

## A Study on Bovine Tuberculosis among Cattle in North Karnataka

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### ABSTRACT

The Prevalence of tuberculosis among cattle caused by *Mycobacterium bovis* a member of *Mycobacterium tuberculosis* complex is described in this paper. The disease is of socio-economic and public health importance and is of great significance to international trade of animals and animal products. This disease not only poses a threat to the economy of developing countries but also remains a problem in developed countries. During the period of eight years between 2002-03 to 2009-10, a total of 3286 cattle in 36 organized farms spread across Belagavi, Dharwad and Bagalkote districts of north Karnataka, were screened using single intradermal tuberculin test. Overall percentage prevalence of tuberculosis among cattle was found to be 1.76. Percentage prevalence of tuberculosis was higher in female (1.85%) than in male (0.78%). As regards to the breed wise percentage prevalence, it was higher in Holstein Fresian breed (HF) (2.66 %) than Jersey (1.37 %) cattle and there are no positive cases among Indigenous breed of cattle. In conclusion as a result of this study, the pattern of bovine tuberculosis among cattle revealed a gradual decline in the overall percentage prevalence, over a period of eight years from 2002-03 to 2009-2010 and so also in males, females, HF and Jersey breeds.

**Key words :** Prevalence; Bovine tuberculosis; cattle; North Karnataka.

### INTRODUCTION

Tuberculosis is an infectious disease occurring in several animal species including domestic and wild animals as well as humans (Grange 2001; Pavlik *et al.* 2002). *M. bovis* is the main contributing mediator of human intestinal tuberculosis in those developing countries of the world, where bovine milk had not often been pasteurized properly before use [Bonsu *et al.* 2000]. Aerosol exposure to *M. bovis* is considered to be the most frequent route of infection among cattle, but infection by ingestion of contaminated material, contact with infected animals and other wildlife, also occurs (OIE Manual. 2016). The disease is often subclinical and clinical signs are not clinically distinctive, hence in the live animal it could be diagnosed on the basis of delayed hypersensitivity reactions or by laboratory analysis. The prevalence of bovine tuberculosis is variable in different parts of the world and it varies from region to region and even from one farm to another in same locality (Javed *et al.* 2006). Review of literature reveals paucity of data about the

prevalence of bovine tuberculosis in animal population in North Karnataka and more so in cattle population. The present research study was undertaken with the following objectives (i) to determine the prevalence of bovine tuberculosis among cattle with respect to sex and breed (ii) to determine the overall prevalence of bovine tuberculosis among cattle in North Karnataka and (iii) to study the temporal pattern or the trend of the disease during the study period in North Karnataka.

### MATERIALS AND METHODS

The present study was carried out during 2002 to 2010 including a total 3286 cattle (excluding calves less than 6 months of age and cattle in advanced pregnancy) of either sex and different breeds, in 36 organized farms spread across three districts of North Karnataka, viz, belagavi, Dharwad and Bagalkot.

**Bovine tuberculin PPD:** Bovine tuberculin PPD was procured from Indian Veterinary Research Institute (IVRI), Izatnagar.

**Single intradermal test (SID):** Single intradermal test was performed on all the 3286 cattle in the neck region as per the instructions of the manufacturer. Site of injection in the middle of the neck was cleaned and shaved. Thickness of a fold of the skin is measured by using Vernier Caliper. 0.1 ml of bovine tuberculin Purified Protein Derivative (PPD) was injected intradermally (200 international units or 100 µg) using tuberculin syringe and needle. Thickness of the skin was again measured after 72 hours of injection. An animal with an increase of 4 mm or more in skin thickness was considered as a positive reactor.

## RESULTS AND DISCUSSIONS

Over a period of eight years, 3286 Cattle in 36 organized farms located in three districts of North Karnataka namely Belgavi, Bagalkote and Dharwad, were screened using SID. The results of the same are presented in Table: 2, 3 and 4.

### Overall Prevalence of tuberculosis:

Out of 3286 cattle screened by SID test, 58 animals were positive reactors indicating the overall percentage prevalence of 1.76 (Table-2). Results of the present study are in agreement with Phaniraja *et al.* (2010), Neeraja *et al.* (2014), Hareesh Didugu *et al.* (2016), who have recorded a prevalence of 2.4 per cent, zero and 0.87 per cent respectively and also Mukherjee (2006) who recorded 0.0(1990), 1.7(1992) & 0.54 per cent (1993), but it differs from others (Aneesh Thakur *et al.* 2010, Mukesh Kumar Thakur, 2015), who have recorded a prevalence of 14.3 per cent and 13.12 per cent respectively in India, Awah *et al.* (2012) reported 4.67 per cent in Cameroon, Ghumman *et al.* (2013) and Noorrahim *et al.* (2015), recorded 11.71 and 5.75 per cent respectively in Pakistan, Mondal *et al.* (2014) recorded 5.9 per cent in Bangladesh, Mohammed Nega *et al.* (2012) recorded 7.1 per cent in Ethiopia and Ivania Moine *et al.* (2014), in Mozambique recorded 39.65 per cent in bovines.

Variation in the prevalence of tuberculosis could be due to the variation from year to year and geographical variation either within each country or

between the countries (Javed *et al.* 2006). Prevalence of tuberculosis also varied from region to region and even from farm to farm in the same region (Imtiaz Khan *et al.* 2008). Prevalence of tuberculosis is influenced by many factors such as geographical situation of a country, its temperature, hygienic status of humans and management of animals and enforced regulatory laws in Public Health and Veterinary Public Health sectors and also due to size of the sample, type of diagnostic test used, stock density and husbandry system (Mahmud *et al.* 2014).

### Year wise Prevalence of tuberculosis:

Over a period of eight years, 3286 Cattle were screened by using SID test and the results were given in Table-2. The overall percentage prevalence during 2002-03, 2003-04, 2004-05, 2005-06, 2006-07, 2007-08, 2008-09 and 2009-10 was 8.85, 2.73, 1.01, 0.84, 0.22, zero, zero and 0.19, respectively. Based on the results of SID during the period of study, a gradual decline in the percentage prevalence of tuberculosis among cattle was observed from 2002-03 to 2008-2009 and during the year 2009-10 one animal out of 518 was found positive. Similar observation of declining trend has been made by Ghumman *et al.* (2013) over a period of four years from 2006-07 to 2009-10.

Variation in the percentage prevalence of tuberculosis among cattle could be due to the variation from year to year and geographical variation either within each country or between the countries (Javed *et al.* 2006). The declining trend observed in the present study could be attributed to the periodic screening removal/culling of positive reactors, improved management or hygienic practices on the farm.

