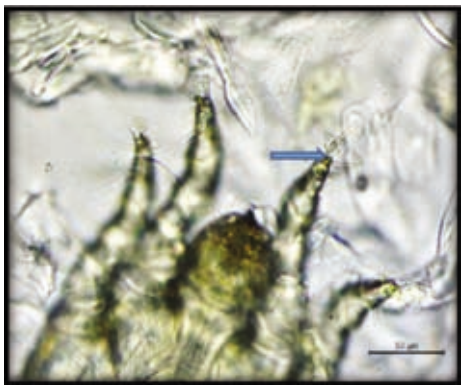

Fig. 1. *C. texanus* with cup shaped suckers on unsegmented pedicel of first and second pairs of legs

Fig. 2. *C. texanus* with short seta 1(a) and long seta 2(b)

Fig. 3. Crusty alopecia on limbs

Fig. 4. Group I; A- Before treatment; B- After treatment

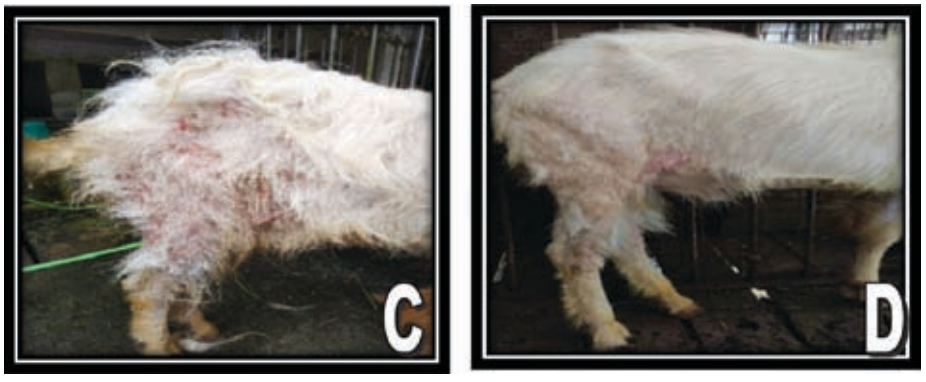

Fig. 5. Group II; C- Before treatment; D- After treatment

In the present study, haemogram of the infested goats revealed lower mean haemoglobin concentration and per cent lymphocytes compared to healthy goats. Total leukocyte count and per cent neutrophils, monocytes revealed significant increase compared to control group (Table1). These observations were also noted by Marimuthu *et al*. (2015).

The infested animals did not show any significant changes in the serum zinc, copper and glucose concentrations compared to the control group (Table 2).

Animals treated topically with permethrin or lime sulphur were clinically normal by 20-40 days after the initial treatment. The reduction of mite count in group I and group II were similar (Table 3). Anaemia, neutrophilia, monocytosis and lymphocytopenia observed prior to the treatment in the infested goats became normal at the end of treatment. Serum zinc and copper concentrations after treatment

Table 1. Comparison of haemogram of goats infested with *C. texanus* before and after treatment

Sl. No	Parameters	Uninfested goats (n=6)	Group I (n=6)		Group II (n=6)	
			Before treatment	After treatment	Before treatment	After treatment
1	Hb (g/dL)	9.63 ± 0.29	7.98 ± 0.88 ns	9.38 ± 0.97 *	8.02 ± 0.34 ns	8.93 ± 0.53 ns
2	VPRC (%)	28.12 ± 0.84	25.02 ± 2.33 ns	28.65 ± 2.76 *	23.77 ± 1.68 ns	27.37 ± 1.34 ns
3	TEC ($\times 10^6$/µL)	15.96 ± 0.47	13.91 ± 1.19 ns	16.7 ± 1.77 ns	14.61 ± 0.79 ns	16.63 ± 1.23 *
4	TLC ($\times 10^3$/µL)	12.22 ± 0.55	15.93 ± 2.2 ns	14.97 ± 2.85 ns	16.32 ± 1.56 ns	13.13 ± 0.68 ns
5	Neutrophils (%)	35.83 ± 0.6	45.5 ± 3.17 ns	40.5 ± 3.22 ns	44 ± 2.72 ns	35.67 ± 0.62 *
6	Lymphocytes (%)	62.33 ± 0.88	45.67 ± 3.12 **	56.33 ± 3.42 ns	50.33 ± 1.91 **	61.83 ± 0.7 **
7	Monocytes (%)	0.33 ± 0.21	3.17 ± 0.6 **	0.83 ± 0.17 **	0.83 ± 0.31 **	0.5 ± 0.22 ns
8	Eosinophils (%)	1.5 ± 0.22 ns	5.83 ± 2.32 ns	2.33 ± 0.21 ns	4.83 ± 1.35 ns	2.00 ± 0 ns

** Significant at 0.01 level (P<0.01); * Significant at 0.05 level (P<0.05); ns Non-significant (P>0.05)

Table 2. Comparison of serum biochemical parameters of goats infested with *C. texanus* before and after treatment

Sl. No	Parameters	Uninfested goats (n=6)	Group I (n=6)		Group II (n=6)	
			Before treatment	After treatment	Before treatment	After treatment
1	Glucose (mg/ dL)	76.34 ± 5.75	86.8 ± 10.38 ns	81.99 ± 12.36 ns	93.78 ± 7.41 ns	93.22 ± 5.68 ns
2	Zinc (µg/dL)	132.57 ± 34.64	92.03 ± 32.19 **	154.23 ± 22.08 ns	68.79 ± 10.63 **	128.67 ± 14.59 *
3	Copper (µg/dL)	45.92 ± 5.32	44.5 ± 8.46 ns	55.56 ± 7.68 ns	45.08 ± 6.72 ns	66.08 ± 7.09 *

** Significant at 0.01 level (P<0.01); * Significant at 0.05 level (P<0.05); ns Non-significant (P>0.05)

Table 3. Comparison of mite count between Group I and Group II goats

Day	Group I (n=6)	Group II (n=6)	Z-value
0th day	2.83 ± 0.40	2.17 ± 0.54	0.989^{ns}
10th day	1.00 ± 0.37	0.50 ± 0.34	1.042^{ns}
Z-value	2.333* (0.020)	2.232* (0.026)	

* Significant at 0.05 level (p<0.05); ns Non-significant (p>0.05)

were increased, indicating the adequate nutritional anti -oxidant level in the body and health of the integumentary system.

Jesse *et al*. (2016) observed persistence of lesions even after the injection with ivermectin @ 200 µg/kg BW S/C for three weeks. Therefore, they continued the treatment with amitraz bath for three weeks and lesions were resolved. Hence, it was postulated that the systemic injection with ivermectin alone is not at all effective in the treatment of chorioptic mange and the use of topical acaricidal agents along with inj. ivermectin was suggested (Jesse *et al*., 2016).

In the present study, both treatment protocols caused recovery of the clinical symptoms of caprine chorioptic mange (Fig. 4 and Fig.5). However, a skin scraping after 1 year revealed the presence of mites in the goat treated with permethrin.

Conclusion

From the microscopical examination of skin scrapings of infected goats the aetiology of caprine chorioptic mange was identified as *C. texanus* based on their morphological characters. It was the first report of *C. texanus* in goats of Kerala. Chorioptes are generally having small round body and unsegmented pedicel with cup shaped suckers. The *C. txanus* are having short opisthosomal setae 1 and long setae 2. In the present study, crusty erythematous alopecia was noted predominantly on the lower legs. Reduction in haemoglobin concentration and per cent lymphocytes along with elevation in the values of the total leukocyte count, per cent neutrophils and per cent monocyte were observed in affected goats. Values of serum glucose, zinc and copper were normal. Topical application of permethrin or limesulphur along with parenteral ivermectin and vitamin A supplements are equally effective for the therapeutic management of caprine chorioptic mange.

Conflict of interest

The authors declare that they have no conflict of interest.

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Egg quality and fatty acid profile of yolk in eggs from Athulya, native and IWN x native crossbred layers under backyard system#

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Abstract

Backyard poultry production forms the basis for transforming the rural poultry sector from subsistence to a more economically productive base. The current study was conducted to evaluate the egg quality of eggs produced by Athulya, native and IWN x Native crossbred layers under backyard system. At 36 weeks of age, one egg from each group of birds was collected for fatty acid estimation of egg yolk and 20 eggs from each group was collected at 40 weeks of age for assessing the haugh unit score, yolk colour, shell thickness and shell colour. The beneficial polyunsaturated fatty acid content was higher in native bird eggs. The eggs from Athulya and native chicken had better internal quality in terms of haugh unit and yolk colour score. The shell thickness of eggs was similar among three groups. The egg shell colour was white in Athulya, tinted to brown in native and tinted in IWN x Native crossbreds and the shell colour in native and crossbreds exhibits more consumer preference.

Keywords: *Backyard, fatty acid profile, egg quality*

Running title: Egg quality and fatty acid profile of yolk

Rural backyard poultry provides nutritional supplements in the form of valuable nutrients through eggs. In a free-range chicken system, grass, herbs, insects that live in soil and plants

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enrich chicken food and they may assimilate natural nutrients in their eggs. The internal and external quality of the egg is an important trait in both intensive and extensive system of poultry production. The egg quality can be affected by many factors such as breed, feed, temperature, storage, rearing conditions, diseases etc. Eggs with higher haugh unit score possess better internal quality. The darker yolk coloured eggs has more consumer preference and this colour of yolk is affected mainly by xanthophyll content of the feed. Eggs with optimum shell thickness indicates a better external quality of the egg and egg shell colour is an important trait that influence the consumer acceptability and marketability of eggs. The consumption of unsaturated fatty acids is associated with the reduction of undesirable cholesterol and ω-3 polyunsaturated fatty acids are relevant nutrients in egg yolks and are considered as essential fatty acids. The fatty acid content, internal and external quality of eggs can vary in different breeds of chicken when reared under backyard system. The objective of the present study is to evaluate the egg quality and fatty acid profile of yolk in eggs from Athulya, native and IWN x Native birds reared under backyard system.

Materials and methods

Experimental layout

The study was conducted using three group of layer birds namely, Athulya, native and IWN x Native under backyard system of rearing. The experiment birds were reared under deep litter system up to 14 weeks of age. Thirty households were selected and a total of fifty birds were distributed from each group so that each household received a set of five birds. At 36 weeks of age, one egg from each group was randomly selected and a total of three eggs was utilised for fatty acid estimation of yolk. The collected eggs as samples for fatty acid estimation was analysed at Animal Feed Analytical and Quality Assurance Laboratory (AFAQAL), Namakkal, TamilNadu by gas chromatography method. At 40 weeks of age, 20 eggs from each group was collected and was utilised to assess the haugh unit score, yolk colour score, shell thickness and shell colour.

Estimation of egg quality and fatty acid content of egg yolk

Two eggs from each household were randomly collected and a total of 20 eggs were subjected to measure the internal qualities of egg. The eggs were carefully broken without damaging the albumen and yolk for studying the internal qualities. The height of albumen was measured using Ame's tripod stand micro meter. The yolk colour score was assessed using DSM yolk colour fan. Shell thickness was measured using screw gauge micro meter and expressed in mm. Haugh unit score was calculated from height of albumen and weight of respective eggs.

The formula for calculating the Haugh unit score is given below

$$\text{Haugh unit} = 100 \times \log (H - 1.7W^{0.37} + 7.6),$$

where H stands for height of albumen (mm) and W stands for weight of eggs (g).

One egg was randomly selected from each treatment group at 36 weeks of age and the sample egg were subjected to fatty acid profile estimation using Gas chromatography as per O'Fallon *et al*. (2007). Results were expressed as percentage. The difference in relative content of saturated and unsaturated fatty acids of the eggs was recorded.

Statistical analysis

Data collected on egg quality parameters during the experiment were statistically analyzed by means of one-way ANOVA using SPSS Version 24.0. The statistical analysis on fatty acid profile of egg yolk was not done as the data was obtained from pooled samples.

Results and discussion

Fatty acid content of egg samples

The fatty acid content of Athulya, native and IWN x Native crossbred eggs at 36 weeks of age is listed in the table 1.

Fatty acid content of egg yolk is affected by the hen's diet. Higher inclusion

Table 1. Fatty acid profile of Athulya, native and IWN x Native crossbred eggs at 36 weeks of age

Parameters	Treatment groups		
	Athulya	Native	IWNx Native
Myristic acid (%)	2.08	1.77	1.10
Palmitic acid (%)	29.98	29.58	32.65
Stearic acid (%)	11.08	9.94	8.72
Behenic acid (%)	4.03	4.74	3.13
Oleic acid (%)	24.33	26.38	34.85
Palmitoleic acid (%)	5.76	5.96	3.57
Arachidonic acid (%)	0.08	0.03	0.08
Linoleic acid (%)	9.11	11.89	7.69
Eicosapenteanoic acid (%)	5.86	6.22	4.79
Docosahexaenoic acid (%)	3.78	3.34	3.21
Linolenic acid (%)	0.12	0.09	0.17
ω-6 (%)	9.19	11.92	7.77
ω-3 (%)	9.76	9.65	8.17
ω-6:ω-3	1.06	0.809	1.05
Total SFA (%)	47.17	46.03	45.60
Total MUFA (%)	30.09	32.24	38.42
Total PUFA (%)	18.95	21.57	15.94

Table 2. Egg quality parameters of eggs from Athulya, native and IWN x Native crossbred layers at 40 weeks of age are listed below

Group	Haugh Unit score	Yolk colour score	Shell thickness (mm)	Shell colour
Athulya	75.33^{b}±2.47	8.37^{b}±0.33	0.35±0.02	White
Native	73.18^{b}±2.98	7.79^{b}±0.26	0.33±0.01	Tinted to brown
IWN x Native	64.41^{a}±2.60	6.74^{a}±0.30	0.33±0.00	Tinted

Mean values bearing different superscripts differed significantly within a column ($p<0.05$)

of monounsaturated fatty acids and polyunsaturated fatty acid than saturated fatty acid in human diet can reduce cholesterol level. In the present study, the fatty acid contents in the eggs of all the three groups did not differ much. The PUFA content was higher in eggs of native birds. The saturated fatty acid value was lower in IWN x Native birds. The MUFA value was higher in IWN x Native crossbreds. The difference in fatty acid content of eggs might be due to breed effect.

In agreement with the present findings, Sarma *et al*. (2018) reported that the most favourable PUFA ratio was higher in Desi chicken eggs of Assam. Contrary to this, Arsha (2019) recorded a higher SFA and PUFA content in eggs of Athulya (48.30 and 15.65) than native birds (46.37 and 13.04) and a lower MUFA value in Athulya (33.78 and 4.61) than native birds (38.89 and 5.15) under intensive system of rearing. Cherian *et al.* (2002) recorded a similar palmitoleic acid content as that of IWN x Native bird. From the nutritional point of view, the ω6: ω3 ratio is better in eggs from free range (Simcic *et al*., 2011). The lower ω6: ω3 ratio in Desi chicken eggs indicated its superiority over other eggs in reducing cardiovascular risks (Sarma *et al*., 2018).

Egg quality traits

The Haugh unit score of an egg is a measure of albumen quality and a higher Haugh unit score indicates a better internal egg quality. There are many factors such as age, breed or strain of hen, dietary ingredients, storage conditions and possible diseases, which affect

haugh unit values (Roberts, 2004). In the present study, it was found that the birds from IWN x Native crossbreds (64.41) produced eggs with lower albumen height and haugh unit values at 40 weeks of age indicating poor internal egg quality compared to eggs from Athulya and native birds. The lower internal egg quality in IWN x Native crossbreds is in agreement with reports of Harikrishnan *et al.* (2019) in the same crossbred. The present findings of difference in internal egg quality of the three groups can be attributed to the genotypic differences. The haugh unit score of eggs of Athulya and native birds was almost similar in the present study which is in contrary to findings of Lordelo *et al.* (2017) who reported a lower haugh unit score in eggs of commercial hybrids than native Portuguese breeds.

A darker yolk colour is preferred by egg consumers. The colour of egg yolk is influenced by factors like xanthophyll in the feed (Whiting *et al*., 2019), breed or strain of bird and possible diseases affecting pigment or fat metabolism. In the present study, eggs from birds in Athulya and native group had darker yolk colour. The yolk colour was lighter in eggs laid by IWN x Native crossbreds which was in accordance with the reports of Harikrishnan *et al.* (2019) in the same crossbred. The genotypic influence on yolk colour was also reported by Lordelo *et al*. (2017) in which yolk colour in the eggs of native chicken was lighter than commercial hybrids. In the present study, birds from all the three groups had access to green under backyard rearing which was the reason for darker egg yolk colour compared to previously reported results for the same group of birds in intensive system (AICRP, 2016).

There are many factors such as age, breed or strain of bird, feed, ambient temperature and diseases that affect shell thickness (Sreenivas *et al*., 2013). Eggs with shell thickness ≤0.30 mm are considered as thin shelled eggs. In the present study, the egg shell thickness in all the three groups were similar and optimum. This indicates that the feed resources available in the households were sufficient to meet the calcium requirements of these birds for proper egg shell formation. The egg shell thickness in the present study was higher than previously published data for the same breeds in intensive system by AICRP (2016) but lower than egg shell thickness reported by Harikrishnan (2019) which might be due to variation in feeding, housing and ambient temperature.

Even though egg shell colour is not an indicator of nutritive value, it is an important factor influencing consumer preference (Cavero *et al*., 2008). Generally, egg shell colour is a breed characteristic but the intensity of the shell colour can vary within the breed or strain. In the present study, the egg shell colour was white in Athulya, tinted to brown in native and tinted in crossbred birds. The difference in egg shell colour observed among the groups in the present study was due to different genotype of the birds.