### Sex wise Prevalence of tuberculosis:

Sex wise prevalence of tuberculosis among cattle, is presented in Table.3. In all 255 males and 3031 females were screened during the study, of which two males and 56 females were found positive reactors for tuberculosis by SID, contributing to a prevalence of 0.78 per cent and 1.85 per cent respectively. On comparison, between the male and female, it was observed that the percentage prevalence of tuberculosis

among female cattle was higher than male cattle. Our observation is in corroboration with the earlier researchers (Salgado *et al.* 2009; Phaniraja *et al.* 2010; Nwanta *et al.* 2011; Arshad *et al.* 2012; Mahmud *et al.* 2014; Mukesh Kumar Thakur 2015; Noorrahim *et al.* 2015).

Year wise prevalence of tuberculosis during the period of study both in male and female revealed a decreasing trend from 2002-03 to 2009-10. Perusal of literature did not reveal any temporal pattern of prevalence of tuberculosis among male and female. Hence this could be considered as the first record.

**Breed wise Prevalence of tuberculosis:**

The results are presented in Table. 4. A total of 1727 HF, 873 Jersey and 686 Indigenous cattle were screened during the study period, of which 46 HF and 12 Jersey cattle were positive for tuberculosis by SID where as all the indigenous cattle were found negative. The overall percentage prevalence among HF was 2.66, Jersey was 1.37 and that of Indigenous cattle was zero. Breed wise comparison of the percentage prevalence, revealed that the percentage prevalence of tuberculosis was higher among HF than Jersey breed where as percentage prevalence was zero among Indigenous cattle. Similar observations were made by earlier researchers (Phaniraja *et al.* 2010; Mukesh Kumar Thakur 2015; Noorrahim *et al.* 2015).

Analysis regarding the temporal pattern of the disease within each breed revealed a gradual reduction in the percentage prevalence of the disease from 2002-03 to 2009-2010.

**Conclusion:**

The present study revealed (i) the overall percentage prevalence of bovine tuberculosis among cattle in the study area i.e., three districts of North Karnataka is 1.76, (ii) prevalence is higher in female cattle than in males, (iii) prevalence is higher in HF breed of cattle than Jersey and Indigenous breed and (iv) as far as the temporal pattern of the disease is concerned, in the study area, a declining trend was observed during the study period of eight years from

2002-03 to 2009-10. Our observations are restricted to three districts of North Karnataka.

The present study, therefore, recommends a wide screening of cattle to assess the infection/ carrier status that should enable to institute effective control measures in view of global prevalence of tuberculosis and zoonotic importance of mycobacterium *spp.*

**Table. 1. Classification of animals**

Total No. of animals tested	Sex		Breed		
	Male	Female	HF	Jersey	Indigenous
<b>3286</b>	255	3031	1727	873	686

**Table-2: Results of SID among Cattle (overall)**

Year	No. tested	No. Positive	% Positive
2002-03	384	34	8.85
2003-04	366	10	2.73
2004-05	592	6	1.01
2005-06	716	6	0.84
2006-07	457	1	0.22
2007-08	212	0	0
2008-09	41	0	0
2009-10	518	1	0.19
<b>Total</b>	<b>3286</b>	<b>58</b>	<b>1.76</b>

**Table-3: Prevalence of tuberculosis among cattle with respect to sex**

Year	Male			Female		
	No. tested	No. Positive	% Positive	No. tested	No. Positive	% Positive
2002-03	02	00	00	382	34	8.90
2003-04	44	01	2.27	322	09	2.79
2004-05	21	00	00	571	6	1.05
2005-06	58	01	1.72	658	5	0.76
2006-07	55	00	00	402	01	0.25
2007-08	09	00	00	203	00	00
2008-09	14	00	00	27	00	00
2009-10	52	00	00	466	1	0.21
<b>Total</b>	<b>255</b>	<b>02</b>	<b>0.78</b>	<b>3031</b>	<b>56</b>	<b>1.85</b>

**Table-4: Prevalence of tuberculosis among cattle with respect to breed**

Year	HF			Jersey			Indigenous		
	No. tested	No. +ve	% +ve	No. tested	No. +ve	% +ve	No. tested	No. +ve	% +ve
2002-03	219	27	12.32	133	07	5.26	32	00	00
2003-04	238	08	3.36	85	02	2.35	43	00	00
2004-05	220	04	1.82	198	02	1.01	174	00	00
2005-06	380	05	1.32	217	01	0.46	119	00	00
2006-07	236	01	0.42	66	00	00	155	00	00
2007-08	134	00	00	54	00	00	24	00	00
2008-09	24	00	00	06	00	00	11	00	00
2009-10	276	01	0.36	114	00	00	128	00	00
<b>Total</b>	<b>1727</b>	<b>46</b>	<b>2.66</b>	<b>873</b>	<b>12</b>	<b>1.37</b>	<b>686</b>	<b>00</b>	<b>00</b>

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## Efficacy of Short Term Avikesil-S® with Cloprostenol for Estrus Synchronization in Jamnapari Goats\*

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### ABSTRACT

Estrus synchronization is a popular ART and cascade of hormonal protocols have been tried to achieve maximum reproduction in goats. The present study was aimed to evaluate the reproductive efficacy of estrus synchronization in Jamnapari does using intravaginal sponges containing 350 mg natural progesterone (Avikesil-S®) *in situ* for seven days and 125 µg Cloprostenol sodium *i.m.* at the time of sponge withdrawal. All the ten animals under treatment exhibited estrus within 33.40±3.27 h and duration of estrus was 23.00±2.20 h. After allowing the does in estrus for natural mating, 90 per cent pregnancy rate was observed at 35 days gestation through ultrasound. Further, all pregnant does delivered after gestation period of 150.00±1.00 days. Average litter size, single birth, twin birth, sex ratio (male:female) and birth weight of kids were 1.11, 88.88 per cent (8/9), 11.11 per cent (1/9), 50:50 and 4.10±0.22 kg, respectively. Avikesil-S® intravaginal sponge for seven days along with Cloprostenol injection on day of sponge removal proved efficient estrus synchronisation in Jamnapari goats.

**Key words :** Jamnapari, Goat, Estrus synchronization, Avikesil-S®.

### INTRODUCTION

Profitability and sustainability of any livestock farm solely depend on the unfaltering reproduction. Low reproductive efficiency due to long post-partum anestrus, variability in estrous cycle and kidding interval is considered as a major constraint in goat breeding. Assertive results of estrus synchronisation in ameliorating low reproductive efficiency have made it a prerequisite for high economic efficiency in intensive goat farming (Yotov *et al.*, 2016).

Estrus synchronization, key technology for managing production systems, allows concentration of mating and parturition at suitable times to take advantage of niche markets, feed supplies, labor and raising price trends. Treatment with progestagens *viz.*, Medroxy progesterone acetate, Flugesterol acetate, Methylacetoxy progesterone and natural progesterone

for 12-18 days was the conventional method of estrus synchronization in goats. Peer review about follicular dynamics in goats from Menchaca and Rubianes (2003) and Cruz *et al.* (2005) have led to adoption of short term (5-7 days) progestagen treatment along with prostaglandins for successful estrus synchronisation with better fertility rates (Menchaca and Rubianes, 2007; Souza *et al.*, 2011). However, non-availability and economical non-viability of different commercially available progestagens lead to invention of indigenous intravaginal sponge, Avikesil-S® containing 350 mg of natural progesterone by CSWRI, Avikanagar. Avikesil-S® has been exclusively utilised in sheep estrus synchronisation. However, efficacy of the same in goats yet to be evaluated.

Jamnapari is a descript breed of India, found in home tract in and around Rajasthan and Uttarpradesh.

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Recently, commercial goat farms in Karnataka have adopted this breed extensively. Reproductive performance and estrus synchronisation studies on this breed in Karnataka are non-existent. The present study was aimed to analyze the reproductive efficacy of estrus synchronization in Jamnapari does using Avikesil-S® *in situ* for seven days and 125 µg Cloprostenol sodium, on day of sponge removal.

## MATERIALS AND METHODS

The present study was conducted in a commercial goat farm, located at Chinthamani, Chickaballapur district of Karnataka state. A total of 10 multiparous Jamnapari goats aged between 2 and 4 years, weighing 25-35 kg with body condition scores of 2.5 to 3.5, that had delivered at least once and crossed minimum 60 days post parturition were selected for the study. Two fertile bucks aged between 2 and 4 year which had used for mating previously and proved were also included in this study. Prior to the experiment, animals were dewormed with Albendazole. Does were penned into two compartments of five each. Buck and does were separated by sight, smell and vision.

All does were screened for pregnancy by using Real-time B-mode trans-abdominal ultrasound equipment (Honda, HS/2000 with a 5-10 MHz longitudinal transducer, Japan). All selected animals were treated with intravaginal sponge Avikesil-S® left *in situ* for seven days followed by intramuscular injection of 125 µg Cloprostenol sodium. at the time of sponge withdrawal.

Estrus signs were monitored twice in a day for 15 min each time (06:00 and 18:00 hours) daily for five days after sponge withdrawal. Once the hormonal therapy was completed does were kept along with sexually experienced, fertile bucks in the ratio of one buck for five does. Every buck was painted with different natural colours at its brisket region to facilitate the identification of doe which has successfully served. Does with colour mark on their back were considered bred successfully.

The following parameters such as sponge retention rate, estrus rate, interval to estrus and duration of estrus were assessed by the end of estrus. Pregnancy

diagnosis was carried out using real-time b-mode trans-abdominal ultrasound equipment.. Pregnant animals were followed until kidding and gestation period, kidding rate, type of birth, litter size, sex ratio and birth weight were recorded. Sponge retention rate, estrus induction rate, pregnancy rate, kidding rate, single birth and twin birth are expressed as per cent. Whereas, estrus induction interval, duration of estrus, gestation period and birth weight are expressed as mean ± SE in corresponding units of measure.

## RESULTS AND DISCUSSION

Estrus synchronization in goats has been efficiently achieved using different progestagen agents. Intravaginal route of administration of progestagens for short term (5-10 days) has been proved effective with varied success rate. Ritar *et al.* (1990) explained the possibility that some animals where PGF2á was not included in the short term protocol maintained functional corpora lutea at pessary removal, causing failure of estrus synchronization in such animals. Inclusion of prostaglandins has been proved effective in achieving increased efficiency ranging from 74 to 100 per cent (Maffili *et al.*, 2005; Dogan *et al.*, 2008; Fonseca *et al.*, 2009; Pawshe *et al.*, 2013; Cardoso *et al.*, 2018 and Bind *et al.*, 2018) in spite of employing the short term progestagen therapy.

Combination of different progestagens and prostaglandins at varied time combinations have been tried, Maffili *et al.* (2005), Fonseca *et al.* (2009) and Cardoso *et al.* (2018) had used 60 mg MAP impregnated sponges for 5, 6 and 7 days in Anglonubian, Alpine and Toggenburg goats, respectively. Dogan *et al.* (2008) have used 20 mg cronolone impregnated sponges in Turkish Saanen goats. Pawshe *et al.* (2013) and Bind *et al.* (2018) have used natural progesterone impregnated sponges for 11 days in local goats. Majority of the scientific community have adopted prostaglandin injection at 24 h before sponge removal, [Dogan *et al.* (2008) (75 µg cloprostenol sodium), Fonseca *et al.* (2009) (50 µg D-cloprostenol), Cardoso *et al.* (2018) (37.5 µg cloprostenol sodium) and Bind *et al.* (2018) (125 µg cloprostenol sodium)]. However, Maffili *et al.* (2005) had injected prostaglandin on the

first day of sponge insertion. In the present study, very purpose being finding the suitable estrus synchronization protocol for goats in the field contemplating the less labor seeking protocol was justifiable and thus Prostaglandin injection was given during the sponge removal as it was adapted by Pawshe *et al.* (2013). However, present selective method has the additional benefits of reduced costs, labor and less detrimental effects on animal welfare because of reduced animal handling time.

**Table 1: Reproductive parameters observed while estrus synchronisation.**

Parameters	Jamnapari
Sponge retention rate (%)	100
Estrus response (%)	100
Estrus induction interval (hr)	33.40±3.27
Duration of Estrus (hr)	23.00±2.20
Pregnancy rate (%)	90
Gestation period (days)	150.00±1.00
Kidding rate (%)	100
Litter size	1.11
Single (%)	88.88 (8/9)
Twins (%)	11.11 (1/9)
Sex ratio (Male:Female) (%)	50:50
Birth weight (kg)	4.10±0.22

During the study, none of the animals lost vaginal sponge. Wildeus (2000) has peer reviewed concepts of estrus synchronization in sheep and goats and opined rate of sponge retention should be higher than 90 per cent in sheep and goats. Extended duration of intravaginal sponge treatment increases the risk of the sponge's loss (Husein *et al.*, 2007). Factors such as management system, intravaginal sponge texture and consistency, techniques employed in inserting sponge, size of vagina of individual doe and experience of the operator have been reported to influence sponge retention rate (Omontese *et al.*, 2012). Saribay *et al.* (2011) observed 96.2 per cent sponge retention rate in six day protocol which is moderately lower than present study. Similar observations were recorded in Karaca *et al.* (2010), Pawshe *et al.* (2013) and Bind *et al.* (2018) who had adapted protocols ranging from 8 to

14 days. Thus Avikesil® are suitable in shape, size and texture and can be successfully used in goats.