Conclusion

Current findings showed that eggs of native chicken contained more beneficial fatty acids. Athulya and native chicken eggs under backyard rearing showed better internal quality than IWN x native chicken eggs. The shell thickness was optimum for three groups of birds and the shell colour was favourable for native and IWN x native birds regarding consumer preference.

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Conflicts of interest

There were no conflicts of interest reported by the authors.

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A SWOT analysis of rabbit farming in Kerala[#]

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Abstract

A survey was undertaken for SWOT (Strength, Weakness, Opportunity and Threats) analysis of rabbit production system in Thrissur and Malappuram districts of Kerala. Sixty rabbit units were selected randomly from the rabbit farmers of the two districts. The rabbit units were classified as small (1-10 doe unit) and medium (>10 doe unit). Thirty farms from each category were selected for SWOT analysis to assess internal and external factors affecting the viability and sustainability of rabbit farms in Kerala. The SWOT factors had Kendall's 'W' value ranging from 0.005 to 0.017 and 0.006 to 0.069 respectively for the small and medium rabbit farms. Based upon outcomes, most important strengths and opportunities could be combined to formulate a functional strategy that can vitalize the rabbit production systems.

Keywords: *Rabbit farming, SWOT analysis, Kerala.*

Running title: A SWOT analysis of rabbit farming in Kerala

Domestic rabbit (*Oryctolagus cuniculus*) is a potential, unexploited, micro-livestock species that possesses a lot of positive features such as high reproductive efficiency, early sexual maturity, short gestation length and short generation interval. Rabbit husbandry has great potential to improve the socio-economic status of the rural poor people. The SWOT analysis of rabbit production system will help to understand the functionality, stability, sustainability and viability of the rabbit farm.

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During the year 2014, the FSSAI (Food Safety and Standards Authority of India) did not include the Leporids in the list of animal species for human consumption, which restricted the slaughter of rabbits thereby resulting in drop in supply and demand for rabbit meat. In April 2016, the FSSAI reinstated the rabbit meat under Food Safety and Standards (Food products standards and food additives) Regulations, 2011. Rabbit production has revived after a period of decline caused by these policy restrictions imposed by the government. Hence a systematic approach is needed for the best exploitation of the rabbit farming system during the revival period. The practices of the top 10 per cent of the most successful farmers with appropriate refinement can be adopted by others for improving their production and income.

Materials and methods

Strength, weakness, opportunity and threats in rabbit farming among the different class of farmer group were studied and suggestions were put forward for the improvement of rabbit rearing in the study area. The methodology for this study applied exploratory research design via structured questionnaire and the Likert method of summated ratings (Murphy and Dooley, 2000 and Sansidar and Reddy, 2012).

Data sets included details about attributes related to strengths, weaknesses, opportunities and threats of the commercial rabbit operations. Terms were appropriately defined and explained to each rabbit farmer prior to the commencement of the survey. In the first phase of the survey, the respondents were requested to list the most critical SWOT-related issues. In the second phase, participants were to rank (rate) each entry on a Likert scale from 1 (not important), 2 (somewhat important), 3 (very important) and 4 (extremely important). A cross-validation step involved presenting preliminary outcomes for consensus, while noting areas of disagreement.

Analysis of SWOT data was done via non-parametric statistics using Kendall's coefficient of concordance (Kendall's W) to determine the extent to which the ranking of SWOT attributes was in accord (agreement) among respondents. The calculation of Kendall's W applied the following formulae (Legendre, 2005)

$$W = \frac{12S}{m^2(n^3 - n)} \qquad S = \sum_{i=1}^{n} (R_i - \overline{R})^2$$

$$Ri = \sum_{j=1}^{m} r_{ij} \qquad \overline{R} = \frac{1}{n} \sum_{i=1}^{n} R_i$$

S = Sum of Squared deviations

m = Number of judges or raters

n = Number of objects

Ri = Total rank given to object i

$\overline{R}$ $\overline{R}$ = Mean value of total rank

r_{ij} = Object i is given the rank r_{ij} by judge number j.

Results and discussion

SWOT analysis of rabbit farms presented in the Table 1. Out of the attributes mentioned in the strengths, first ranking was received for the ability of rabbits to utilize forages in both small and medium rabbit farms. Other attributes arranged in the order of decreasing rank included high prolificacy of does, low incidence of diseases and usage of renewable resources. These attributes were perceived and ranked as strengths of rabbit farms. Kendall's 'W' value for strengths of small and medium rabbit farms were 0.013 and 0.069, respectively. These results were in close agreement with Oseni *et al*. (2016), who reported that the strengths were basic assets of the rabbit rearing enterprise. Low-cost feeding system, usage of locally available biomass and integration of crop-livestock production system were the strengths of rabbit production system.

Out of the attributes mentioned in the weakness, top most ranked attribute was the lower productivity followed by higher production cost, poor quality inputs and rabbits treated as pets. Kendall's 'W' value for weakness of small and medium rabbit farms were 0.012 and 0.013, respectively. These results were in close agreement with Oseni *et al*. (2016), who

Table 1. SWOT analysis of Rabbit farms

Variables	Category of farm					
	Small rabbit farm			Medium rabbit farm		
	Mean	SE	Kendall's 'W' test Mean Rank	Mean	SE	Kendall's 'W' test Mean Rank
Strength						
1. Ability of rabbits to consume forages	2.67	0.211	2.58	2.87	0.218	2.90
2. High Prolificacy of rabbit does	2.60	0.189	2.53	2.50	0.178	2.43
3. Low disease incidence	2.50	0.202	2.58	2.47	0.196	2.55
4. Use of renewable resources (e.g. wood bamboo and forages) as production inputs	2.40	0.207	2.30	2.23	0.190	2.12
			Kendall's 'W'=0.013			Kendall's 'W'=0.069
Weakness						
1. Low overall productivity	2.57	0.213	2.62	2.57	0.190	2.60
2. High production costs e.g. breeding stocks, quality cages, etc.	2.50	0.208	2.60	2.50	0.196	2.55
3. Poor quality inputs	2.40	0.218	2.40	2.40	0.228	2.55
4. Rabbits seen more as "pets" than as livestock	2.40	0.201	2.38	2.27	0.225	2.30
			Kendall's 'W'=0.012			Kendall's 'W'=0.013
Opportunities						
1. Incomes and nutrition securities to households raising rabbits	2.53	0.208	2.48	2.60	0.228	2.60
2. Acceptance across ethnic and religious lines	2.50	0.218	2.60	2.43	0.196	2.55
3. Low investment costs especially for smallholder units	2.47	0.213	2.52	2.37	0.222	2.40
4. Multiple products (weaners, breeding stocks or fryers sold live, slaughtered, frozen or grilled meat, etc.	2.33	0.221	2.40	2.33	0.216	2.45
			Kendall's 'W'=0.005			Kendall's ' W'=0.006
Threats						
1. Low consumption and marketing challenges	2.60	0.212	2.62	2.53	0.190	2.70
2. Potential in poverty alleviation unrecognized	2.53	0.202	2.65	2.47	0.213	2.63
3. No government policy on rabbit production	2.33	0.194	2.33	2.20	0.222	2.40
4. Inappropriate solutions to constraints of production (e.g. nutrition, genetics, housing, etc.)	2.30	0.221	2.40	2.17	0.198	2.27
			Kendall's 'W'=0.017			Kendall's 'W'=0.031

reported that these attributes were liabilities of rabbit production which can affect the growth, development and expansion of rabbit farm.

From the attributes mentioned in the opportunities, highest ranked attribute was the income and nutrition securities to households in both small and medium rabbit farms. This was supported by Owen *et al.* (2005) who suggested rabbitry contributes to poverty alleviation. Other attributes arranged in the order of decreasing rank included acceptability of rabbit meat

across all ethnic and religious taboos and low-cost investment and multiple products from rabbits. Kendall's 'W' value for opportunities of small and medium rabbit farms were 0.005 and 0.006, respectively.

In the attributes related to threats, highest ranking was obtained for the lower consumption and challenges in marketing followed by unawareness of rabbit production in poverty alleviation, no government policy regulations regarding rabbit project and inappropriate solutions to constraints faced by the rabbit farmers. Kendall's 'W' value for opportunities of small and medium rabbit farms were 0.017 and 0.031, respectively.

Conclusion

After SWOT ranking and analysis, weaknesses can be overcome by taking advantage of opportunities and strengths. All the weaknesses and threats could be resolved and overcome by the strategy of implementing successful rabbit projects. Based upon outcomes, most important strengths and opportunities could be combined into a functional strategy that vitalizes the rabbit production systems.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Occurrence of *Escherichia coli* in cloacal samples of broiler chicken from Kollam and Kottayam districts#

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Abstract

Foodborne pathogens like E. coli are considered as the major causes of foodborne illness in humans worldwide. The present study was undertaken to determine the occurrence of E. coli in cloacal samples of broiler chicken from Kollam and Kottayam districts. The occurrence of E. coli in cloacal samples from broiler chicken was 76.5 per cent from Kollam and 79 per cent from Kottayam through culture techniques. Out of the total 400 cloacal swab samples collected from broiler chicken, 77.8 per cent were positive for E. coli. The samples which were subjected to conventional culture techniques were further analysed for PCR confirmation. The study revealed that, 56.5 and 67 per cent samples were positive for E. coli from Kollam and Kottayam, respectively. An overall occurrence of 61.8 per cent out of 400 samples were confirmed for E. coli by PCR. One Health approach can be used as a suitable tool to combat the foodborne zoonotic diseases, since it is an integrated, multidisciplinary, holistic approach. Proper implementation of biosecurity measures in farms is mandatory to control foodborne zoonotic diseases.

Keywords: *E. coli, broiler chicken, one health*

Running title: Occurrence of *Escherichia coli* in cloacal samples

Foodborne illnesses are usually infectious or toxic in nature caused by bacteria, viruses, parasites or chemical substances, entering the body through contaminated food or water. It is often caused by ingestion of eggs, raw or undercooked meat, fresh produce and dairy products

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contaminated by norovirus, *Campylobacter* spp., non-typhoidal Salmonella and pathogenic *E. coli* (WHO, 2015). *Escherichia coli* causes diarrheal diseases, which account for more than four per cent of the total daily global disease burden every day. It also leads to 1.8 million deaths every year, among which 90 per cent were children (Yulistiani and Praseptiangga, 2019). They are the most important foodborne pathogens of *Enterobacteriaceae* family, which causes foodborne illness transmitted mainly through poultry products. Hence, the present study was designed with an objective to find the occurrence of *E. coli* in cloacal samples of broiler chicken.

Materials and methods

The present study was undertaken to determine the occurrence of *E. coli* in cloacal samples of broiler chicken and molecular confirmation of the positive isolates. A total of 400 cloacal swab samples from broiler chicken were collected from different farms of Kollam and Kottayam districts of Kerala. From each district 200 samples were collected for a period of 12 months from October 2019 to November 2020.

After sampling, the cloacal swabs were aseptically placed in tubes containing peptone water. The samples were brought to the laboratory in a thermocool container within 24 h for further isolation and identification by conventional culture techniques. The samples in peptone water were streaked on to MacConkey agar (MCA) incubated at 37°C for 24 h. Followed by plating of lactose fermenting colonies onto EMB agar and incubated at 37°C for 24 h. Colonies with typical characteristics of greenish metallic sheen with dark centre were selected for further confirmation by PCR. The boiling and snap chilling technique was used for the preparation of DNA template (Lee *et al*., 2009).

The molecular reagents and chemicals used in the study were procured from Sigma (Bangalore), Thermo Scientific (Mumbai) and Sigma- Aldrich (USA). The reagents and chemicals used for the PCR were PCR reaction buffer (10X), Taq DNA polymerase (1U/µL), dNTP mix (2.0 mM), $MgCl_2$ (25mM), forward and reverse primer set (100 pmoles/µL) and sterilised milliQ water. The materials used for submarine agarose gel electrophoresis were Tris Boric acid EDTA (TBE), electrophoresis buffer (1X), agarose gel (1.5 %), gel loading buffer (6X), safe DNA staining solution and molecular weight marker (50 bp ladder).

Oligonucleotide primers targeting the *uid*A for *E. coli* were used in the study. The target gene that was detected by PCR and the primer sequences used (Alqahtani *et al.,* 2015) in the study with some modifications are

Table 1. Primers used for the PCR identification of *E. coli*

Sl. No	Primers	Sequence	Size (bp)
1.	*uid*A F	5' TGGTAATTACCGACGAAAACGGC 3'	162 bp
	*uid*A R	5'ACGCGTGGTTACAGTCTTGCG 3'	

Table 2. Components of PCR mixture for amplification of *uid*A gene

Sl. No	Name of reagents	Quantity (µL)
1.	Template DNA	2.0
2.	PCR buffer (10X)	2.5
3.	$MgCl_2$ (25mM)	1.0
4.	*Taq* polymerase (1U/µL)	0.5
5.	dNTP mix (2.0 mM)	1.0
6.	Forward primer (*uid*A) (100 pmoles/µL)	1.0
7.	Reverse primer (*uid*A) (100 pmoles/µL)	1.0
8.	Nuclease free water	16.0
Total		25.0

Table 3. Temperature and cyclic conditions for *uid*A gene

Sl. No	Steps	Conditions	No. of cycles
1.	Initial denaturation	94°C for 10 min	
2.	Denaturation	94°C for 40 sec	35 cycles
3.	Annealing	55°C for 60 sec	
4.	Extension	72°C for 50 sec	
5.	Final extension	72°C for 5 min	

Table 4. Occurrence of *E. coli* in broiler chicken by culture techniques

Sl. No.	District	Total samples analysed	Positive samples		*Chi*-square	p-value
			Number	Per cent		
1	Kollam	200	153	76.5	0 .361^{ns}	0.548
2	Kottayam	200	158	79.0		
Total		400	311	77.8		

p< 0.05 – significant, ^{ns}- Non significant

Table 5. Occurrence of *E. coli* in broiler chicken by PCR

Sl. No.	District	Total samples analysed	Positive samples		*Chi*-square	p-value
			Number	Per cent		
1	Kollam	200	113	56.5	4.668^{s}	0.031
2	Kottayam	200	134	67.0		
Total		400	247	61.8		

p< 0.05 – significant, ^{s}- significant

shown in Table 1. The components of reaction mixture for one reaction are shown in Table 2. The annealing temperature used for the primers of *uid*A gene was 55°C as depicted in Table 3. The PCR products were stained with SYBR safe dye and detected by submarine gel electrophoresis.

Results and discussion

Isolation and identification of E. coli

In the present study, isolation and identification of *E. coli* was done from cloacal swabs of broiler chicken. Similar type of samples were used in various studies conducted by Zinnah *et al*. (2007) from Mymensingh and Ejeh *et al.* (2017) from Nigeria. During the study, the samples were collected in peptone water and further streaked on EMB agar for the selective isolation of *E. coli.* The colonies with characteristic metallic sheen with black centre were identified as *E. coli.* Biochemical tests were used for further characterisation of the organism. This was same as that of the study conducted by Samanta *et al*. (2014) from West Bengal, India, and Akond *et al*. (2009) from Bangladesh.

Occurrence of E. coli in Broiler Chicken by Culture Techniques

The occurrence of *E. coli* in cloacal swabs of broiler chicken from Kollam district was 76.5 per cent and that of Kottayam district was 79 per cent. Altogether 77.8 per cent overall occurrence from both districts were observed. Statistical analysis using Pearson *Chi*-square test revealed that, there is no significant difference (p>0.05) between the occurrence of *E. coli* in broiler chicken from Kollam and Kottayam (Table 4). A study conducted by Kwoji *et al*. (2019) from Maiduguri reported 77.05 per cent occurrence of *E. coli* from broiler chickens, which is in accordance with the present study.

Eze *et al.* (2013) from Nigeria and Stella *et al.* (2016) from Brazil investigated the occurrence of *E. coli* in cloacal swabs from broiler chicken. The study reported 37 and 60 per cent *E. coli* occurrence, which was low when compared to the results of the present study. Also 100 per cent occurrence of *E. coli* in cloacal swabs of broiler chicken were recorded in a study by Wibisono *et al*. (2020) from Blitar, Indonesia.

Escherichia coli is a normal innocuous inhabitant of gastrointestinal tract of man and animals, hence the chance of occurrence is more. Even though they are the common inhabitant in gastrointestinal tract, some of them are pathogenic in nature and are responsible for causing various foodborne illness. Immunocompromised hosts and poultry are negatively affected with pathogenic *E. coli.* In humans *E. coli* responsible for various conditions like endocarditis, meningitis, urinary tract infection, septicemia, epidemic diarrhoea, whereas in case of poultry it causes omphalitis, cellulitis, yolk sac infection, swollen head syndrome, coligranuloma, and colibacillosis. Although most of them are non-pathogenic, they are used as indicators of faecal contamination (Akond *et al*., 2009). Unhygienic and poor management may contribute to the high occurrence of *E. coli* organisms in poultry.

Confirmation of E. coli by PCR

Molecular confirmation of *E. coli* in the current study was done by targeting *uid*A gene. Bej *et al*. (1991) from Connecticut used this gene as a suitable PCR target for *E. coli.* The study by Abdelrahman *et al*. (2008) also revealed that, PCR could be used as a routine diagnostic technique for rapid detection of *E. coli* using *uid*A gene primers.

In this study, PCR confirmed 56.5 and 67 per cent samples as positive for *E. coli* from Kollam and Kottayam districts, respectively. The occurrence of PCR confirmed *E. coli* isolate in broiler chicken from Kollam and Kottayam differ significantly ($p< 0.05$). Altogether, an occurrence of 61.8 per cent *E. coli* positive samples were detected from both districts by PCR (Table 5).