Synchronization treatment was initiated irrespective of the estrous cycle stage and none of the does showed estrus while the intravaginal sponges were *in situ*. Therefore, it is accepted that the dose of progestagen in the Avikesil-S® delivered from the vagina during treatment was sufficient to suppress the preovulatory discharge of pituitary gonadotropins. In spite, many workers have observed 74 to 100 per cent estrus induction rate using progestagens and prostaglandin combination, none have reported 100 per cent estrus induction using progestagen sponge for short period of seven days. This demonstrates that 7-day protocols with Avikesil-S® intravaginal progesterone sponges provided adequate surge of endogenous gonadotropins to initiate the sequence of hormonal cascade of events resulting in efficient estrus synchronization program.

In the present study, all animals treated had exhibited estrus achieving tight synchronization of estrus within 33.39±3.26 h after termination of the treatment. Fonseca *et al.* (2009) and Cardoso *et al.* (2018) observed 80 and 74 per cent estrus induction in Alpine and Anglonubian goats. Pawshe *et al.* (2013) observed shorter estrus induction interval (17.41±1.31 h), than the duration recorded in the present study(33.40±3.27 h), however, the present finding was within the range of that reported by Dogan *et al.* (2008), Fonseca *et al.* (2009), Cardoso *et al.* (2018) and Bind *et al.* (2018) who observed estrus induction interval of 39.6±1.20, 53.50 ± 18.10, 24.47 ± 8.71 and 41.87±0.64 h, respectively.

In goats, estrus duration is said to be 30-40 h (Noakes *et al.*, 2001). Estrus duration of 38.3 ± 14.7, 53.5 ± 18.1 and 34.25 ± 1.06 h were recorded in artificial insemination studies by Maffili *et al.* (2005), Fonseca *et al.* (2009) and Bind *et al.* (2018), respectively. Whereas, our observation of estrus duration, 23.00±2.20 h, is in acceptance with Dogan *et al.* (2008) and Cordoso *et al.* (2018) who had also adopted natural mating like in our study and reported 31.8 ± 2.4 and 24.47 ± 8.71 h estrus duration, respectively. On the other hand, estrus duration

observed in this study agrees with previous reports where copulation was shown to significantly reduce estrus duration from 20 to 50 per cent (Romano, 1993) due to the effect of mechanical stimulation of penis against vaginal fornix (Romano, 1994). Romano *et al.* (2000) even observed increased fertility rate when a sterile copula was permitted by a vasectomized teaser prior to the artificial insemination.

Pregnancy rates achieved directly indicates the success rate of any estrus synchronization protocol. Though it depends on the many external factors, mimicking the physiological endocrine milieu in case of estrus synchronization protocols is the premier factor. Natural mating with fertile male has given satisfactory results compared to artificial insemination in previous studies, where 12.5 to 75 per cent pregnancy rate have been observed in estrus synchronization protocols using progestagen and prostaglandin (Maffili *et al.*, 2005; Dogan *et al.*, 2008; Fonseca *et al.*, 2009; Pawshe *et al.*, 2013; Bind *et al.*, 2018; Cardoso *et al.*, 2018). However, 90 per cent pregnancy rate observed in our study proves the efficiency of present estrus synchronisation protocol in Jamnapari goats.

Gestational period tend to vary among the breeds, parity, type of birth and sex of fetus. Miah *et al.* (2016) and Hassan *et al.* (2010) have reported  $150.88 \pm 0.85$  and  $152.8 \pm 17.50$  days of gestation period, respectively in Jamnapari goats reared and bred under natural conditions. In present study  $150.00 \pm 1.00$  days gestation period is in accordance with earlier observation and concentrated kidding within ten days observed in our study is beneficial to adopt efficient dam and kid management practices.

All nine pregnant goats delivered without any gestational complications, thus giving the kidding rate of 100 per cent, litter size of 1.11, single and twin births in 88.88 and 11.11 per cent of cases, respectively. According to previously available data Jamnapari goats observed to have a litter size of  $1.59 \pm 0.11$  and  $1.70 \pm 0.60$ ; single, twins and triplets of, 46.8, 46.8 and 6.25 per cent of cases and 32.90, 58.20 and 8.80 per cent of cases, respectively by Miah *et al.* (2016) and Hassan *et al.* (2010), which are higher than our observation. Litter size is a reproductive trait which proved to be

associated with the genetic potential, management practices and nutritional status of the flock. Furthermore, use of GnRH or PMSG in estrus synchronization protocol is proved to increase the litter size (Titi *et al.*, 2010). The objective of the study was to check the efficiency of the economically cheaper protocol without including additional hormones.

Sex ratio was 50:50, exactly equal numbers of male and female kids were born in present study which was almost similar to the observation made by Hassan *et al.* (2010), 53.2 males: 46.8 females (*per cent*) under field condition in larger population. Birth weight of kids was  $4.10 \pm 0.22$  kg which is higher than the previous observation by Hassan *et al.* (2010) under natural breeding and grazing practices which is attributed to the better management practices adopted in our study.

In conclusion, the use of Avikesil-S® containing 350 mg natural progesterone for seven days and  $125 \mu\text{g}$  *i.m.* injection Cloprostenol sodium on the day of sponge removal is a suitable method of estrus synchronization for Jamnapari goats in Southern Karnataka. The better estrus synchronization and the high fertility rates can be obtained in the similar agro-ecological zones.

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## Epidemiology of Otitis in Dogs in and Around Bangalore

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### ABSTRACT

A total of 15766 dogs presented to Veterinary College Hospital, Hebbal, Bangalore during the period of six months from January 2014 to June 2014 were examined for clinical signs suggestive of otitis. Out of the 15766 dogs presented for investigation, 840 dogs were diagnosed to be affected with otitis giving an overall prevalence rate of 5.32 % and higher occurrence of otitis was recorded in dogs in the age group of 1-4 years (42.85 %). German Shepherds were the most affected (28.57%) among the different breeds and the incidences were higher in male dogs (64 %) as compared to female dogs (36%). Dogs with erect ears (50%) were highly prone for otitis and majority of the dogs had unilateral otitis infection (67.85 %) as compared to bilateral infection (32.15 %). Clinical examination revealed that 100, 92.85, 82.14, 85.71, 71.42, 46.42, 25.00 and 17.85 percent of dogs had aural pruritus, shaking, pain, discharge with odour, inflammation, erythema with thickening and head tilt respectively.