Hossain *et al*. (2008) and Nazir (2004) from Bangladesh reported 60 and 62.5 per cent occurrence of *E. coli,* which is in perfect tune with the present study. A low occurrence of 36.11 per cent noticed in cloacal swabs of broiler chicken by Andrews and Aswathy (2019) from Wayanad, Kerala. Compared to the present results, highest occurrence of 90.7 and 100 per cent were reported by Saidani *et al*. (2017) from Tunisia and Meguenni *et al*. (2019) from Central Algeria, respectively.

Escherichia coli is the most frequently seen pathogen associated with foodborne disease outbreaks, which is often identified by β-glucuronidase enzymatic activity or by detection of *uid*A gene by PCR. Since this house keeping gene found in almost all *E. coli* (Bej *et al*., 1991), it was considered as a suitable PCR target in this study. According to the report by Fratamico (2003), PCR based techniques was more sensitive than culture technique. This also revealed the importance of PCR in the present study.

Conclusion

From the study, it was concluded that an overall occurrence of 77.8 per cent samples were positive for *E. coli* by culture techniques. The samples which were subjected to conventional culture techniques were further analysed for PCR confirmation. The study revealed that, 61.8 per cent samples were confirmed for *E. coli* by PCR. Since *E. coli* is one among the common foodborne pathogens, there is a need for surveillance and control of this organism. A multifaceted One Health approach can combat foodborne diseases. This includes collaborative approach by various disciplines including human medicine, veterinary medicine, epidemiology, environmental specialist, public health institutes and epidemiological surveillance agencies. Upgradation and proper implementation of biosecurity measures are mandatory to control the spread of foodborne zoonotic pathogens.

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Conflicts of interest

There were no conflicts of interest reported by the authors.

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B- mode ultrasonographic biometry of cataractous eyes in dogs#

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Abstract

Cataract is one of the main reasons interfering with examination of the eye in many species of animals. As the opaque lens obscures visualization of the posterior segment of the eye, evaluation of the structures in the posterior segment becomes impossible. B-mode ultrasonography of the eye plays a major role in such conditions to evaluate the posterior segment and to evaluate the biometry of the intraocular structures. In the current study, dogs with cataracts of various stages were subjected to ocular ultrasonographic evaluation and biometry of ocular structures. Biometry for ocular parameters like axial length of the eye (D1), vitreous chamber depth (D2), lens diameter (D3) and lens depth (D4) were recorded in all the dogs with cataractous lens. The ultrasonographic appearance of the lens varied in echogenicity with the stage of the cataract. The changes in the cortex and nucleus part of the lens were also imaged and recorded. B-mode ultrasonography was useful in ruling out structural pathologies related to the vitreous and retina prior to cataract surgery.

Keywords: *Cataract, B-mode ocular ultrasonography*

Running title: B- mode ultrasonographic biometry of cataractous eyes in dogs

The lens is a transparent structure that allows the light to focus on the retina (Patil *et al*., 2014). Cataract is one of the main reasons for vision impairment in dogs and humans (Santosh *et al*., 2019). Cataract is defined as opacity of the lens due to any physical or chemical assaults and the only change it undergoes is opacification unlike other organs (Magrane, 1971). The lens is

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made up of protein concentration of 33% of its total weight with most of those proteins being transparent and water-soluble (Shahzad *et al.,* 2012).

B-mode ultrasonography could be used in cataract where the posterior segment cannot be visualized (Brunell, 2014). It is one of the non-invasive, real time, rapid diagnostic imaging techniques which allows evaluation of internal structures of the eye (Ganesan and Iyer, 2018 and Feliciano *et al.*, 2013). Diagnostic ocular ultrasound is a two-dimensional imaging technique used to determine anatomical standards and pathological alterations, especially when ocular and intraocular opacities prevent the assessment of the posterior segment of the eye (Vali and Razeghi, 2019 and Philip *et al*., 2017). A probe with frequency of 7.5-10 MHz can be used to ocular ultrasonography (Kumar, 2012). In the current study, various intraocular parameters were measured in dogs with different stages of cataract and different changes associated with the morphology of the lens were also documented.

Materials and methods

A total of 55 dogs which were presented with various ocular affections to the University Veterinary Hospitals, Mannuthy and Kokkalai were selected for the study. All the dogs were selected irrespective of breed, sex and age. Detailed physical and clinical examinations were carried out in all the dogs which included recording of rectal temperature, pulse rate (per min), rate of respiration (per min), capillary refill time and colour of the conjunctival mucous membranes. Dogs were grouped into five categories based on their age for the ease of the study and discussion (Table 1).

Ocular ultrasonography was performed using ultrasound machine (MY LAB™ 70 VETXV e-Saote, SpA, Italy.) with 7.5-10 MHz microconvex probe. The probe was placed in the thumb compartment of a new examination glove which was filled with copious amount of coupling gel, for every scan. The glove was secured to the probe with a rubber band placed around to avoid spilling of the gel. This set up functioned as a standoff pad to obtain clear images of the anterior structures, reduce surface artifacts and minimalize the contamination to the cornea. In all the dogs, trans-corneal scan was adopted wherein the probe was placed on the cornea directly and gentle pressure was applied to maintain contact. Prior to the scan, topical desensitization was achieved with a drop of 0.5% proparacaine hydrochloride eye drops (Oproxy ® (Proparacaine hydrochloride 0.5% eye drops, Ophtho Remedies Pvt. Ltd., Allahabad) twice at five minutes interval. The animals were restrained in lateral recumbency or sternal recumbency or while standing with manual restraint. Eye lids were held open manually in all the dogs during the scan.

In every scan, following parameters were measured and recorded in both left and right eyes. Ocular parameters were recorded in the horizontal plane to have a wider diameter of the eye to visualise all the intraocular structures. Biometrical parameters recorded were, axial length (D1) which was the distance between the echoes of the posterior face of the corneal surface and the retina, vitreous chamber depth (D2) which was the distance between echoes of the posterior capsule of the lens and the retina, lens diameter (D3) which was the distance between echoes of the opposite points of lens equator *i.e.* lateral and medial zonules of the ciliary body and lens depth (D4) was the distance between echoes of the anterior and posterior capsules of the lens. All the measurements were recorded in millimeters by using in-built measurement facility of the scanner. Biometry was recorded by placing the cursor exactly in the center of the specified locations (Fig. 1).

The obtained data of ocular biometry of different types of cataracts were analyzed by statistical software SPSS 24.0 using Student's t test. The values of $P < 0.05$ and less were considered statistically significant.

Table 1. Age-wise grouping of the dogs

Group	Age
Group I	0 months to one year
Group II	Two to five years
Group III	Six to nine years
Group IV	Ten to 12 years
Group V	13-15 years

Table 2. Age and type wise distribution of cataract

Age	Immature cataract		Mature cataract		Hypermature cataract		Overall	
	No. of dogs	%	No. of dogs	%	No. of dogs	%	No. of dogs	%
Group I (0 - 1 Year)	1	16.7	2	3	0	0.0	3	11.1
Group II (2 - 5Years)	4	66.7	6	31.3	2	40.0	12	44.4
Group III (6 –9Years)	1	16.7	6	43.8	2	40.0	09	33.4
Group IV (9-12Years)	0	0	2	12.5	1	20.0	03	11.1
Total	6	100.0	16	100.0	5	100.0	27	100.0

Table 3. Comparison of biometrical measurements (in mm) among different type of cataracts

Variable		Immature	Mature	Hypermature	F-value
Right eye	**Axial Length (D1)**	17.82 ± 0.4^{b}	20.2 ± 0.41^{a}	18.58 ± 0.38^{b}	7.268**
	Vitreous depth (D2)	8.92 ± 0.35	10.03 ± 0.58	10.16 ± 0.69	0.791^{ns}
	Lens diameter (D3)	12.92 ± 0.77	11.66 ± 0.5	12.8 ± 0.36	1.393^{ns}
	Lens depth (D4)	6.93 ± 0.49	6.66 ± 0.58	6.22 ± 0.5	0.181^{ns}
Left eye	**Axial Length (D1)**	17.62 ±0.51^{b}	19.66 ± 0.3^{a}	19.02 ± 0.47^{a}	6.412**
	Vitreous depth (D2)	8.6 ± 0.43	9.04 ± 0.41	10.32 ± 0.84	1.755^{ns}
	Lens diameter (D3)	13.1 ± 0.66^{ab}	12.12 ± 0.37^{b}	13.9 ± 0.19^{a}	3.405*
	Lens depth (D4)	7.58 ± 0.34	7.15 ± 0.44	7.14 ± 0.18	0.212^{ns}

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*
Means having different letter as superscript differ significantly

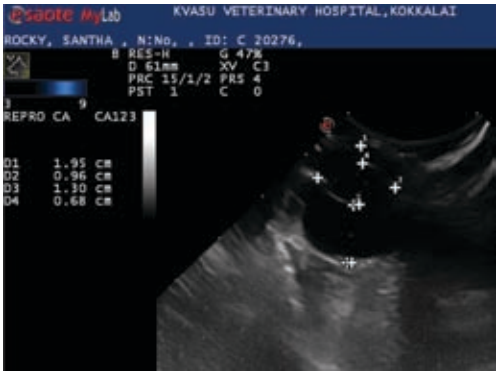

Fig. 1. D1: Axial length of the globe
D2: Vitreous chamber depth
D3: Lens diameter D4: Lens depth

Results and discussion

All the 27 dogs were restrained in sternal or lateral recumbency which was also reported by Boroffka (2006). Topical anaesthesia achieved with 0.5% proparacaine hydrochloride yielded sufficient desensitization long enough for the scanning procedure (Kumar, 2012 and Herring *et al.,* 2005). Trancorneal approach gave clear and sharper images with clear visualization of the anterior and posterior segment of the eyes which was also reported by Toni *et al.* (2013) and Labruyere *et al.* (2008).

In the B-mode scan of the eye, two fluid filled cavities (anterior chamber and vitreous chamber) were anechoic in appearance. The cornea could be visualized as a thin curvilinear hyperechoic line parallel to the probe in all the dogs. Iris was found in continuation with the globe and ciliary body in the form of a single line. The lens appeared anechoic in normal eyes with curvilinear anterior and posterior capsules being echogenic (Kumar, 2012). Under the posterior margin of the lens, vitreous body was seen as an anechogenic chamber, filled with vitreous humor. Vitreous was bordered anteriorly by posterior capsule of the lens and posteriorly by posterior wall

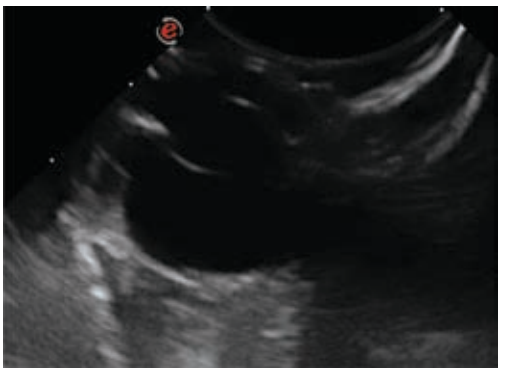

Fig. 2. Immature cataract

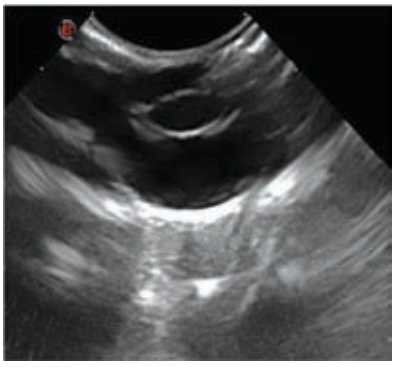

Fig. 3. Mature cataract

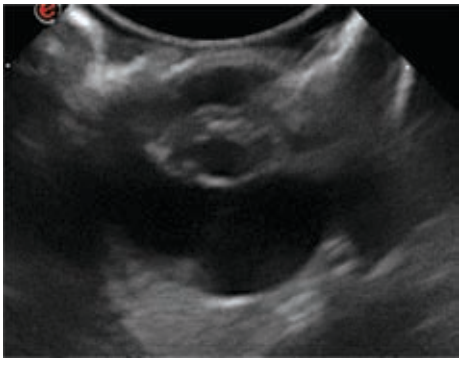

Fig. 4. Hypermature cataract with cortical changes

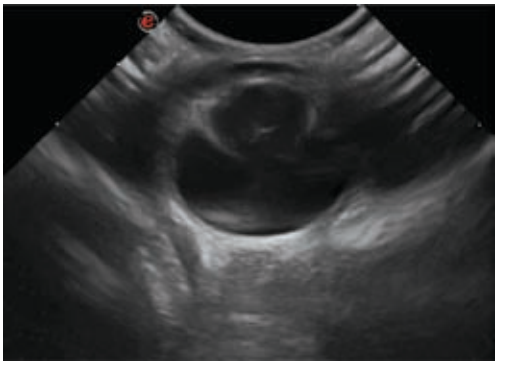

Fig. 5. Intumescent cataract

of the eye ball. At the posterior wall of the eye ball, the optic disc could be imaged as a thick hyperechoic structure which was evidently more echogenic than adjacent structures (Toni *et al*., 2013). The retina, choroid and sclera were visualized as a single hyperechoic line. Ultrasonographic appearance of the immature, mature and hypremature cataract were imaged and documented in the Fig.2-5.

Out of the 27 dogs, 12 of them belonged to the Group II with (44.4 per cent), eight dogs belonged to the Group III (33.4 per cent) and three dogs each in Groups I and IV (11.1 per cent). Among the 27 dogs, 16 with mature cataract, six dogs with immature cataract and five dogs with hypermature cataract. Occurrence of the type of cataract in different age groups were depicted in the Table 2. Santosh *et al.* (2019) reported highest incidence of mature cataract (85.96 per cent) followed by incipient cataract (7.02 per cent), immature cataract and hypermature cataract (3.51 per cent each).

For the six immature cataracts, Mean ± SE (mm) of D1, D2, D3 and D4 were 17.82 ± 0.4, 8.92 ± 0.35, 12.92 ± 0.77 and 6.93 ± 0.49, respectively for the right eye and 17.62 ± 0.51, 8.6 ± 0.43, 13.1 ± 0.66 and 7.58 ± 0.34, respectively for the left eye.

For the 16 mature cataracts, Mean ± SE (mm) of D1, D2, D3 and D4 were 20.2 ± 0.41, 10.03 ± 0.58, 11.66 ± 0.5 and 6.66 ± 0.58 for the right eye and 19.66 ± 0.3, 9.04 ± 0.41, 12.12 ± 0.37 and 7.15 ± 0.44 for the left eye, respectively for 16 mature cataracts.

For the five hypermature cataracts, Mean ± SE (mm) of D1, D2, D3 and D4 were 18.58 ± 0.38, 10.16 ± 0.69, 12.8 ± 0.36 and 6.22 ± 0.5, respectively for the right eye and 19.02 ± 0.47, 10.32 ± 0.84, 13.9 ± 0.19, 7.14 ± 0.18, respectively for the left eye. The mean biometrical values were depicted in the table 3.

In the biometry of various ocular parameters, there was significant difference

in the axial length of the eyes with mature cataract when compared with that of immature and hypermature cataracts in the left and right eyes ($p< 0.05$ level) which was also reported by Kumar (2012). There was significant difference in the lens diameter of the hypermature and mature cataracts in the left eyes. These findings were similar to the reports made by Kumar (2012).

There was no significant difference in the rest of the parameters which was similar to the observations made by (Ingale, 2018, Silva *et al.,* 2010). One case of intumescent cataract was recorded with increased depth of the lens (8.7 mm) which was in accordance with Ganesan and Iyer (2018). The dog with intumescent cataract did not have elevated glucose levels which was not in accordance with the reports made by Ganesan and Iyer (2018) that diabetes mellitus could be a leading cause for intumescent cataracts. There was significant increase in the echogenicity of the lens capsule with progression of stage of the cataract which was also observed by Diaz (2004).

Conclusion

B-mode ultrasonography is an essential aid in evaluating the cataractous eye where the posterior segment could not be visualized in conventional ophthalmic examination. It is required to assess the structural integrity of the other intraocular structures prior to the cataract surgery. Measuring the biometry of intraocular structures in cataractous eyes helps to maintain a database to compare with the echobiometry of intraocular structures of normal eyes.

Acknowledgement

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Conflict of interest

The authors declare that they have no conflict of interest.

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Haemato-biochemical and blood gas changes in bovines under multimodal anaesthesia#

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Abstract

The study was conducted in six crossbred female cattle aged nine months to five years and weighing between 82-375 kg, to evaluate haematobiochemical and blood gas changes in during multimodal general anaesthesia. The animals were premedicated by intravenous administration of butorphanol (0.05mg/kg body weight) immediately followed by xylazine (0.02 mg/kg body weight). On achieving sedation, the animals were controlled in left lateral recumbency and induction of anaesthesia was carried out by intravenous administration of ketamine and midazolam at the dose rate of 4.0 and 0.2 mg/kg body weight respectively. Endotracheal intubation was performed and maintenance of general anaesthesia was carried out using isoflurane in 100% oxygen. The variation in total erythrocyte count, total leukocyte count, volume of packed red cells, platelet count and haemoglobin were non-significant before and after anaesthesia. A non-significant lymphocytopaenia with neutrophilia and mild variations in aspartate aminotransferase and alanine aminotransferase enzyme levels were also noticed. Blood pH reduced significantly ($p<0.05$) after induction of anaesthesia and returned to baseline values after recovery whereas $PvCO_2$ ($p<0.05$) and base excess ($p<0.01$) values increased significantly after induction of anaesthesia. The blood bicarbonate did not alter significantly following induction of anaesthesia.

Keywords: *Multimodal anaesthesia, blood gas changes*

Running title: Haemato-chemical changes in bovines under multimodal anaesthesia

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Maintenance of anaesthesia with inhalant anaesthetics in ruminants is considered to be superior to injectable techniques, with respect to safety and early recovery (Cantalapiedra *et al.,* 2000). The level of analgesia provided by inhalant anaesthesia can be improved by inclusion of drugs like opioids such as butorphanol which is an opioid agonist-antagonist and has good sedative and analgesic properties. Ketamine, though an excellent agent for induction of anaesthesia, causes tonic - clonic seizure like activity when used without preanaesthetic sedation. Addition of α_2 adrenoreceptor agonists like xylazine in the anaesthetic protocol is found to overcome this and enhance sedation, analgesia and muscle relaxation. Midazolam, a short acting benzodiazepine, when administered with ketamine reported to result in better muscle relaxation and analgesia than xylazine with less cardiopulmonary side effects in clinical setting. Combination of α_2 agonist with opioid could provide reliable and uniform sedation (Malik *et al*., 2011). A balanced anaesthesia by combination of these four drugs is more beneficial as the dosage of individual drugs can be reduced and hence the toxicity. The effect of these drugs on haematobiochemical and blood gas parameters during general anaesthesia in adult cattle is reported.

Materials and methods

Six crossbred female cattle aged nine months to five years with body weight ranging from 82-375 kg were subjected to elective surgical procedures. All the animals were numbered I to VI and were subjected to standard preanaesthetic preparation. Food was withheld for 24 hours and water for 12-18 hrs prior to surgery. The animals underwent surgical procedures such as herniorrhaphy with onlay grafting (animal I), excision of interdigital fibroma (animal II), transfixation pinning (animal III), excision of cutaneous mass (animal IV) and umbilical herniorrhaphy (animals V and VI).

The combination of inj.xylazine[1] and inj.butorphanol[2] were administered as premedicants at the dose rate of 0.02 and 0.05 mg/kg bodyweight respectively intravenously.

Once the sedation was achieved, the animals were controlled in left lateral recumbency and induction of anaesthesia was carried out by intravenous administration of Inj.ketamine[2] (4.0 mg/kg body weight) immediately followed by Inj.midazolam[2] (0.2 mg/kg body weight). Upon the induction of anaesthesia, the trachea was intubated with a cuffed endotracheal tube of appropriate size and was connected to semi closed circuit of a large animal anaesthetic machine[2] and the anaesthesia was maintained with isoflurane[2] (2-4%) in 100% oxygen. The onset of sedation was appreciated by symptoms viz; reduced alertness, lowering of head, drooping of eyelids, salivation and in coordinated gait.

The animals, from premedication to recovery from anaesthesia, were monitored by observing physiological and anaesthetic parameters at 15 minute interval. Blood samples were collected before administration of preanaesthetics and after recovery from anaesthesia and were subjected to estimation of haematological parameters *viz;* total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocyte count (DLC), haemoglobin concentration (Hb), platelet count (PLT) and volume of packed red cells (VPRC) and serum biochemical parameters viz; alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also studied. Venous blood was collected in heparinized vials *viz.* before premedication, after induction of anaesthesia, 15 minutes after commencement of isoflurane anaesthesia and after recovery for

6. Inj. xylazine – Indian Immunologicals Ltd. Hyderabad, Telangana
7. Inj. butorphanol – Neon Laboratories Ltd. Mumbai
8. Inj. ketamine – Troikaa Pharmaceuticals Ltd. Ahmedabad, Gujarat
9. Inj. midazolam – Troikaa Pharmaceuticals Ltd. Ahmedabad, Gujarat
10. Mallard 2800 C – AB Medical Technologies, USA
11. Isoflurane – Raman and Weil Pvt. Ltd. Mumbai
12. epoc® Blood analysis system - Siemens Healthineers, India

Table 1. Haematological and serum biochemical parameters before and after multimodal anaesthesia in bovine (n=6)

Parameters	Before anaesthesia	After anaesthesia	t-value	P-value
TLC (103 /µL)	17.75 ± 2.98	17.02 ± 2.73	2.125^{ns}	0.087
TEC (106 /µL)	6.32 ± 0.23	6.06 ± 0.4	1.286^{ns}	0.255
HGB (g/dL)	7.35 ± 0.43	6.92 ± 0.49	1.06^{ns}	0.338
VPRC%	22.13 ± 1.85	21.3 ± 2.39	0.758^{ns}	0.483
PLT (103 /µL)	371.83 ± 33.18	364.5 ± 22.37	0.458^{ns}	0.666
LYM%	64.63 ± 6.99	62.75 ± 8.53	0.576^{ns}	0.589
MON%	3.65 ± 0.62	3.9 ± 1.12	0.401^{ns}	0.705
GRA%	31.82 ± 6.48	32.45 ± 8.17	0.152^{ns}	0.885
ALT (IU /L)	30.12 ± 2.97	31.36 ± 3.35	1.192^{ns}	0.287
AST (IU /L)	73.66 ± 10.62	77.66 ± 14.06	0.949^{ns}	0.386

Analysis done by students 't' test using SPSS version 24.0
ns Non-Significant (P>0.05)

Table 2. Changes in blood gas parameters during different stages of multimodal anaesthesia in cattle (n=6).

Parameters	Before induction	After induction of GA	15mins after ET intubation	After recovery	F-value (P-value)
pH	7.52 ± 0.05^{a}	7.42 ± 0.03^{ab}	7.40 ± 0.02^{b}	7.51 ± 0.04^{ab}	3.916* (0.030)
pCO2(mmHg)	35.02 ± 2.95^{b}	43.93 ± 3.39^{ab}	48.02 ± 2.78^{a}	34.97 ± 4.36^{ab}	4.166* (0.025)
pO2(mmHg)	116.35 ± 19.2	113.28 ± 23.26	119.4 ± 13.19	89.05 ± 24.89	0.622^{ns} (0.612
cHCO3(mmol/L)	23.4 ± 2.93	28.28 ± 0.45	30.45 ± 0.75	28.98 ± 0.80	4.120^{ns} (0.077)
BE(ecf) (mmol/L)	2.90 ± 0.37^{c}	4.05 ± 0.16^{b}	5.75 ± 0.83^{ab}	6.50 ± 0.46^{a}	10.093** (0.004)

Analysis done by repeated measures ANOVA followed by Least Significant Difference method using SPSS version 24.0

*** Significant at 0.01 level (P<0.01); * Significant at 0.05 level(P<0.05); ns Non-Significant (P>0.05)*

Means having different letter as superscript differ significantly within a row

estimation of blood gas parameters like blood pH, partial pressure of carbon dioxide, partial pressure of oxygen, blood bicarbonate and base excess values using automatic blood gas analyser[1]. The changes in haematobiochemical and blood gas parameters during the anaesthesia were analysed statistically.

Results and discussion

The mean total erythrocyte count (10^6/µL) was 6.32 ± 0.23 and 6.06 ± 0.4 before administration of preanaesthetics and after recovery from anaesthesia respectively (Table 1). TEC values reduced non-significantly after anaesthesia. The finding was in agreement with that of Hikasa *et al.* (2000) in sheep and Muchalambe *et al.* (2020) in cattle. The mean total leukocyte count (10^3/ µL) among animals decreased non-significantly after recovery than that before anaesthesia and was 17.75 ± 2.98 and 17.02 ± 2.73 respectively (Table 1). The decrease in leukocyte count after anaesthesia could be attributed to ketamine administration (Cullen and Van Belle, 1975). The TLC values were elevated which could be due to pre-existing subclinical infection. The mean value of haemoglobin concentration (g/dL) was 7.35 ± 0.43 and 6.92 ± 0.49, the mean value of platelet count (10^3/ µL) was 371.83 ± 33.18 and 364.5 ± 22.37 and the mean volume of packed red cells (%) was 22.13 ± 1.85 and 21.3 ± 2.39 before

administration of pre anaesthetics and after recovery from anaesthesia respectively (Table 1). The decrease in haematocrit, platelet count and haemoglobin values observed on recovery from anaesthesia was not significant when compared to pre surgical values. Kilic (2008) reported significant decrease in haemoglobin concentration in calves after detomidine – midazolam – ketamine anaesthesia. Kumar *et al.* (2014) also made a similar observation with ketamine-midazolam anaesthesia in buffalo calves. Muchalambe *et al.* (2020) reported significant decrease in haemoglobin and platelet concentration upon administration of midazolam - propofol and xylazine – propofol combinations as induction agents for isoflurane anaesthesia in cattle. Decrease in VPRC was reported following xylazine administration in calves (Picavet *et al.*, 2004) and in goat (Ahmad and Shukla, 2011) and with isoflurane anaesthesia in sheep (Hikasa *et al.,* 2000). Kilic (2008) and Muchlambe *et al.* (2020) attributed the decreased fluid shift to the intravascular compartment from extravascular compartment to maintain normal cardiac output as the cause for reduction in haematocrit values. Pooling of circulatory blood cells in the spleen due to decreased sympathetic activity could be another cause for reduced packed cell volume, total erythrocyte count, total leukocyte count, platelet count and haemoglobin (Kilic, 2008). However the Hb and VPRC values were below the normal physiological range before and after anaesthesia, which could be attributed to the history of haemoprotozoan infection in two cattle under the study.

The mean lymphocytes count (%) was 64.63 ± 6.99 and 62.75 ± 8.53 at the two observations respectively. The mean granulocytes count (%) was 31.82 ± 6.48 and 32.45 ± 8.17 and mean monocytes count (%) was 3.65 ± 0.62 and 3.9 ± 1.12 before administration of pre anaesthetics and after recovery from anaesthesia, respectively (Table 1). Lymphocytopaenia along with granulocytophilia observed after anaesthesia in the present study was non-significant which could be attributed to the stress caused by administration of pre medicants and induction agents followed by stimulation of adrenal gland (Singh *et al.,* 2013).

Serum alanine aminotransferase and aspartate aminotransferase exhibited insignificant increase before and after anaesthesia. The mean value of alanine amino-transferase level (IU/L) was 30.12 ± 2.97 and 31.36 ± 3.35 and aspartate aminotransferase (IU/L) was 73.66 ± 10.62 and 77.66 ± 14.06 before administration of pre anaesthetics and after recovery from anaesthesia respectively (Table 1). Lower blood circulation to the liver after administration of anaesthetics could be the cause for non-significant increase in liver enzymes after anaesthesia (Malik and Singh, 2007).

The mean blood pH among the studied animals were 7.52 ± 0.05, 7.42 ± 0.03, 7.40 ± 0.02 and 7.51 ± 0.04 before premedication, after induction of anaesthesia, 15 minutes after commencement of isoflurane anaesthesia and after recovery from anaesthesia respectively (Table 2). A significant ($p<0.05$) decrease in pH following induction and commencement of isoflurane anaesthesia was noticed which could be due to respiratory depression and subsequent respiratory acidosis (Benato *et al.,* 2013). Significant decrease in arterial pH was noticed in calves by Picavet *et al.* (2004) and Kilic (2008) following xylazine – guaiphenesin - ketamine and dexmedetomidine – midazolam - ketamine anaesthesia respectively. Harmanjeet *et al.* (2013) also observed significant reduction in blood pH in buffaloes under general anaesthesia. The pH value was marginally elevated above the normal physiological limit before the anaesthesia and fluctuated within the normal limits during the anaesthesia.

The mean blood P_vCO_2 (mmHg) in the study was 35.02 ± 2.95, 43.93 ± 3.39, 48.02 ± 2.78 and 34.97 ± 4.36 before premedication, after induction of anaesthesia, 15 minutes after commencement of isoflurane anaesthesia and after recovery from anaesthesia respectively(Table 2). A significant increase ($p<0.05$) in $PvCO_2$ was noticed after induction of anaesthesia and commencement of isoflurane anaesthesia from the baseline values in the present study. Similar changes in $PaCO_2$ values were reported by Hikasa *et al.* (2000) in sheep, Harmanjeet *et al.* (2013) in buffaloes, Capak *et al.* (2017) in dogs. Hypercapnia observed in the present could be due to alveolar

hypoventilation and positioning of the animals during surgical procedure (Benato *et al.* 2013). The mean blood P_vO_2 (mmHg) was 116.35 ± 19.2, 113.28 ± 23.26, 119.4 ± 13.19 and 89.05 ± 4.89 before premedication, after induction of anaesthesia, 15 minutes after commencement of isoflurane anaesthesia and after recovery from anaesthesia respectively (Table 2). Even though there was a drastic reduction after the recovery, it was not significant statistically. The decrease in P_vO_2 values after induction of anaesthesia may be due to the pressure on diaphragm by viscera while placing the animal on lateral recumbency. The increase in the P_vO_2 values fifteen minutes following commencement of isoflurane anaesthesia observed in this study may be due to the high concentration of oxygen inspired from the fresh gas supply provided after intubation. These observations were in agreement with those made by Picavet *et al.* (2004). The P_vO_2 value was decreased after extubation, probably due to withdrawal of the oxygen on weaning from the anaesthesia machine.

The mean blood -$cCHO_3$ (mmol/L) was 23.4 ± 2.93, 28.28 ± 0.45, 30.45 ± 0.75 and 28.98 ± 0.80 before premedication, after induction of anaesthesia, 15 minutes after commencement of isoflurane anaesthesia and after recovery from anaesthesia respectively (Table 2). The variations were not significant statistically though slight elevation was noticed during post induction of anaesthesia upto recovery. The increase in bicarbonate values noticed during the anaesthesia could be a compensatory mechanism to mitigate the respiratory acidosis caused by increase in P_vCO_2 (Benato *et al.,* 2013).

The mean blood base excess (ecf) (mmol/L) was 2.90 ± 0.37, 4.05 ± 0.16, 5.75 ± 0.83 and 6.50 ± 0.46 before premedication, after induction of anaesthesia, 15 minutes after commencement of isoflurane anaesthesia and after recovery from anaesthesia respectively. There was a significant increase ($p<0.01$) in base excess value after induction of anaesthesia. However the increase was well within the normal physiologic range. The findings of the present study were in contradiction with observations made by Picavet *et al.* (2004) who observed non-significant increase in base excess value in calves. The increase in base excess values could be due to respiratory acidosis caused by an increase in P_vCO_2 value during anaesthetic maintenance (Benato *et al.,* 2013).

Conclusion

In the present study, the haematological, serum biochemical and blood gas parameters were within the normal range, suggesting that the anaesthetic combinations used in the present study did not produce any adverse effect on body systems.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Epidemiological investigation for brucellosis in dogs of Thrissur#

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Abstract

India is endemic to bovine brucellosis, and there is a high potential for transmission of disease from ruminants to dogs. A total of 18 bitches belonging to five different breeds at different stage of abortion (30 days to 65 days of gestation) were selected for this study. Majority of them were showing abortion (88.89 per cent) at 45 to 65 days of the gestation. Microscopic examination of Stamp stained smear obtained from the aborted foetal stomach contents revealed red coccobacillary organisms suggestive of Brucella spp.in three cases. RBPT on paired sera samples on day of presentation and three weeks after abortion showed agglutination within four minutes in five out of 18 female dogs. DNA extracted from the aborted tissues of a RBPT positive Labrador dog yielded amplicons of 193 base pair specific for Brucella spp. on PCR. The results obtained from this study stress the need for screening dogs for canine brucellosis in the current brucellosis surveillance and control programmes.

Keywords: *Bovine brucellosis, abortion, zoonotic disease, stamp staining, RBPT, PCR*

Running title: Epidemiological investigation for brucellosis in dogs of Thrissur

Canine brucellosis is an infectious disease of dogs caused by *Brucella* spp. (*B. canis*, *B. abortus, B. suis and B. melitensis)* (Wanke, 2004). Hollett (2006) opined that canine brucellosis is one of the major causes of abortion and infertility in dogs and possessed a potential health hazard to humans. The Center for Food Security and Public Health (CFSPH, 2018) reviewed that canine brucellosis could end the reproductive life of a breeding animal. The present study was carried out realising the importance of diagnosing a zoonotic disease in the current pandemic situation and paucity of information on canine brucellosis in Kerala.

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Materials and methods

A total of 18 female dogs with a history of abortion at different stages of gestation presented to the University Veterinary Hospitals, Kokkalai and Mannuthy were selected for this study.

On the day of presentation, impression smears from foetal stomach contents were collected and examined by stamp staining method (Alton *et al*., 1988). Paired serum samples were collected from selected animals on the day of presentation and after 3 weeks of abortion for serological studies using Rose Bengal plate test (RBPT).

Stomach contents of aborted foetus and post abortion vaginal discharges collected in sterile containers was used for extraction of Deoxyribonucleic acid (DNA). Brucella genus specific polymerase chain reaction (PCR) was performed from the extracted DNA samples using the forward primer (JPF) sequence (5'-GCGCTCAGGCTGCCGACGCAA-3') and the reverse primer (JPR) sequence (5'-AC CAGCCATTGCGGTCGGTA-3'), at a denaturation temperature of 94°C for 4 minutes, followed by 35 cycles at 94°C for 60 seconds, 60°C for 60 seconds, and 72°C for 60 seconds and one final extension at 72°C for 3 minutes (Leal-Klevezas *et al*.,1995).

Results and discussion

Canine brucellosis holds the nickname, "The Great Imposter" (Bramlage *et al*., 2015), because the clinical signs of infection could mimic many other diseases. In India, the first report of Brucella infection in dogs was that of Pillai *et al.* (1991) from Small Animal Clinic of the Madras Veterinary College, Chennai.