### INTRODUCTION

Otitis externa is the most common disease of the canine ear canal. It is aesthetically disagreeable to the owner and cause severe discomfort to the dog. Extension of infection to the internal ear can result in deafness. The disorder involves an acute or chronic inflammation of the epithelium of the external auditory meatus, sometimes involving the pinna. The estimated incidence ranges from 4 to 20 per cent (Muller and Schmitz, 1983). Clinical signs include erythema, discharge, desquamation of the epithelium, pain and pruritus. Unfortunately, many cases of the otitis remain unresolved and become progressively refractory to therapy (Radlinsky and Mason, 2006). The objective of the present study was to know the epidemiological pattern of otitis in dogs in and around Bangalore.

### MATERIALS AND METHODS

A total of 15766 dogs presented to Veterinary College Hospital, Hebbal, Bangalore during the period of six months from January 2014 to June 2014 were examined for clinical signs suggestive of otitis such as erythema, presence of exudate, foul odour, head shaking / tilt and evidence of pain on palpation were

selected for our study. The detailed description of the dog with respect to age, breed, gender, ear type, ear affected (unilateral/bilateral) were

### RESULTS AND DISCUSSION

The present study was undertaken to record the prevalence of otitis in dogs with respect to age, breed, gender, ear conformation and ear type, presented to Veterinary College Hospital, Hebbal, Bangalore for the period from January 2014 to June 2014. The results pertaining to the study are given below.

**Prevalence :** Out of the 15766 dogs investigated, 840 dogs were diagnosed as otitis giving an incidence rate of 5.32 %. Similar findings have been reported by Chaudhary and Mirakhur, (2003), who have reported prevalence of 4.92 per cent. However contrary to the findings of our study, Ettinger and Feldman, (2000), have recorded higher prevalence of otitis ranging from 25-70 per cent in dogs. The lower incidence of otitis in the present study may be attributed to good health care provided by the pet owner.

**Age :** Age wise incidence of otitis revealed that 60, 360, 270, 90 and 60 dogs were affected with percent

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positivity of 7.14, 42.85, 32.14, 10.71 and 7.14 in the age group of less than one year, 1-4, 4-7, 7-10 and more than 10 years respectively. The results were in agreement with Mahendran *et al.*, (2007), who recorded that dogs aged between one to six years were highly prone for otitis. However, several workers have reported that incidence of otitis is not affected by the age of the affected animals (Nuttal, 1998).

**Breed :** German Shepherd was the most affected 240 (28.57%), followed by Labrador Retriever 210 (25.00%), Mongrels 120 (14.28%), Dachshund 60 (7.14%), Cocker Spaniel 60 (7.14%), Rottweiler 20 (7.14%), Golden Retriever 30 (3.57%), Lhasapso 30 (3.57%) and Pomeranian 30 (3.57%). The results were in concurrence with Lakshmi and Tirumala Rao, (2013) and Mahendran *et al.*, (2007). Fernandes *et al.*, (2006) also reported that the highest incidence of otitis was observed in Poodles followed by Mongrels, Cocker Spaniels and German Shepherd. The higher incidence of otitis in German Shepard is may be due to susceptibility of the breed, long deep ears and hyperactivity of its cerumen producing glands (August, 1998).

**Gender :** Of the 840 dogs, 538 (64%) males and 302 ( 36%) females were affected with otitis. The present finding is in accordance with the reports of various workers who also suggested higher incidence of otitis in male dogs (Chaudhary and Mirakhr, 2003). The higher incidence in males may be due to the fact that androgen hormones tend to increase sebum production, which appears to be a predisposing factor to flare up latent infection, whereas estrogens in females elicit an opposite response (Sandeep Kumar *et al.*, 2014). The other possible reason for higher incidence in males under the study may be that owners generally prefer male dogs as companion animals and the number of cases presented to the hospital is predominantly males as compared to females.

**Ear type :** Of the 840 affected, 420 (50.00%) had erect ears, 270 (32.14%) had semi erect ears and 150 (17.85%) dogs had drooped / pendulous ears. The results were in agreement with the findings of Cafarchia *et al.*, (2006) who reported otitis to be more common in breeds of dogs having erect and hairy ears

followed by pendulous ears. The higher incidence of otitis in breeds with erect ears under the study may be due to the fact that upright pinna appears to expose the ear canal of the animals to external factors resulting in predisposing the animal to otitis as compared to pendulous ears (Muller and Schmitz, 1983). However, contrary to the results of our study several authors have reported that dogs with pendulous ears are more susceptible to otitis as compared to erect and semi erect ears (Lakshmi and Tirumala Rao, 2013).

**Ear affected :** Of the 840 affected, 570 ( 67.85%) dogs had unilateral ear infection, while 270 ( 32.15%) had bilateral ear infection. The results were in agreement with Lakshmi and Tirumala Rao, (2013) who reported unilateral infection to be more common.

**Clinical examination of patient :** The clinical investigation of 840 dogs affected with otitis revealed that, all the 840 (100%) dogs had aural pruritus exhibited by ear scratching, 780 (92.85%) dogs showed ear shaking, 690 (82.14%) had evinced ear pain, 720 (85.71%) dogs had foul odour ear discharge, 600 (71.42%) had inflammation of the ear, 390 (46.42%) dogs had erythema and thickening of pinna, 210 (25%) dogs had head tilt and 150 (17.85%) dogs had swelling at the base of the ear. Similar observations were made by Dixit *et al.*, (2006) suggested that the most common clinical signs of otitis in dogs were erythema, pain, discharge, foul odour, loss of appetite, head tilting shaking of the ear, ulceration and bleeding.

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## Retrospective Study on Incidence of Long Bone Fractures in Goats

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### ABSTRACT

The present study was carried to evaluate the incidence of long bone fractures in goats presented to Department of Veterinary Surgery and Radiology, Veterinary College, Bidar by analyzing the data of past eleven years (2007 - 2017) in relation to age, sex and type of fracture. A total of 9349 cases were presented for surgery of which 1653 were cases of surgery in goats. It was found that the overall incidence of long bone fractures among all surgical problems of goats was 8.77 % (n = 145). Female goats were more commonly affected (66.21 %) than males (33.79 %). The incidence of fractures was most common in goats between 6 to 12 months of age (35.17 %) followed by those of below 6 months to one year (32.41 %). The incidence of fractures in goats between one to two years of age was 17.24 % and in goats above two years (15.17 %) of age. 75.17 % of the goats had simple fractures and the rest had compound fractures. Hind limbs were found to be more commonly involved (54.25 %) than fore limbs. The most commonly affected bones were metatarsal (27.59 %) and metacarpal (24.82 %) followed by tibia (22.06 %). Oblique fractures were more common (42.76 %) than transverse type (38.3%). Majority of the long bone fractures in goats were due to automobile accidents (31.72 %).