Behzadi and Mogheiseh (2011) reported that all breeds of sexually mature, reproductively active dogs are equally infected by *Brucella* spp. In this study, 18 bitches of five different breeds (Labrador, Rottweiler, Beagle, Spitz, German shepherd dog) at different stage of abortion (30 days to 65 days of gestation) were included. Majority of the abortions (88.89 per cent) were mid to late term (45 to 65 days) of the gestation as described by Wanke (2004) for canine brucellosis (Fig. 1).

The Stamp stained smears obtained from the aborted foetal stomach contents on microscopic examination revealed red coccobacillary organisms against the blue background suggestive of *Brucella* spp. in three cases.

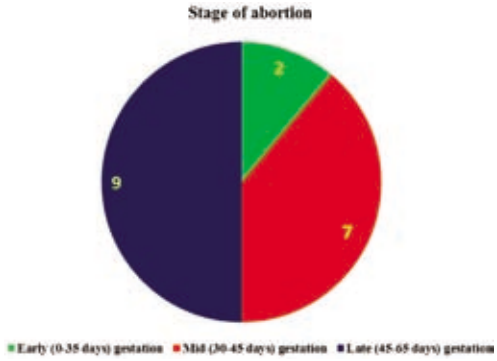

Fig. 1. Stage of abortion in dogs with history of abortion

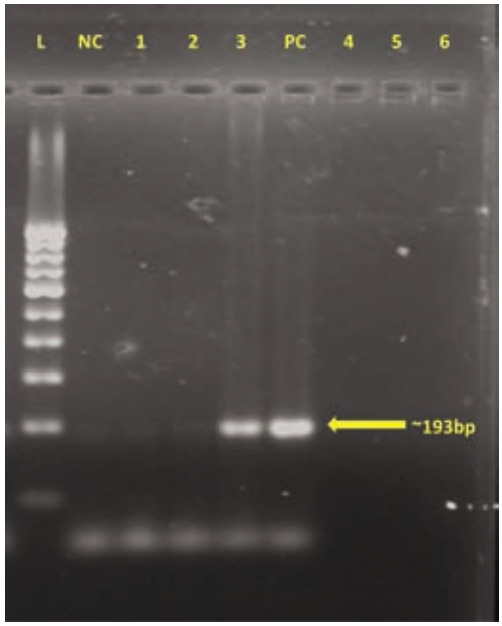

Fig. 2. Agar gel electrophoresis of genus specific PCR of *Brucella* spp. (L- Lane, 100 bp ladder; NC – Negative control; PC- Positive control)

Baek *et al.* (2003) suggested RBPT as the main serological screening test for the diagnosis of brucella infection in suspected dogs with history of abortion. Out of 18 paired serum samples collected, five samples (27.78

per cent) showed agglutination on RBPT using *B. abortus* S99 antigen within four minutes.

Brucella genus specific PCR was performed as described by Leal-Klevezas *et al.* (1995), which yielded an amplicon size of 193 base pair (Fig. 2). Thus, Brucella infection could be confirmed from the DNA samples extracted from the uterine discharges of a RBPT positive Labrador dog.

Canine brucellosis should be considered in the differential diagnosis whenever there is a history of abortion in female dogs (Carmichael, 2018). Cosford (2018) recommended that all male and female dogs should be routinely tested serologically before mating. In brucella tested positive cases, it is advised to neuter or castrate such dogs even though they are of high genetic value. Alamian and Dadar (2020) stressed the need for systematic screening and epidemiological investigations on canine brucellosis among companion animals in order to improve brucellosis surveillance and control programs. Mol *et al*. (2020) clearly demonstrated that the diagnosis of canine brucellosis remained a challenge even when multiple tests were employed.

In this study, a combination of RBPT and PCR, which is an easy, economic and reliable technique helped in proper diagnosis of the case.

Conclusion

To conclude, all dogs of reproductive age should be regularly screened for brucellosis. All urogenital infections in animals should be treated with antibiotics only if required and should be in accordance with the culture and sensitivity pattern of antibiotics. Animals tested positive for brucella infection should not be used for further breeding purposes.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Radiographic assessment of pulmonary metastatic lesions in superficial cutaneous and mammary neoplasms in dogs#

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Abstract

The present study was carried out to assess the pulmonary metastatic lesions in cases of canine superficial and mammary neoplasms presented to the University Veterinary Hospitals, Mannuthy and Kokkalai, Kerala Veterinary and Animal Sciences University during a twelve month period from February 2019 to February 2020. Twenty-four cases of neoplasms in dogs consisting of 12 cases of superficial neoplasms and 12 cases of mammary neoplasms were subjected to fine needle aspiration cytology (FNAC) for the confirmation of malignancy of neoplasm and three-view thoracic radiographs were taken to assess the pulmonary metastatic pattern. The results were correlated with the malignancy detected in histopathological analysis after excisional biopsy. The most commonly observed pulmonary metastatic lesions were pulmonary nodules followed by pulmonary micronodules, miliary nodules, and pulmonary mass. These lesions were more evident in malignant mammary neoplasms especially in tubulopapillary carcinoma, ductal carcinoma, and medullary mammary carcinoma with spatial arrangements more in the perihilar region followed by caudodorsal, midventral, and cranioventral area of lung parenchyma. In case of superficial neoplasms miliary patterns and pulmonary microndules were mostly detected as pulmonary metastatic pattern in soft tissue sarcoma, round cell tumour, and malignant fibrohistiocytoma.

Keywords: *Superficial neoplasm, mammary neoplasm, pulmonary metastasis, computed thoracic radiography*

Running title: Pulmonary metastatic lesions in cutaneous neoplasms in Dogs

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Neoplasms are regarded as one of the most common diseases encountered in the pet population with reasons attributed to the drastic changes in the hormonal and environmental conditions. Canines are prone to develop neoplasms twice as frequently as humans (Rungsipipat *et al*., 2003). Skin neoplasms including that of subcutaneous tissue and adnexa are commonly observed in dogs followed by mammary neoplasms (Klopfleisch *et al*., 2010). The lung is the common site for metastasis in malignant mammary and superficial neoplasms and these metastatic changes may manifest as solitary or multiple pulmonary nodules in the parenchyma or as lymphangitic or endobronchial metastasis (Jung *et al*., 2004). Even though computerised tomography (CT) was found to be more sensitive than radiography for detection of pulmonary metastasis, radiography had a positive predictive value of 83 to 94 per cent in identifying thoracic metastatic lesions (Armbrust *et al*., 2012). Lateral thoracic radiographs alone can cause increased radio opacity which could lead to reduced contrast between pulmonary parenchyma and metastatic nodules located in these lobes. As a result, focal pulmonary consolidations or nodules may not be visible. Hence, the three- view thoracic radiographs (lateral and ventrodorsal/ dorsoventral views) are necessary for detecting early pulmonary metastasis for clinical staging of animals with cancer (Raditic and Bartges, 2014).

Materials and methods

Animals under study were divided in two groups of twelve animals each; group I with superficial cutaneous neoplasms, designated as A_1 to A_{12} and group II with mammary neoplasms as B_1 to B_{12}. All cases were subjected to three- view thoracic radiographic evaluation (right lateral, left lateral and ventrodorsal views) using a 200 mA X-ray machine with computed radiography system. The shortest exposure time, highest kVp and low milliampere × second (mAs) (6-12.5 mAs) was used to minimize the effects of motion. Thoracic radiographs were interpreted for size, morphology, area of distribution of pulmonary metastatic lesions, and atypical pulmonary metastatic lesions. The pulmonary metastatic lesions were categorised as miliary nodules of <2 mm, pulmonary micronodules with 2-7 mm, pulmonary nodule ranging from 7-30 mm, and pulmonary mass with >30 mm diameter. The distribution patterns of pulmonary metastases were recorded as single or multiple lesions in the perihilar, cranioventral, midventral and caudodorsal region of lung parenchyma as described by Mai *et al.* (2008). Atypical features such as tumour embolism (spray paint lesion), bronchial mineralization, calcification, cavitation signs, feeding vessel sign, haemorrhage and secondary pneumothorax (Seo *et al.,* 2001) were also studied. The thoracic radiographic findings on pulmonary metastasis were correlated with the histopathology of the neoplasms.

Results and discussion

Specific pulmonary metastatic lesions and histopathological findings that were detected in the study are depicted in Table 1 and the radiographic features of metastasis including size, distribution of pulmonary metastases and atypical metastatic features are described in Table 2. The most commonly observed pulmonary metastatic lesions were pulmonary nodules followed by pulmonary micronodules, miliary nodules, and pulmonary mass in both groups. The most common pulmonary metastatic nodular pattern in superficial neoplasms were pulmonary micronodules and reticulonodular pattern (Fig. 1.A, 1.B, 2.A and 2.B, respectively) and that in mammary neoplasms were pulmonary nodules followed by pulmonary micronodules, miliary nodules, and pulmonary mass. Depending on the source and type of tumour, the pulmonary metastases could develop a wide range of radiographic appearance (Franquet *et al*., 2020). Lung consolidation might lead to the formation of pulmonary masses (>30 mm size), which were observed in both superficial neoplasm (liposarcoma) (Fig. 3.A and 3.B) and mammary neoplasm (medullary mammary carcinoma) (Fig. 4.A and 4.B) in the present study. Similar unusual form of metastatic spread to the lung was characterized by lepidic growth of the tumor cells along the lining of the alveolar walls, resulting in a primary pulmonary adenocarcinoma like appearance (Franquet

Table 1. Correlation between specific pulmonary metastatic lesions and histopathology of neoplasms

Group	Animal Number	Specific pulmonary metastatic lesions (Thoracic radiograph)	Histopathology of Primary Tumour
GROUP-I	A_1	No metastatic lesion, pneumothorax	Sebaceous adenoma
	A_2	No metastatic lesion, pleural effusion, sternal lymphadenopathy	Fibroma
	A_3	Pulmonary micronodule and mixed disseminated alveolar interstitial pattern	Plasma cell tumour
	A_4	Reticulonodular and mixed disseminated alveolar interstitial pattern	Trichoblastoma (solid)
	A_5	Reticulonodular and mixed disseminated alveolar interstitial pattern	Apocrine adenoma
	A_6	Pulmonary micronodules, pulmonary nodules	Sebaceous adenocarcinoma
	A_7	Pulmonary micronodules pulmonary nodules, pulmonary mass, sternal lymphadenopathy	Liposarcoma
	A_8	Pulmonary micronodules and reticulonodular pattern, sternal lymphadenopathy	Squamous cell carcinoma
	A_9	Pulmonary micronodules, pulmonary nodules, pulmonary mass and reticulonodular pattern	Malignant fibrohistocytoma
	A_{10}	Miliary nodules, pulmonary micronodules and reticulonodular pattern	Squamous cell carcinoma
	A_{11}	Pulmonary micronodules pulmonary nodules, sternal lymphadenopathy	Squamous cell carcinoma
	A_{12}	No metastasis	Hepatoid gland carcinoma
GROUP-II	B_1	Pulmonary nodule and pulmonary mass, pleural effusion, sternal lymphadenopathy	Ductal carcinoma
	B_2	Pulmonary micronodules, Pulmonary nodules and reticulonodular	Tubulopapillary carcinoma
	B_3	Miliary nodules, pulmonary micronodules and reticulonodular pattern, pleural effusion	Ductal carcinoma
	B_4	Pulmonary nodule	Tubulopapillary carcinoma
	B_5	Pulmonary micronodule and reticulonodular pattern	Ductal carcinoma
	B_6	Miliary nodules, pulmonary micronodules and mixed disseminated alveolar interstitial pattern, pleural effusion, pneumothorax	Solid carcinoma
	B_7	Pulmonary nodule	Spindle cell sarcoma
	B_8	Pulmonary nodule	Ductal carcinoma
	B_9	No metastasis, pneumothorax	Fibroadenoma
	B_{10}	Mixed disseminated alveolar interstitial pattern	Ductal carcinoma
	B_{11}	Pulmonary mass, pleural effusion, sternal lymphadenopathy	Medullary mammary carcinoma
	B_{12}	Pulmonary nodule	Ductal cell carcinoma

et al., 2020). In the present study, most of the metastatic lesions were located in the perihilar region followed by the caudodorsal, midventral and cranioventral areas. The maximum number of metastatic lesions were observed uniformly all over the lung as reported by Gowthami (2017). Other metastatic patterns such as interstitial disseminated reticulonodular pattern and mixed disseminated alveolar interstitial pattern observed in right lateral view were 33.33 per cent and 16.67 per cent respectively in Group I and 25 per-cent and 16.67 per cent in Group II. The atypical metastatic lesions such as calcification, secondary pneumothorax and cavitation signs were also observed during advanced stages of metastasis (ductal cell

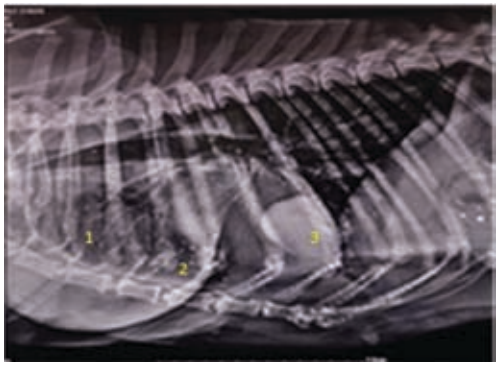

Fig. 1. A. Skiagram of throrax of a dog showing Pulmonary miliary nodules (1), pulmonary micronodules(2) at cranioventral region and pulmonary mass (3) at caudoventral region of lung parenchyma in right lateral view (Case A_9- diagnosed as fibrohistiocytoma)

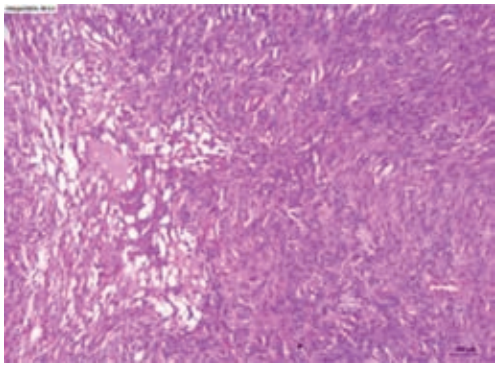

Fig 1. B. Malignant fibrohistiocytoma (Case A_9)
Presence of numerous spindle cells and mononuclear histiocytoid cells (H&E ×200)

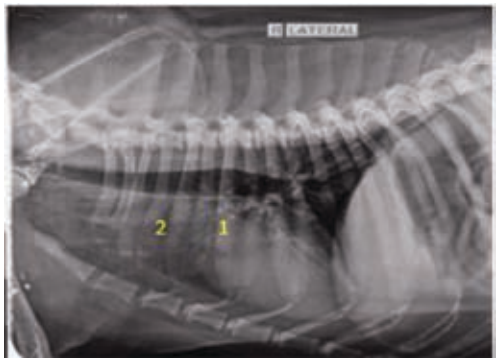

Fig. 2. A. Skiagram of thorax of a dog showing pulmonary micronodules (1) and mixed disseminated alveolar interstitial pattern (2) in the right lateral view (Case A_3- diagnosed as plasma cell tumour)

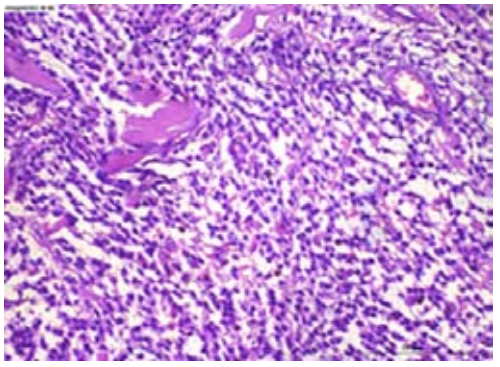

Fig 2. B. Plasma cell tumour (Case A_3)
Presence of sheets of round cells with hyperchromatic and eccentric nuclei (H&E ×200)

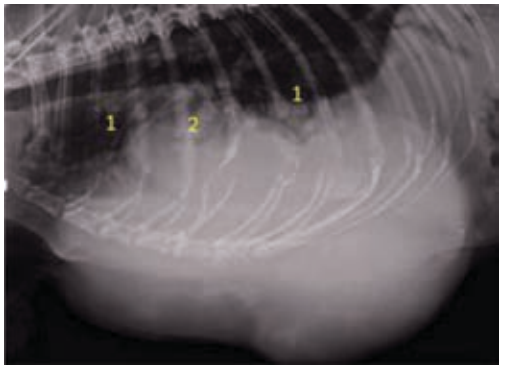

Fig. 3. A: Skiagram of thorax of dog showing pulmonary nodules (1) (Size – 6.1 – 28.32mm) in the cranioventral, perihilar, caudo ventral and midventral areas of lung parenchyma and probable feeding vessel sign (2) in the right lateral view (Case A_7 diagnosed as liposarcoma)

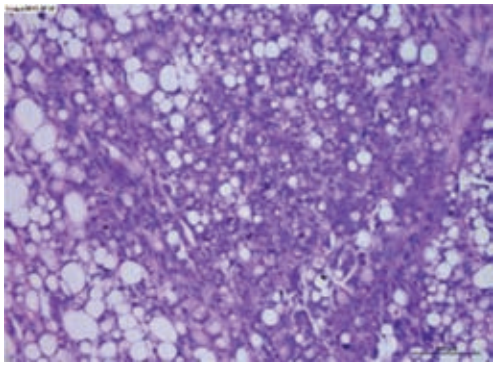

Fig. 3. B: Liposarcoma (Case A_7)
Presence of anaplastic and pleomorphic cells of highly variable morphology, large bizzare multinucleated cells and intracytoplasmic fat vacuoles (H&E ×200)

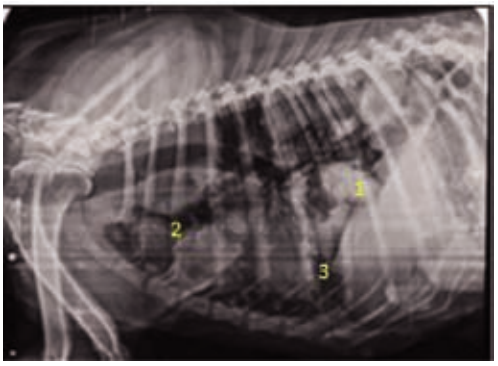

Fig. 4. A: Skiagram of thorax of a dog showing diffused pulmonary masses (Size >30 mm) (1), probable cavitation signs (2) and secondary pneumothorax (3) in right lateral view (Case B_{11} diagnosed as medullary mammary carcinoma)

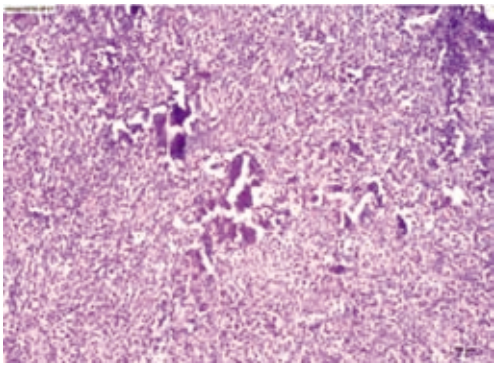

Fig. 4. B: Medullary mammary carcinoma (Case B_{11})
Carcinomatous growth composed of sheets and groups of large pleomorphic, polyhydral or oval cells having larger nuclei. Stroma shows hyalinisation and haemorrhage (H&Ex200)

Table 2. Radiographic assessment of pulmonary metastatic lesions in dogs with superficial (Group –I) and mammary (Group–II) neoplasms

Sl. No	Type of lesion	Group I			Group II		
Based on Size (Number of animals)							
		RL	**LL**	**VD**	**RL**	**LL**	**VD**
1	Miliary nodules <2 mm	3	3	1	2	2	0
2	Pulmonary micronodule (2-7 mm)	7	8	3	4	4	1
3	Pulmonary nodule (7-30 mm)	2	4	2	6	4	3
4	Pulmonary mass (>30mm)	1	1	1	2	1	1
Based on distribution pattern (Number of animals)							
1	Interstitial disseminated reticulonodular pattern	4	3	1	3	3	1
2	Mixed disseminated alveolar interstitial pattern	2	1	0	2	2	1
Atypical features (Number of animals)							
1	Tumour embolism	-	-	x	-	-	x
2	Calcification	2	2	x	3	3	x
3	Cavitation signs	2	1	x	2	2	x
4	Secondary pneumothorax	1	2	x	3	3	x
5	Feeding vessel sign	-	-	x	2	2	x

RL - Indicates right lateral view
LL - Indicates left lateral view
VD - Indicates ventrodorsal view
X - Indicate the lesions that were not identified on that view

carcinoma, medullary mammary carcinoma and liposarcoma). Pneumothorax was observed in two cases of mammary neoplasms (B_6 and B_9) and one superficial neoplasm (A_1). Spontaneous pneumothorax might be the initial sign of pulmonary metastasis and Seo *et al.* (2001) proposed mechanisms as the formation of bronchopleural fistula due to tumour necrosis. Weerakkody and Niknejad (2019) reported a "feeding vessel sign", which consisted of a distinct vessel leading directly to a nodule or a mass, which was suspected in two cases of advanced pulmonary metastasis (ductal cell carcinoma and medullary mammary carcinoma).

Conclusion

Thoracic radiography was found to be reliable, cost and time effective diagnostic procedure for identification of initial or advanced stages of pulmonary metastasis

associated with superficial and mammary neoplasms. Even though thoracic radiography is the primary diagnostic choice for pulmonary metastasis, there are limitations for using this as confirmatory diagnosis; most of the soft tissue nodules of diameter less than 0.5mm could not be done by thoracic radiography. Diagnosis of feeding vessel signs, cavitation, tumour embolism and haemorrhage around the nodule needed further advanced techniques such as CT or MRI.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Insights into the role of *Moringa oleifera* in ameliorating acetaminophen induced hepatotoxicity in Nile tilapia, *Oreochromis niloticus*

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Abstract

The study was aimed to investigate the hepato-protective effect of Moringa oleifera in *Nile tilapia (Oreochromis niloticus) exposed to acetaminophen. Fishes exposed to sub-lethal concentration of acetaminophen for 96 hours, were fed on feed incorporated with moringa leaf, for 21 days. Histological studies of liver of fish fed with M. oleifera leaf incorporated feed , for 21 days after 96-hour acetaminophen exposure showed significant reparative changes when compared to the control. The experiment indicate that dietary supplementation of moringa leaf had hepatoprotective effect in Nile tilapia exposed to acetaminophen.*

Keywords: *Acetaminophen*, *Moringa oleifera, histopathology, hepatoprotection*

Running title: Role of *Moringa oleifera* in ameliorating acetaminophen induced hepatotoxicity

The presence of pharmaceuticals and active pharmaceutical ingredients (APIs) in the environment have become an emerging problem in recent decades (Heberer, 2002). Acetaminophen (N-acetyl-para-aminophenol, paracetamol, APAP) is one of the most widely used over-the-counter analgesic and antipyretic agent. Although acetaminophen has a good safety profile at therapeutic levels, it can cause severe hepatic and renal damage when administered in high dose in both in experimental animals and in humans (Agrawal and Khazaeni, 2020; Ghosh and Sil, 2007). Liver being the major organ involved in detoxification shows alterations in response to exposure to harmful components. Conventional hepatoprotective drugs used for the treatment of such adverse reactions are often inadequate. Traditional herbal drugs are significant repositories of chemical constituents which are hepatoprotective in nature. There is an increasing demand for phyto-drugs and therefore it is essential to explore new medicinal plants to ameliorate the hepatotoxic effects of acetaminophen.

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Moringa oleifera, commonly known as drum-stick, is found mostly in Asia, Africa and South America. It is also known as miracle tree due to its medicinal properties. All parts of this tree such as seed, leaf, root, bark, flower etc. have medicinal value. *Moringa oleifera* is used as an anti-inflammatory, antioxidant, antidiabetic, antiproliferative, anti-ulcer, hypolipidemic and hepatoprotective agent (Al-malk and El Rabey, 2015; Saini *et al*., 2016; El Rabey *et al*., 2018; Elbakry *et al*., 2019). The main objective of this study was to examine the ameliorative and hepatoprotective effect of *M.oleifera* leaves in acetaminophen induced toxicity in Nile tilapia.

Materials and methods

Moringa oleifera leaves collected locally from Ernakulam district, were washed in clean water and dried under shade for 7 days. Using a mechanical homogenizer the leaves were powdered. The fine powder was stored in a clean and dry container at room temperature. Acetaminophen exposed fishes were fed with feed mixed with drum stick leaf powder, at a rate of 2.5g/day/kg for 21 days.

The male *O. niloticus* of mean length 15 ± 1 cm weighing about 100 g were collected from fish farm at Maliankara, Ernakulum district in Kerala, India. The fishes were brought to the laboratory and acclimatized for a week (pH 6.5, temperature 28°C, dissolved oxygen 3.73mg/L).

The entire experiment was divided into two phases. In the first phase, a control group of 15 fishes after acclimatization, was kept in dechlorinated tap water (Group I). Another group of 15 fishes (Group II) were kept in water containing 1/5th LC_{50} of acetaminophen (0.33 ml of acetaminophen per litre of water) for 96 h. The xenobiotic level was maintained constant, after renewal of water every day. After four days, 5 fishes each from Group I and II were sacrificed (Rema, 1995), liver dissected and separately pooled. The liver tissue, washed and wiped thoroughly with blotting paper to remove blood and other body fluids, was then fixed in Bouins fluid for 24 h and processed further to form the wax blocks. Blocks with the tissue were cut into thin section of five micrometer thickness and stained with haematoxylin and eosin. The sections were observed under phase contrast microscope with attached photomicrography (NIKON ECLIPSE 80 i) under 400 X magnification.

During the second phase, the remaining 10 fishes from Group II were again grouped into two and was subjected to feeding study. One group was fed on normal fish feed (proximate composition: protein 30%, fat 5%, fiber 5.5% and moisture 11.5%) and formed the control for the second phase of experiments. The second group was fed with f normal fish feed incorporated with moringa leaf powderfor 21 days. After the period of feeding, liver was carefully dissected out and processed as described earlier.

Results and discussion

In the present study, liver of control group showed normal arrangement of hepatic parenchyma with centrally placed nucleus and clear cytoplasm. Each hepatocyte had clear spherical nucleus. Hepatocytes were located among sinusoids and arranged as cords (Fig.1-A) Generally healthy teleost fish showed normal architecture with a typical parenchymatous appearance without pathological abnormalities (Vicentini *et al*., 2005; Figueiredo *et al*., 2007).

Liver of fish exposed to 1/5th LC_{50} of acetaminophen for 96 h showed acute congestion (Fig.1-B). In some regions, hepatocytes were hypertrophied with degeneration of cytoplasm . Necrosis of hepatocytes was seen in almost all regions of liver. Distended sinusoidal space with blood is indicative of acute congestion. Blood vessels were also engorged within the pancreatic tissue also. There was vacuolation in the periphery of hepatic parenchyma and extensive congestion with sinusoids severely expanded with blood. Surrounding the sinusoids there was a fatty infiltration. Pancreatic tissues were highly congested and bile duct was hyperplastic. Degeneration of hepatocytes, nuclear pyknosis in most cells and accumulation of metal binding proteins in its nuclei were reported in the liver of cadmium treated *O. niloticus* (Kaoud *et al*., 2011). Kavitha *et al.*(2011) reported

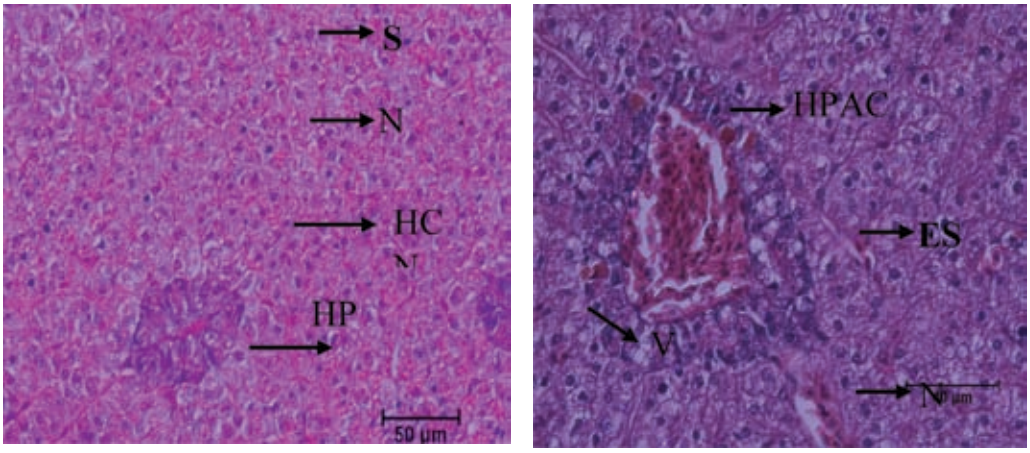

Fig. 1. Histological changes in liver of *Nile tilapia* in first phase of experiment

A-normal liver (control) showing uniform arrangement of hepatocytes (HC) with normal nucleus (N), normal sinusoids (s) and normal hepatopancreas (HP). **B**. acetaminophen treated liver ($1/5^{th}$ LC_{50} 96 h) showing acute congestion in hepatopancreas (HPAC), expanded sinusoid (ES), vacuolation (V) and necrosis (N).

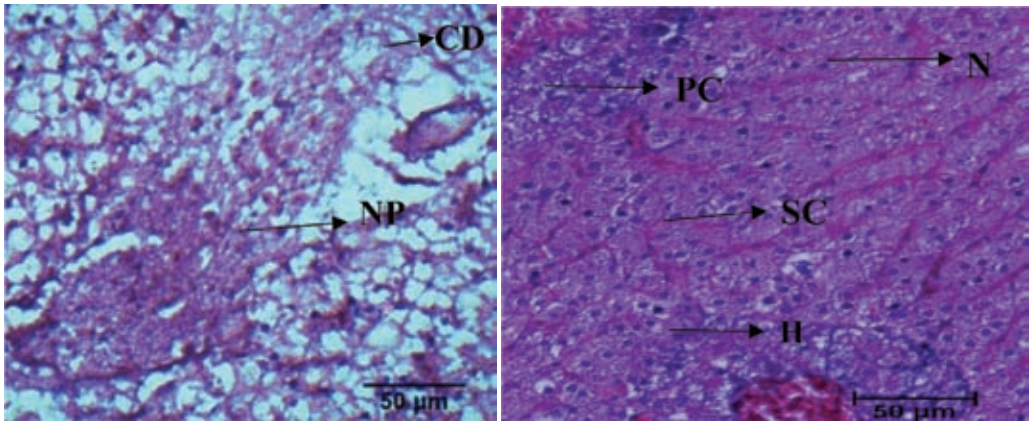

Fig. 2. Histological changes in liver of Nile tilapia in the second phase of experiment

A. Paracetamol treated control liver showed cell death (CD) and nuclear pyknosis (NP). **B.** Liver of moringa leaves fed group showed comparatively mild sinusoid congestion (SC), pancreatic congestion (PC), normal nucleus (N) and normal hepatocytes (H)

hepatocellular vacuolation and blood vessel congestion in *O. mossambicus* exposed to acetaminophen at 500mg/Kg orally. Hepatic injuries like degeneration of vacuoles in the cell and necrotic aggregation of cells that was associated with oxidative stress, were observed in *Danio rerio* exposed to acetaminophen (Jyotsna, 2016).

The liver of control group in the second experiment (acetaminophen treated) showed diffused degenerative changes like extensive pancreatic and hepatocyte congestion. Signs of cell death could be observed with most cells Nuclear pyknosis was a consistently observation. Cytoplasmic architecture was also completely lost compared with the test group. Blood vessels were highly congested and filled with blood. Erythrocyte infiltration into sinusoids was observed. Loss of reticular fibres, which cross links and act as a supporting mesh in soft tissues of liver, was a common feature (Fig. 2. A). Liver is one of the main organs which regulates many important metabolic functions and hepatic injury directly affects metabolic functions of the liver (Mitra *et al*., 1998).

Histopathological evaluation plays a major role in the assessment of the harmful effects of xenobiotics (Reddy and Rawat, 2013). Van der Oost *et al*. (2003), reported that histopathological examinations have long

been recognized to be reliable biomarkers of fish exposed to stress. Hepatotoxicity is one of the very common disorders resulting in serious debilities ranging from severe metabolic disorders to mortality (Patel *et al*., 2008). Paracetamol is considered as a safe drug, if consumed at correct dose rates. But overdose may lead to dangerous and fatal conditions (Penna and Buchanan, 1991), as this drug is hepatotoxic at high concentrations. The liver is one of the major organs responsible for the metabolism of endogenous and exogenous compounds and one of the first target organs to toxic insults (Cao *et al*., 2016).

Compared to control group, liver of fish fed with feed containing moringa leaves, showed lesser signs of hepatotoxicity. In moringa leaf treated group, hepatocytes had normal arrangement with prominent nucleus. Hepatic cells maintained their round polygonal shape. The size of the hepatic cells reflects their physiological functional state. In some regions the cells showed mild enlargement, mild nuclear hypertrophy and sinusoids showed minute swelling. Mild pancreatic congestion and mild vacuolation were also observed in some regions (Fig. 2. B). Uma *et al*. (2010) and Fakurazi *et al*. (2012) observed that *M. oleifera* leaves were able to protect acetaminophen-induced liver damage by decreasing liver enzymes and hepatic lipid peroxidation as well as by increasing antioxidant enzyme levels. Many plants and their products have the potential to protect liver from toxic effects of hepatotoxins. This is due to the presence of active constituents such as alkaloids, sterols and flavonoids which have free radical scavenging activities (Anwer *et al*., 2008). In our study also, the tissues of liver of moringa fed fish showed considerable reparative changes. Ross and Kasum (2002) reported, that plant secondary metabolites such as flavonoids have much antioxidative, anti-inflammatory, anti-proliferative, radical-scavenging activity and pro-apoptotic effects in various cell types. Tekle and Sahu (2015) investigated the ameliorative effects of moringa flower on *O. niloticus* subjected to *Aeromonas hydrophila* induced stress. Moringa leaves have efficient antioxidant properties (He *et al*., 2018). The beneficial functions of *M.oleifera* are closely associated to its high content of phytochemicals such as flavonoids, glucosinolates, phenolic acids and isothiocyanates (El-Hadary and Ramadan, 2019).

Conclusion

The present study emphasises the protective role of moringa leaves against acetaminophen induced damages in liver tissue of *Nile tilapia*. Administration of moringa leaves partially restored the general structure of the liver. Moringa leaves may be considered as a potential source of natural antioxidants and phytochemicals against hepatotoxicity.

Conflict of interest

The authors declare that they have no conflict of interest.