**Keywords :** Fracture, goats, incidence, bone

### INTRODUCTION

Goat is one of the major livestock reared in India due to its docile nature and low cost of production. People rear goats by letting them free for grazing in nearby grasslands and pastures, during which they meet with automobile accidents, attack by dogs and maliciously get hit by stones or sticks and encounter fracture of long bones. Because of their curious nature, they also attempt impossible jumps resulting in severe unpredictable forces on bone, leading to complex and comminuted fractures (Singh *et al.*, 2006). Fractures of long bones are the most common orthopaedic problem in goats (Singh and Nigam, 1981; Philip *et al.*, 1998 and Kumar, 2005). A search of literature revealed dearth of literature regarding the incidence of long bone fractures in goats. Hence the present study was undertaken to study the incidence of long bone fractures in goats.

### MATERIALS AND METHODS

The present study was carried out to study the incidence of long bone fractures in goats in relation to

age and sex of the animal and type of fracture by analyzing the data of past eleven years (Jan 2007 – Dec 2017) of clinical cases presented to the Department of Veterinary Surgery and Radiology, Veterinary College, Bidar. A total of 9349 surgical cases of different species were treated during the period of study. Among these, 1653 were surgical cases of goats of which 145 (8.77 %) were cases of long bone fracture in goats. The year wise incidence of long bone fractures in goats is presented in table 1.

### RESULTS AND DISCUSSION

The study on surgical cases of goats brought for treatment during the period of study revealed that overall incidence of long bone fractures among all surgical conditions treated in goats was 8.77 %. This may be attributed to the unmonitored grazing of goats in urban areas where the goats are left to graze on their own unaccompanied by the owner, which makes them vulnerable to automobile accidents, trauma by fall from height, malicious hitting and dog bites. Arora (1996) reported that the overall incidence of fractures

in goats was 12.23%. Philip *et al.* (1998) concluded that fractures were the most common orthopaedic problem in goats (11.2%). Tambe *et al.* (2012) in a retrospective study of fracture cases found that 5.5 % of the total presented fractures were of goats.

**Etiology of fracture:** Various causes for long bone fractures were recorded in the study. Most of the fractures reported in the study were due to automobile accidents (31.72 %) followed by malicious attempts (26.89 %), falling from height (14.48 %) and dog bite (2.75 %) as shown in Table 2 and plate I. However, in 35 (24.13 %) cases the exact cause of fracture was unknown. The inquisitive nature of goats and free grazing system of rearing might have contributed to the incidence of fractures. Singh *et al.* (2017) opined that an acceptable reason behind the higher incidence of fracture in goats due to automobile accident might be, congregation of nomadic, semi nomadic and rural population keeping goats towards the urban periphery with availability of least grazing area due to which movement of the animals takes place in the urban area. Moreover, violent hitting of goat by stick, by the people may also be responsible for causing fracture of long bones. Dass *et al.* (1985) observed calcium deficient soil as a major predisposing factor of caprine fractures in the hilly terrains of Chottanagpur region, the immediate causes being fall from height and automobile accidents. Aithal and Singh (1999) observed that road accidents and falling from height to be the major causes for bovine and caprine fractures. Virkar (1999) reported that majority of the long bone fractures (55.38%) in goats were due to automobile accidents. Singh *et al.* (2008) found that trauma due to automobile accidents followed by falling from a height and external violence were the major cause of long bone fractures in goats. According to Kushwaha *et al.* (2011), the major cause for fractures in goats was being hit by something, followed by falling from a height and external trauma. Tambe *et al.* (2012) reported that the majority of the animals were presented with the history of road accidents (51.5%), followed by other reasons like falling, dog bite and fighting.

**Sex wise incidence:** The study showed that the incidence of long bone fractures in goats was more in

females (66.21 %) than in males (33.79 %). Out of 145 animals, 96 were females and 49 were male animals. Higher incidence of fractures in females is due to the fact that female goats are kept for longer period in the herd for production of offsprings and males are generally sold for meat purpose. Similarly, Singh *et al.* (1983), Arora (1996), Virkar (1999), Kumar (2005), Dandekar (2007), Singh *et al.* (2008), Tambe *et al.* (2012) and Gupta (2015) have observed that fractures are more common in female goats than in males. On the other hand, Philip *et al.* (1998) recorded the incidence of fractures was more in males (55.7 %) than in females (44.3 %). Singh *et al.* (2017) opined that the incidence of fractures in males can be attributed to the fact that, males are more active than females, which predispose them to the factors responsible for causing the fracture.

**Age wise incidence:** Out of the 145 goats presented with long bone fractures, 47 goats were aged less than 6 months (32.41 %), 51 goats were between 6 months to one year of age (35.17 %), and 25 goats were between one to two years (17.24 %) and 22 were above two years (15.17 %) as shown in Table 3 and plate II. The study revealed that incidence of fracture is more common in younger animals. The higher incidence in goats below one year of age may be due to their delicate body and inquisitive nature, which predisposes to malicious insults or automobile accidents resulting in fracture. Our findings are in concurrence with those of Ganesh *et al.* (1994), Arora (1996), Aithal and Singh (1999), Virkar (1999) and Kumar (2005) who have observed higher frequency of long bone fractures in goats below one year of age. According to Kushwaha *et al.* (2011) a higher incidence of fractures was recorded in goats below nine months of age. Gupta (2015) studied age wise distribution of fractures in goat and found that incidence of fractures in goats between six months to one year age was 50 %, whereas, the incidence of fractures in goats below six months and above one year of age was 25 %. In contrast, Singh *et al.* (1983) surveyed 511 clinical cases of different species and observed that a major proportion of fractures were recorded in the age group of one to three years.

**Bone affected:** In the present study, fractures were found to be more common in hind limbs (54.25 %) than in fore limbs (45.74 %). Similarly, Patil *et al.* (1991) Arora (1996) and Singh *et al.* (2008) recorded that fractures were more in hind limbs (62.96%) than fore limbs. Dandekar (2007) reported that fractures in hind limb (63.33%) were more than forelimb (36.66%) in goats. Singh *et al.* (1983) opined that most of the fractures were caused by automobile accidents, where the animals were most likely to get injury from behind as the animals were slow to react from their hind quarters. Contrarily, Kumar (2005) noticed that forelimbs were more commonly involved in long bone fractures in goats, metacarpus being the commonly affected bone.

The incidence was 27.59 % for metatarsal, 24.82 % for metacarpal, 22.06 % for tibia, 11.03 % for radius-ulna, 7.58 % for humerus and 06.89 % for femur as illustrated in table 4 and plate III. Thus the incidence of long bone fractures was highest in metatarsal bone followed by metacarpal bone in goats. Dass *et al.* (1985) observed that metacarpal and metatarsal were most commonly fractured long bones in goats. Singh *et al.* (2008) opined that the lack of protective musculature around metatarsal and metacarpal bones can be well correlated with higher incidence of fractures involving these bones. In contrast, Singh and Nigam (1981) and Singh *et al.* (1983) found higher frequency of fractures in femur (32 %) followed by tibia (21.7 %), metacarpals (15.3%), metatarsals (10.2%), phalanges (6.4 %), humerus (5.1 %) radius and ulna (3.8 %) and pelvic bones (2.6 %) in goat. Patil *et al.* (1991) reported that the frequency of tibial (23.2 %), metacarpal (23.2 %) and metatarsal (23.2 %) fractures were almost equal in number. Arora (1996) reported a higher incidence of tibial (25.17%) and metacarpal bone (21.67%) fractures in goats. Tambe *et al.* (2012) reported that the incidence of fracture was 27.5 % in tibia followed by radius and ulna, metatarsal and metacarpal bone. Lower incidence was recorded for femur. Gupta (2015) reported highest incidence of fracture in tibial bones (62.50%) followed by humerus, radius ulna, femur (12.50%). Singh *et al.* (2017) opined that more number of fractures in tibia

may be attributed to tendency of goats to suddenly flee from source of trauma like automobile or projectile stick. Being caudal part of the body, there are more chances of tibia to be trapped with a source of trauma. However, Aithal and Singh (1999) observed that femur sustained highest number of fractures in sheep and goats.

**Type of fracture :** It was found that majority of the goats i.e. 109 out of 145 (75.17 %) had simple fractures of long bones, whereas the rest had compound type. Oblique fractures were more common (42.76 %), followed by transverse type (38.3 %). Long bone fractures other than those of cannon bones constituted a significant proportion (47.58 %) and this can be attributed to higher incidence of oblique fractures, where the bones are oriented at an angle. As opined by Singh *et al.* (2017) a plausible explanation of high incidence of oblique fracture might be, when a force less than optimal breaking force of bone, acts tangentially on any object, it get distributed unproportionately with more force on near cortex and less force on far cortex leading to break of the nearby cortex and tear in the cortex which is away, thus creating oblique fracture in goats. Metatarsal and metacarpal fractures were 52.41% of all and the perpendicular orientation of these bones might be a contributing factor to a significant fraction of transverse type (38.3 %). Patil *et al.* (1991) studied 471 clinical cases of fractures and reported that most of them were diaphyseal and either oblique or transverse in nature. Arora (1996) found that most of the long bones fractures in goats were oblique (36.02%) or transverse (34.55%), and diaphyseal in nature. Aithal and Singh (1999) noticed that oblique type constituted the major proportion of long bone fractures in sheep and goats. Kumar (2005) opined that oblique and transverse fractures were the most common type in goats. Tambe *et al.* (2012) reported that mid-shaft diaphyseal fractures were more in goats. Singh *et al.* (2008) reported that metatarsal and metacarpal bones had the highest incidence of fractures, with transverse type being more common followed by oblique, multiple, epiphyseal, impacted and avulsion fractures. Kushwaha *et al.* (2011) opined that most of the fractures were of simple oblique type at the mid-shaft of the bone.

**Table 1: Incidence of long bone fractures in goats treated at Dept. of Veterinary Surgery and Radiology, Veterinary College, Bidar during Jan 2007 – Dec 2017**

Sl.No.	Year (Jan – Dec)	Number of surgical cases treated	Number of surgical cases of goats treated	Number of long bone Fractures in goats
1	2007	631	80	6
2	2008	1098	168	7
3	2009	762	130	12
4	2010	1532	184	7
5	2011	703	114	10
6	2012	849	133	6
7	2013	742	146	6
8	2014	721	163	8
9	2015	975	221	32
10	2016	918	241	32
11	2017	418	73	19
<b>Total</b>	<b>9349</b>	<b>1653</b>	<b>145</b>	

**Table 2: Incidence of etiology of long bone fractures in goats.**

S. No	Cause	Number of animals	Percentage of incidence (%)
1	Automobile accidents	46	31.72
2	Malicious injuries	39	26.89
3	Falling from height	21	14.48
4	Dog bite	04	02.75
5	Unknown causes	35	24.13

**Table 3: Age wise incidence of long bone fractures in goats.**

S. No.	Age	Number of animals	Percentage of incidence (%)
1	Less than 6 months	47	32.41
2	6 months to one year	51	35.17
3	One to two years	25	17.24
4	Above two years	22	15.17

**Table 4: bone wise incidence of fracture.**

S. No.	Limb involved	S. No.	Bone involved	Number of animals	Percentage of incidence (%)
I	Hindlimbs (54.25%)	1	Metatarsal	40	27.59
		2	Tibia	32	22.06
		3	Femur	10	06.89
II	Forelimbs (45.74 %)	4	Metacarpal	36	24.82
		5	Radius-ulna	16	11.03
		6	Humerus	11	07.58

Plate I : Pie diagram showing contribution of various etiological factors for fractures in goats in the study (%)

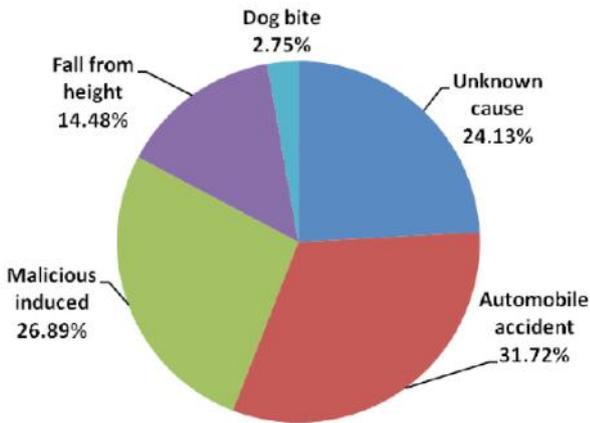


Plate II : Pie diagram showing age wise incidence of long bone fractures in goats (%)