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In vitro maturation of goat oocytes selected using Brilliant cresyl blue staining

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Abstract

The present study was conducted to assess the developmental competence of goat oocytes selected using Brilliant cresyl blue (BCB) staining. Goat ovaries were collected from the slaughtered animals with unknown reproductive history. The oocytes retrieved by aspiration technique were selected based on morphology and subjected to BCB staining. Brilliant cresyl blue staining is based on the activity of glucose-6-phospahte dehydrogenase (G6PDH) enzyme synthesised by the oocytes. The cytoplasm remains blue in oocytes that have finished the growth phase (BCB+) while the growing oocytes remain colourless (BCB-). The stained and unstained oocytes were subjected to in vitro maturation separately to assess cumulus cell expansion index and polar body extrusion. A total of 206 culture grade oocytes were subjected to study, out of which, 76.75 ± 2.38 per cent of oocytes showed positive to BCB staining and 23.21 ± 2.38 per cent were negatively stained. Significantly higher maturation rate was observed in BCB+(92.89 ± 2.37%) oocytes than BCB-(29.72 ± 2.46%). The present study concluded that BCB staining can be used for selecting goat oocytes with good cytoplasmic maturation for further in vitro embryo production.

Key words: *Goat oocytes, in vitro maturation, Brilliant cresyl blue*

Running title: *In vitro* maturation of goat oocytes selected using Brilliant cresyl blue staining

Selection and identification of good quality oocytes is the critical step for *in vitro* embryo production which in turn ensures better *in vitro* maturation (IVM) and blastocyst formation (Egerszegi *et al*., 2010). Generally, the oocyte quality is determined by the morphological characters like number of cumulus cell layers and uniformity of the cytoplasm. A homogeneous population of oocytes with the same morphological characters differ in their developmental competence. Thus, BCB staining is proposed to assess the cytoplasmic competence which is predominantly responsible for oocyte competence.

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Brilliant cresyl blue is a vital stain that allows to determine the intra cellular activity of the glucose-6-phospahate dehydrogenase (G6PDH) which is an enzyme synthesised in the cytoplasm of growing oocytes but remains low in the oocytes that have finished their growth phase. As the enzyme is active during growth phase, immature oocytes reduce BCB to a colourless compound whereas, oocytes that have finished their growth phase show decreased activity of the G6PDH and their cytoplasm remain blue (Shabankareh *et al*., 2014). Studies in different farm species revealed that BCB selected oocytes were having uniform distribution of mitochondria, more lipid content and also high glutathione level all indicating good developmental competence (Ghoneimy *et al*., 2017). The purpose of this study was to use BCB staining as a selection tool to identify more competent goat oocytes having good cytoplasmic maturation for IVM and further processing for *in vitro* embryo production.

Goat ovaries transported to the laboratory within two hours of slaughter were washed several times in normal saline and removed excess tissue debris to reduce contamination. The visible surface follicles were aspirated and their contents were collected using a 10mL disposable syringe attached to 20G needle. Oocyte collection medium (OCM) was used for retrieving cumulus oocyte complexes (COCs). Preliminary selection criteria for COCs were based on morphological characteristics like uniformity of cytoplasm and compactness of cumulus cells (Shioya *et al*., 1988). The COCs were graded according to the number of cumulus layers present around the oocyte (Koeman *et al.,* 2003) as Grade-A (≥3 cumulus layers), Grade-B (1-2 cumulus layers), Grade-C (denuded) and Grade-D (expanded cumulus). Grade A and B oocytes were selected for further *in vitro* studies.

The washed oocytes were subjected to BCB (B-5388-Sigma) staining by incubating at a concentration of 26µM at 37°C for 90 min. The stained oocytes were washed three times in maturation medium and grouped as BCB+ (blue coloured) and BCB- (colourless) depending on the blue colouration of cytoplasm (Fig.1). *In vitro* maturation was carried out separately for BCB+ and BCB- oocytes. Microdroplets of 100µL maturation medium were prepared and 20 to 25 oocytes were transferred to each microdroplet under mineral oil. Incubation was carried out at 38.5ºC in a humidified atmosphere under 5 per cent CO_2 for 27h.

After IVM, the maturation status of oocytes was assessed based on the degree of cumulus cell expansion and extrusion of first polar body in the perivitelline space. A total of 265 goat oocytes were collected from 353 follicles from 94 goat ovaries and classified based on the number of cumulus cell layer and uniformity of cytoplasm. Number for follicles obtained per ovary was 3.44 ± 0.26 and the number of COCs per ovary was 2.84 ± 0.19 with an oocyte recovery rate of 75.07 per cent. The maximum yield of COCs were obtained for grade A (48.43 ± 2.49%) followed by grade B (27.23 ± 2.22%). The yield of grade C and D were 12.28 ± 2.43 and 12.03 ± 2.46 per cent respectively. Since, only Grade A and B were considered as culture quality oocytes, they were selected for further *in vitro* studies. Out of 206 culture grade oocytes selected, 76.75 ± 2.38 per cent of oocytes showed positive to BCB staining and 29.72 ± 2.46 per cent oocytes were negative to BCB staining (Table 1). Maturation was assessed by cumulus cell expansion index following IVM. Most of the matured BCB+ oocytes were having a cumulus expansion index of grade one and two, indicating good quality and BCB- oocytes were having more per cent of unexpanded oocytes of grade zero, indicating poor quality oocytes. The overall maturation rates of BCB+ oocytes were found to be 92.89 ± 2.37 and 29.72 ± 2.46 per cent, respectively (Table 1). On statistical analysis, there was significant ($p<0.05$) differences between the maturation rate of BCB+ and BCB- oocytes.

Table 1. Yield and *in vitro* maturation rate of BCB selected goat oocytes

BCB Staining	Yield (%)	*In vitro* maturation rate (%)
BCB+	76.78 ± 2.38[a]	92.89 ± 2.37[a]
BCB-	23.21 ± 2.38[b]	29.72 ± 2.46[b]

*** Significant at 0.01 level*

Means having different letter as superscript differ significantly within a column

Improvement in the oocyte screening can be achieved by the application of BCB staining in different species. The G6PDH activity was associated with oocyte diameter, lipid content, mitochondrial distribution and high glutathione content and those oocytes were having good cytoplasmic maturation and higher developmental competence (Wu *et al*., 2007). The percentage of BCB+ oocytes were higher than BCB- oocytes as described by several authors (Mohammadi-Sangchehmeh *et al*., 2014 and Abazari-kia *et al*., 2014). Usually, the oocytes were selected on the basis of morphological criteria alone, but oocytes that appear similar in morphology might vary in their developmental capabilities post insemination. Hence, application of an additional selection tool to choose oocytes with good cytoplasmic maturation, such as BCB staining would improve the IVEP outcome. In the present study, a higher yield of BCB+ oocytes than negative could be due to the fact that a preliminary selection was done based on morphology. Rodríguez-González *et al*., (2003) observed more maturation rate for BCB+ oocytes than BCB-. Cumulus expansion is the indicator of the ability to provide energy, transport of the messenger molecules or mediate the effects of hormone during the period of maturation and presence of cumulus investment increases the fertilisation and embryo development rate compared with that of denuded oocytes (Tange *et al*., 2004).

Good maturation rate indicates the quality oocytes and therefore good developmental competence for further *in vitro* developments. The study revealed that the goat oocytes selected by BCB staining (BCB+ oocytes) had a higher per cent of maturation rate when compared to BCB- oocytes.

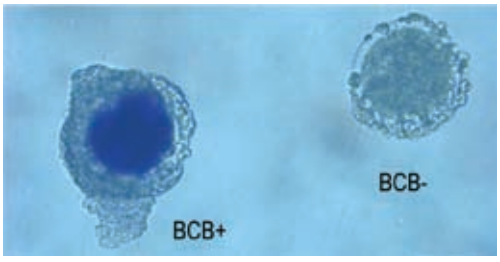

Fig. 1. Brilliant cresyl blue staining of goat oocytes (200X)

Summary

The oocytes selected by this method (BCB+) showed higher degree of cumulus cell expansion and maturation rate than BCB- oocytes. Brilliant cresyl blue being a non-invasive stain, the selected oocytes can further be used for *in vitro* fertilization and embryo production. Hence, this staining method can be used as an effective tool for the selection of good quality goat oocytes for *in vitro* embryo production.

Conflict of interest

The authors declare that they have no conflict of interest.

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Coagulation profile in two nephropathic dogs#

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Abstract

Coagulatory abnormalities are common in renal dysfunction in humans. The studies on coagulatory abnormalities in renal failure in dogs are limited. The present paper deals with coagulation profile in acute and chronic kidney disease in dogs. The haemostatic defects observed in acute renal dysfunction included thrombocytopaenia, prolonged capillary bleeding time (CBT), elevated D-Dimer and hypoantithrombinemia which indicated a hypercoagulable state. Prolongation of prothrombin time (PT), activated partial thromboplastin time (aPTT), elevated D-Dimer concentration and hypoantithrombinemia in chronic kidney disease indicated the presence of hypocoagulable state.

Key words: *Coagulation profile, nephropathy, dog*

Haemostasis could be defined as a complex process of blood clot formation at the site of vascular damage. The haemostatic system is in a balance between procoagulant factors and anticoagulants. Any imbalance in these factors could result in thrombosis or bleeding. Haemostatic abnormalities in renal diseases are common but not widely studied in dogs.The present paper deals with haemostatic defects in two nephropathic dogs presented to the University Veterinary Hospital, Mannuthy.

The first case was a one year old male Rottweiler dog with a history of viper envenomation four days prior to presentation of the animal in the hospital .The animal was treated with antivenom, Vitamin K and prednisolone at a near by veterinary hospital. The owner reported that the animal was not taking food and water even after the treatment. The owner observed vomiting, red colured urine and reddish spots on the abdomen on the day prior to the presentation. The second case was a nine year old female Labrador retriever with a history of anorexia, black-coloured feaces, anuria, vomiting and blood streaks from mouth since five days.

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Upon examination of the first case with the history of viper envenomation, the animal was dull and depressed. The whole blood was clotted within 20 min. Edema on the eyelids (Fig. 1) and right forelimbs, congested mucous membrane, petechiae (Fig. 2), haemoglobinuria, hyperventilation and anuria was also recorded.whereas in the latter case, ulcerations on the buccal mucosa (Fig. 3), hypersalivation, melena, respiratory distress, congested mucous membrane and subnormal temperature were observed. The haematobiochemical values were represented in the table 1.

Echinocytes and schistocytes were observed in acute kidney injury on blood smear evaluation. On ultrasonography, increased cortical echogenicity of the left kidney (Fig. 4) and splenomegaly was observed in first case with snake envenomation. No corticomedullary distinction with irregular contour of kidney was found in the second case.

Based on the findings such as history, clinical signs, ultrasonographic findings, haematology and serum biochemistry, the first case was diagnosed as acute kidney injury and second case as chronic kidney disease.

Treatment was initiated with Amoxicillin-sulbactum @ 12.5 mg/ kg BW IV BID, proton pump inhibitors (Pantoprazole @ 1 mg/kg BW, IV OD), antiemetics (Ondensetron @ 0.5 mg/ kg IV BID), B complex and fluids for both the cases for two days. Darbepoeitin injection at the rate of 0.25 µg / kg BW SC once was given for chronic renal failure case. Animal diagnosed with chronic kidney disease died on the day two of treatment. The animal with acute kidney injury was advised for dialysis and was not presented for further treatment. The owner reported that the animal died after one week of presentation.

Table 1. Haematobiochemical and coagulation changes of nephropathic dogs

Parameters	Control	Case I	Case II
Total erythrocyte count (*10^6/cmm)	6.36±0.25	3.81	3.76
Haemoglobin (g/dL)	13.06±0.5	9.1	6.7
VPRC (%)	35.81±0.19	21.5	16.9
MCV (fL)	66.00±1.21	56.4	44.9
MCH (pg)	21.56±0.68	23.9	17.6
MCHC (g/dL)	33.95±0.81	42.3	39.6
Total leukocyte count (*10^3/cmm)	10.31±1.21	26.5	7.8
Total neutrophil count (*10^3/cmm)	7.23±1.05	21.5	5.2
Total platelet count (*10^3/cmm)	266.50±28.49	17	48
MPV (fL)	7.51±0.14	6.5	6.8
PCT (%)	0.20±0.02	0.012	0.02
PDW (%)	14.98±0.16	11.4	13.2
ALT (U/L)	46.83± 4.07	228.1	97.1
ALP (U/L)	147.73± 26.16	540	659.5
Total protein (g/dL)	5.71 ±0.29	4.03	4.3
Albumin (g/dL)	2.95±0.10	1.25	1.9
Globulin (g/dL)	2.76± 0.22	2.78	2.4
Creatinine(mg/dL)	0.90±0.01	11.9	26.8
CBT (min)	0.40±0.03	3.3	6
PT (sec)	8.5±0.66	12.2	15
aPTT (sec)	15.46±1.6	19.9	23
ACT (sec)	60±7.7	90	150
FDPs (µg / mL)	0.93±0.26	2.415	2.92
D-Dimer (ng / mL)	58.41±8.60	157	193
AT III (ug / mL)	222.56±34	109	26

Detailed study were conducted in the cases to assess the coagulation status of animals based on platelet morphology and coagulation parameters such as capillary bleeding time (CBT), prothrombin time (PT), activated partial thromboplastin time (aPTT), activated coagulation time (ACT), D-Dimer (DD), FDPs and antithrombin III (AT III) concentration (Table 1).

In the first case with the history of viper envenomation, the acute kidney injury could be due to the renal ischemia secondary tohaemorrhage, hypotension, intravascular haemolysis, enzymatic activities in venom and rhabdomyolysis. Chronic renal failure in the second case might be due to the progressive long standing renal injury which affected all the renal compartments. Microcytic anaemia and hypoproductive thrombocytopaenia observed in nephropathic cases could be due to the erythropoietin deficiency in chronic kidney disease (Rubin and Carr, 2007) or due to the venom mediated bone marrow depression in acute kidney injury (Trompouki*et al.,* 2017). Neutrophilic leukocytosis observed in acute renal failure of this studywas in accordance with Nagel *et al.* (2014) who reported that these changes might be associated with cellular damage, inflammatory changes and cytokine release. Serumbiochemisry changes observed in these cases were similar to Polzin *et al*. (2000) who observed increased creatinine level, hypoproteinemia, hypoalbuminemia and low A/G ratio in renal dysfunction.

The sonographic findings in chronic renal failure were similar to Kumar *et al*. (2011) who reported that small sized kidneys without clear architecture could occur in chronic kidney disease. The most common finding during ultrasound inacute kidney failure was cortical hyperechogenicity which was in accordance with Ozmen *et al*. (2010).

The haemostatic defects observed in nephropathic cases were similar to Dorgalaleh *et al.* (2013) and Mcbride *et al.* (2019) who observed thrombocytopaenia, abnormal BMBT and uraemic bleeding in renal

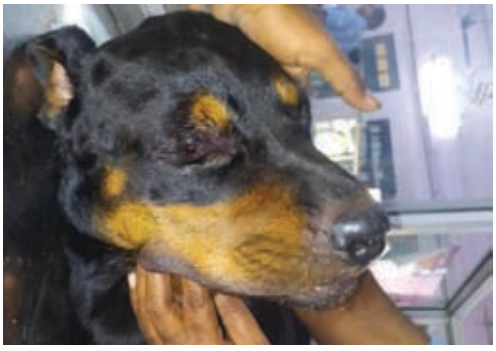

Fig. 1. Swelling of face and eyelids

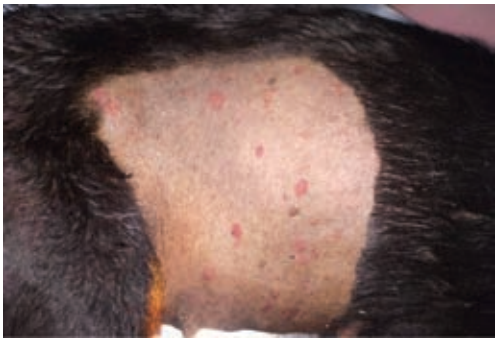

Fig. 2. Ecchymotic areas in lateral abdomen

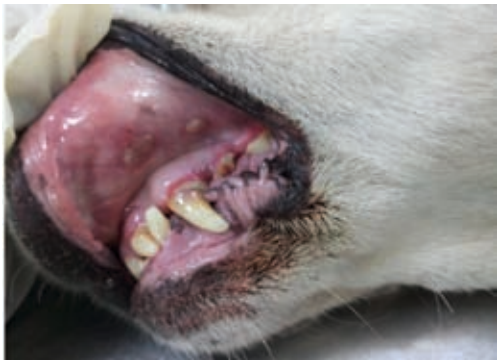

Fig. 3. Ulceration of buccal mucosa

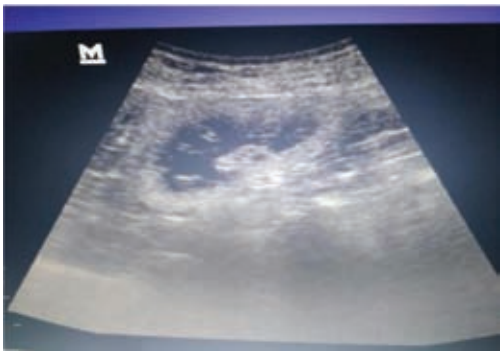

Fig. 4. Increased cortical echogenecity

dysfunction. Compared to the acute renal injury, CBT, PT, aPTT, D-Dimer concentration were more elevated in the chronic kidney disease of this study.The prolonged CBT observed in both these cases might be due to thrombocytopaenia or platelet dysfunction (Mischke, 2014). In acute kidney injury, severe thrombocytopenia with normal PT, aPTT indicated the absence of consumption of clotting factors. Absence of consumption of clotting factors could be due to the prior antivenom treatment (Nayankumar, 2016). But the elevated D-Dimer levels and hypoantithrombinemia recorded in the acute kidney injury could indicated the procoagulant state which increased the risk for developing thrombotic events. Hence the presence of elevated D-Dimer, hypoantithrombinemia, normal PT, aPTT in acute kidney injury indicated the presence of hypercoagulable state (Huang *et al.,* 2017). Presence of prolonged PT, aPTT, elevated D-Dimer and hypoantithrombinemia observed in chronic case indicated a hypocoagulable status.

Thrombocytopaenia was severe in acute kidney injury and moderate in chronic kidney disease. Shistocytes and thrombocytopaenia observed in acute kidney injury due to viper envenomation was in accordance with Vikrant *et al*. (2017) who reported that these findings could indicate microvascularangiopathy secondary to vasculitis. Echinocytes observed in the study was in aggrement with Nayankumara (2016) who reported that phospholipase A2 mediated red blood cell membrane alteration resulted in the formation of echinocytes. Phospholipase A2 in viper venom might cause damage to the platelets, erythrocyte membrane and vascular endothelium and subsequently microangiopathy. Echinocytes observed in chronic kidney disease could be due to dehydration.

Paucity exists in the literature regarding coagulopathic analysis in canine renal diseases. In human patients with stage IV renal failure had thrombocytopaenia, elevated levels of D-Dimer concentration, prolonged bleeding time, prolonged PT and aPTT whereas in stage III renal failure, patients had a normal bleeding time, PT and aPTT but elevated D-Dimer concentration and low platelet count (Shah, 2013). The observation noticed in stage IV by Shah(2013) were similar to chronic kidney disease observed in the present study. The observation noticed in stage III were seen in acute renal injury.

In this study, the D-Dimer concentration observed on the day of presentation in chronic renal failure case was different from disseminated intravascular coagulation criteria (<250 ng / mL).But the guarded prognosis observed in chronic renal failure case could be due to the progression of hypocoagulable state further.

Summary

Based on the findings, haemostatic defects in acute renal failure included thrombocytopaenia, platelet dysfunction and elevation of D-Dimer and hypoantithrombinemia. These observations indicated that the existence of a hypercoagulable state which could lead to thrombotic events in acute renal failure. Wheras in chronic renal failure, prolonged PT, aPTT, elevation of D-Dimer and hypoantithrombinemia indicated the presence of hypocoagulable state.

Conflict of interest

The authors declare that they have no conflict of interest.

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Evaluation of real time PCR for the detection of *Mycobacterium avium* subsp. *paratuberculosis* in faecal samples of cattle

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Abstract

The efficiency and suitability of a MAP F57 based SYBR Green qPCR assay for the detection of Mycobacterium avium subsp. *paratuberculosis (MAP) using a set of known MAP positive (12) and MAP negative (23) DNA samples that were previously identified by conventional IS 900 PCR were assessed. These DNA samples were isolated in our previous study from faecal samples collected from cattle in the livestock farms under government sector with a previous history of Johne's disease. The MAP F57 qPCR was able to identify all the positive samples accurately and rapidly with Cq values ranging from 20-29. The efficiency of qPCR using recombinant plasmid for standard curve was 0.991 and limit of detection was 10 MAP organisms per microlitre of DNA sample.*

Keywords: *Johne's disease, MAP, qPCR, F57*

Running title: Real time PCR detection of *Mycobacterium avium* subsp. *paratuberculosis* in cattle faeces

Johne's disease (JD) is characterised by chronic enteritis and wasting in ruminants. The causative agent is *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The disease is associated with prolonged course and animals may remain infected sub-clinically for several years with low and intermittent shedding of MAP in faeces (Whittington and Sergeant, 2001). Transmission occurs majorly through faeces which contaminates pastures and farm environment. Animals with clinical disease also shed large number of MAP (10^6 MAP cells per gram) in the faeces (Eamens *et al*., 2007). The subclinical form of Johne's disease is difficult to be diagnosed by culture and such animals spread infection silently without manifesting any clinical signs. As the organism is very slow growing and fastidious, PCR based methods for detection have been widely in use and are recommended by OIE (*OIE terrestrial manual*, 2021).

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Most of the diagnostic PCR tests for MAP detection are based on amplification of the MAP specific insertion sequence IS*900,* which is present in multiple copies in MAP genome and hence offers greater sensitivity (Vansnick *et al*., 2004). The MAP F57 gene is unique to MAP and absent in other mycobacterial species. Hence PCR assay targeting this gene offers greater specificity (Poupart *et al*., 1993; Herthnek and Bolske, 2006). Moreover, F57 being a single copy gene is mostly targeted in quantitative real time PCR assays (qPCR) that aim to assess the MAP burden in the clinical samples (Tasara and Stephen, 2005; Irenge *et al*., 2009). Real time PCR methods offer greater sensitivity than bacterial culture (Schonenbrucher *et al*., 2008) and can detect very low numbers less than 10 CFU (Mahony and Hill, 2004; Nelli *et al.,* 2008) of MAP.

In this study, a qPCR assay based on SYBR Green chemistry targeting a 195 bp fragment of MAP F57 gene, developed in our previous study (Chaitanya *et al*., 2019) has been evaluated for its diagnostic efficacy using known MAP positive and negative DNA samples. These DNA samples were isolated in our previous study (Priyanka, 2019) from faecal samples collected from cattle in the government livestock farms with a history of Johne's disease incidence. The DNA extraction kit used was QIAamp DNA stool mini kit (Qiagen). They were identified by IS*900* PCR previously as per the method of Vansnick *et al*. (2004).

A set of twelve MAP IS*900* PCR positive DNA samples and 23 PCR negative samples were chosen for evaluating F57 qPCR. The qPCR assays were performed using an Eppendorf Real Plex Master cycler as described by Chaitanya *et al.* (2019). A standard calibration curve with a series of MAP quantification standards with 10^9 to 10^0 copies of recombinant plasmid carrying F57 target sequence (195 bp) was included in each qPCR run. The DNA (20pg/µL concentration) from known MAP isolate from a previous study (Chaitanya *et al*., 2015) in Tamil Nadu was used as a positive control. All the samples, standards and positive control were run in duplicate. No template control is included to check the specificity of amplification process.

Standard curve of cycle threshold (Cq) values increasing with increase in dilution of MAP F57 plasmid insert was obtained. The Cq values plotted against the dilution factor were found to not deviate much from the mean. The Cq values of standards ranged from 4.2 to 33.1. The least copy number of the plasmid that was able to be detected was 10^1. Slope of the standard curve was –3.297 and the assay efficiency in terms of 'R^2' value was 0.991. From the standard curve, the copy number of F57 plasmid that correspond to each Cq value and the approximate number of MAP organisms in each microlitre of DNA sample could be calculated. This qPCR technique was able to detect as low as 10 MAP per microlitre of the DNA.

In MAP F57 qPCR all the 12 DNA samples that were positive in IS*900* PCR were positive with Cq values ranging from 20 to 29. One of the 23 IS*900* PCR negative samples was considered to be inconclusive or doubtful in qPCR with Cq value 30 and this might be because of high sensitivity of amplification detection in real time PCR when compared to the gel electrophoresis in conventional PCR. Such animals should be tested again after a couple of months with a fresh faecal sample. The Cq values above 35 were considered as negative because all other 22 samples that were IS*900* PCR negative did not show any initiation of amplification curve before 35^{th} cycle. The melt curve analysis confirmed that all the amplicons have acceptable Tm value of 86.8±0.2 indicating the specificity of the assay.

It is evident that this qPCR assay is rapid than conventional PCR. It is suitable for direct detection of MAP in the clinical samples. Developed countries like Australia have adopted real time PCR testing of faecal samples for diagnosis and surveillance of Johne's disease (Plain *et al.,* 2014). However, the assay needs further refinement by inclusion of internal control target for checking the DNA extraction losses and for monitoring PCR amplification conditions. It is well known that DNA extraction procedure may cause reduction in copy number and lead to under estimation of MAP load in the clinical samples. The complex and lipid rich cell wall of MAP results in poor

DNA recovery and PCR inhibitors in faeces affect amplification of the target. Assay should be also evaluated using faecal samples spiked with known number of MAP to find out limit of detection in its actual sense.

Summary

The MAP F57 qPCR assay detected all the 12 IS*900* PCR positive DNA samples as positive with Cq values ranging from 20 to 29 that corresponds to 10^4 MAP copies to 100 copies per microlitre of DNA. The Cq values above 35 were considered as negative. The qPCR assay is rapid and sensitive than conventional PCR detect as low as 10 MAP per microlitre of the DNA.

Conflict of interest

The authors declare that they have no conflict of interest.

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Risk factors associated with subclinical mastitis in dairy cows reared in district Ganderbal, Jammu and Kashmir

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Abstract

Subclinical mastitis is associated with certain risk factors such as age, lactation stage, milk production and parity. A cross sectional study including 135 cross bred Holstein Friesian dairy cows was undertaken from June 2017 to January 2019 in surrounding villages of district Ganderbal in order to identify major risk factors. The overall prevalence of SCM was 81.48%. The highest prevalence of subclinical mastitis associated with various risk factors was 48.1% (early lactation), 44.54% (5-7 years), 49.09% (>10 litres per day) and 30.90% (3rd parity) respectively. In intensive system of rearing, prevalence was 66.36% whereas in extensive 33.63% was found. In present study, single quarter was affected in 61.81% of SCM infections. In milking method, knuckling and stripping showed more incidence of SCM. In present study, moderate form of mastitis was more predominant (40.9%).

Keywords: *Subclinical mastitis, lactation, parity, milk production*

Running title: Association of risk factors in dairy cows

Globally India is the largest milk producer with majority of population dependent on agriculture and animal rearing for earning their livelihood. India has 190 million cattle and 108 million buffaloes with dairy cows accounting for 43% and buffaloes 53% of the total milk production. Bovine mastitis is inflammatory disease of udder and characterized by swelling, pain, fever, redness, heat, clots in milk. Mastitis is a multietiological disease with bacteria being the most common etiological agent. Other than bacteria, others pathogens like mycoplasma, yeast and in minor cases viral

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agents have been found. There are two forms of mastitis, clinical mastitis (CM) and subclinical mastitis (SCM) (Duguma *et al.*, 2014). Subclinical mastitis is the predominant form of mastitis and the disease is present without any noticeable signs, which makes it difficult to diagnose (Ali *et al*., 2017). In SCM, the milk composition is altered with 15-45 decrease in milk production (Halasa *et al.*, 2007). Despite many years of research predisposing factors like improper management, poor hygiene, teat damage, defective milking machines, parity, lactation, breed, age and milk production are associated with incidence of SCM. Effective environmental awareness, better managemental practices with good sanitation procedures can help veterinarians to decrease the prevalence of subclinical mastitis. Because of the diverse agro-climatological conditions in India, it is essential to know the risk factors of SCM in a particular area in order to implement proper planning for therapeutic and control measures. The present study aimed to determine the risk factors associated with SCM in Holstein Friesian cattle.

The present study was carried in different regions of district Ganderbal, Jammu and Kashmir (J & K) with lactating Holstein Friesian dairy crossbred cows being found in the study area during June 2017 to January 2019. In this cross-sectional study, 135 Holstein Friesian crossbred dairy animals were selected with the aim to determine association of risk factors with subclinical mastitis. With visual inspection, the physical examination of milk samples was conducted to determine any color deformity, purity, odor, flakes, clots, blood or any other noticeable difference in appearance. The dairy animals were properly screened for SCM based on clinical examination and CMT and grouped into healthy and subclinical animals.

The overall prevalence of subclinical mastitis was 81.4% in this study. Out of 110 SCM cases, 53, 34 and 23 mastitis milk samples belonged to early (1-3months), mid (4-6months) and late lactation (7-9months) stages. The highest incidence was found in early lactation stage (48.1%) followed by mid lactation (30.90%) whereas lowest prevalence was reported in late lactation stage (20.90%). In present study, dairy cows in the early stage of lactation were affected more with SCM in comparison to mid and late stages of lactation. The present reports are in agreement with previous studies (Gupta *et al*., 2017; Swami *et al*., 2017; Maheshwari *et al*., 2016; Islam *et al*., 2011; Mohana Sundhari, 2010) respectively. The high incidence of SCM during early lactation stages could be due to rapid physiological changes in postpartum tissues of mammary gland, thus leading to constant stress and decreased immune status (Shaikh *et al*., 2018).

The present result indicates that among 110 CMT positive HF dairy cows, 22 cases belonged to 2-4 age group, 49 to 5-7 years, 28 to 8-10 years and 11 to >10 age group respectively. Table 2, represents the relation between SCM and age group. The incidence of SCM in this study was more in 5-7 (44.54%) age group followed by 8-10 (25.45%), 2-4 (20%) and lowest occurrence in > 10 (10%) years of age group. Similar findings of high prevalence in the age group of 5-7 years have been reported (Tiwari *et al*., 2013; Islam *et al*., 2011; Maheshwari *et al*., 2016). The reason for this could be efficient innate defense system in younger animals that render them less susceptible to mastitis infection, whereas reduced milk yield, improper animal handling, decreased milking practice may reduce infection chances in older animals.

The incidence of SCM in this study was more in 5-7 (44.54%) age group followed by 8-10 (25.45%), 2-4 (20%) and lowest occurrence in > 10 (10%) years of age group. Similar findings of high prevalence in the age group of 5-7 years have been reported (Tiwari *et al*., 2013; Islam *et al*., 2011; Maheshwari *et al*., 2016). The reason for this could be efficient innate defense system in younger animals that render them less susceptible to mastitis

Table 1. Number of mastitis quarters affected in study area

No. of quarters affected	No. of animals	Prevalence%
1	68	61.81
2	29	26.36
3	9	8.18
4	4	3.63

Table 2. Incidence between milking method and SCM

		No. of SCM cases	Prevalence %
Milking method	Full hand milking	16	14.54
	Knuckling	60	54.54
	Stripping	27	24.54
	Machine milking	7	6.36

In SCM infected animals, Table 3, respectively, represents the nature of mastitis.

Table 3. Nature of SCM cases in study area

Nature of SCM	No. of SCM cases	Prevalence %
Mild	28	25.45
Moderate	45	40.9
Severe	37	33.63

infection, whereas reduced milk yield, improper animal handling, decreased milking practice may reduce infection chances in older animals.

In SCM infected Holstein Friesian dairy cows, 18 cases belonged to 1-5 litres milk yielding group, 38 to 6-10 and 58 animals to>10 liters per day yielding group. The present study reported highest prevalence in dairy cows with milk yield of >10 litres (49.58%) followed by 6-10 litres (34.1%) and lowest occurrence in >1-5 litres (16.32%). The finding of present study are in agreement with previous reports (Islam *et al*., 2011; Barua *et al*., 2014; Sharma *et al.,* 2018; Tiwari *et al*., 2008). The stress produced by over production and increased loss of micronutrients that have role in immunity, may predispose animals to intramammary infections. Further, a genetic correlation has been found between milk yield and causation of mastitis in dairy cattle.

In dairy cows, the parity-wise cases recorded were 18, 30, 34, 16, 8 and 4 during the parity numbers 1, 2, 3, 4, 5 and 6 respectively. In this study, parity wise incidence of SCM was found to be highest in 3rd parity (30.90%) followed by 2nd parity whereas lowest occurrence was found in 6th parity dairy cows. Maheshwari *et al*., (2016) and Islam *et al*., (2011) recorded similar observations. During 3rd parity there is high yielding stress on udder causes the broadening of teat canal due to the pressure on teat sphincter resulting in increased chance of microbe entry and hence increased the chance of infection (Maheshwari *et al*., 2016).

In intensive system, 73 dairy cows were positive for SCM whereas in extensive system 37 animals were infected. The incidence of SCM infection in intensive and extensive system is shown in Table 5. The prevalence of SCM was more in intensive system (66.36 %) as compared to extensive system (33.63%). Poor hygienic conditions and big herd size with less spacing may be related with high incidence in intensive as compared to extensive. Abdurahman, (2006) reported poor milk hygiene associated with high incidence of mastitis in dairy animals.

In the present study, 135 Holstein Friesian dairy cows were screened for SCM with 110 positive for mastitis. Table 1shows the prevalence of quarters affected. In this study, single quarter showed more incidence of SCM infection (61.81%) followed by two quarters (26.36%). Yohannis *et al*., (2013) have reported similar findings. The high quarter wise prevalence may be due to poor udder health and improper managemental practices.

The relationship between incidence of SCM and milking methods is shown in Table 2. In the present study, knuckling and stripping caused more damage to udder tissues leading to mastitis. This in concordance to report of Suresh *et al*., (2017).The reason for this could be poor hygiene of milker's hand and improper udder washing.

Summary

In dairy cows, the primary risk factors that are associated with occurrence of SCM are lactation, age, increased milk production and parity. The prevalence study indicates that early lactating cows, 5-7 age group, milk production greater than 10 litres per day and dairy animals in 3rd parity were more susceptible to subclinical mastitis. The study also indicated that dairy

cows in intensive system of rearing were more prone to mastitis. This study also revealed that infections in single quarters, knuckling method and moderate form of mastitis were commonly reared in the selected area. To prevent animals from SCM infection, it is important to identify the risk factors of the disease. Management and poor feeding practices may greatly influence the risk factors among dairy cattle. Therefore, preventive measures like cleaning of teat before milking with clean water, washing of milker's hands, farm hygiene, improvement in milk technique and proper diagnostic techniques are fundamentally important in minimizing the mastitis infections.

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Conflict of interest

The authors declare that they have no competing interests

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