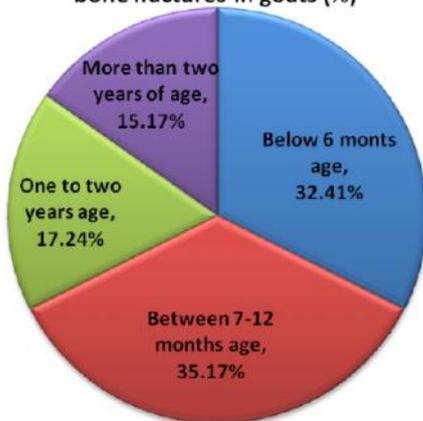
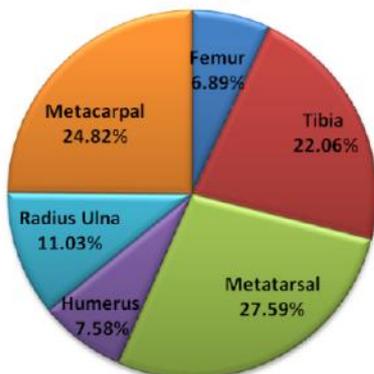


Plate III : Pie diagram showing bonewise incidence of fracture (%)



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## A Report On Parasitic Infection In A Peacock (*Pavo cristatus*)

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### ABSTRACT

A dead peacock was presented to the department of Veterinary pathology, Veterinary College, Hassan for post mortem. The physical examination incidentally revealed tick infestation of the carcass. During post mortem examination round worms were observed in the gizzard. The ticks, worms along with the intestine and its contents were subjected to further parasitological examination. The ticks were identified as *Haemaphysalis* species and the round worms as *Cheliospirura hamulosa*. The adult *Raillietina echinobothrida* tapeworm was found attached to the intestinal mucosa and on examination of the intestinal contents, two species of *Eimeria* oocysts and eggs of cestode were identified.

**Key words:** *Eimeria* oocysts, *Cheliospirura hamulosa*, peacock

### INTRODUCTION

Peacock (*Pavo cristatus*) which is a national bird of India is one of the most recognizable species of peafowl. India is having the highest diversity of peacock species. They usually suffer from parasitic infections, which are among most common problems affecting wild birds, occurring mostly as subclinical conditions but may also cause mortality (Freitas *et al.* 2002). Various factors contribute to infections in peafowls such as unnatural habitat, the human encroachment of the forest lands (Perrins, 1990), availability of vectors, intermediate hosts urbanization etc. Further, peafowls usually predate on various intermediate hosts of parasites that plays pivotal role in transmission of parasitic diseases, particularly helminths. Earlier workers have reported parasitic infections in varying degree in Indian peafowl and other gallinaceous birds viz., *Ascaridia* spp. (Rao *et al.* 1981; Rao and Hafeez 2006), *Raillietina* spp. (Saif *et al.* 2008), *Eimeria* spp. (Bhatia and Pande 1966, 1968). There is a paucity of information on parasitic diseases in peafowl. In the present study, parasites recovered incidentally during postmortem examination of peafowl are described.

### MATERIALS AND METHODS

The peacock was presented to the department of pathology for post mortem examination. During

physical examination ticks were found attached to the beak region. They were collected by using forceps and preserved in 70% alcohol. The ticks were later cleared in liquid phenol and mounted in phenol balsam. During internal examination, the gizzard showed presence of nematodes. The nematodes collected were washed 3-4 times in normal saline to clear the mucus and were fixed in alcohol glycerin, then cleared in lactophenol and mounted in Canada balsam. Segments of tapeworms and scolex were also recovered during examination of small intestine. The tape worm segments along with scolex were carefully rinsed in normal saline and was flattened between the two slides and fixed in 70% alcohol for Borax carmine staining. The staining was carried out as per standard procedure. The faecal sample collected from the intestine was examined by sedimentation and flotation techniques which revealed unsporulated oocysts and tapeworm eggs. Later the unsporulated oocysts were kept for sporulation by using 2.5% potassium dichromate. The micrometry and morphometry was done to appreciate the oocysts.

### RESULTS AND DISCUSSION

The ticks recovered from the peafowl during external examination were identified as *Haemaphysalis* sp (Fig.1). The tick infestation in peacocks cause irritation, anaemia and they also transmit

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haemoprotozoan diseases. The free range birds are more prone for tick infestations because of presence of free living stages in the environment.

The female nematodes recovered from the gizzard were identified as *Cheilospirura hamulosa*. The anterior end of the worm showed buccal capsule leading to muscular and glandular oesophagus and uterus filled with embryonated eggs. The results were in agreement with Menezes *et al.* (2003). During post mortem examination the lining of the gizzard showed small dark ulcerated areas.

The tapeworms recovered during postmortem examination was identified as *Raillietina echinobothrida* (Fig.2). The scolex of the adult tapeworm has four suckers and a rostellum, the suckers were round in shape which is a characteristic feature of *R. echinobothrida*. It was further confirmed by examination of gravid segments with egg packets containing 8-12 eggs. The cestode eggs were detected on faecal sample examination which measured 51.5 x 47mm (Fig.3). The penetration of scolex into the mucosa cause irritation resulting in nodules which can be confused with tuberculosis.. The results were in accordance with Dutta *et al.*, (2013) who reported mixed infection of *Ascaridia* spp. along with *Raillietina* spp. and *Eimeria* spp. in Indian Peafowl (*Pavo cristatus*) of Ramnabagan Mini Zoo, Burdwan, West Bengal, India.

In the present study, two types of oocysts were observed. Based on micrometry, the larger oocysts which were oval in shape with a measurement of 27.5x17.5m (20-28x16-20) were identified as *E. pavonina* (Fig. 4). The oocysts which were of medium size were identified as *E. mayuri* with a size of 17.5x16mm (14-20x14-18) (Fig. 5). In India five

*Eimeria* spp. are reported viz. *E. pavonina* (Banik and Ray, 1964); *E. mandali* (Banik and Ray, 1964); *E. pavonis* (Mandal, 1965); *E. mayuri* (Bhatia and Pande, 1966) and *E. patnaiki* (Ray, 1966) have been described besides *Isospora mayuri* affecting peacock (Pellerdy, 1965). A survey conducted by Jaiswal *et al.*, (2013) in free range blue peacocks revealed five species of *Eimeria* and one species of *Isospora*.. Kathravan *et al.* (2017) reported that *Eimeria* spp were the most common (43%) particularly *E. mayuri* and *E. pavonis*, *Hymenolepis* spp (4.16%), *Ascaridia* spp (6.9%), *Strongyloides* spp (4.16%) and *Strongyles* (2.77%) in wild free ranging peafowls. Mixed parasitic infection was observed in the present case which was also reported by Muraleedharan *et al.*, (1990) and Jaiswal *et al.*(2013). Coccidiosis in free ranging birds is not of great concern but the oocysts concentration builds up in captivity can cause clinical disease in the birds. The micrometry of the oocysts and parasitic eggs were done and tabulated (Table.1).

### CONCLUSION

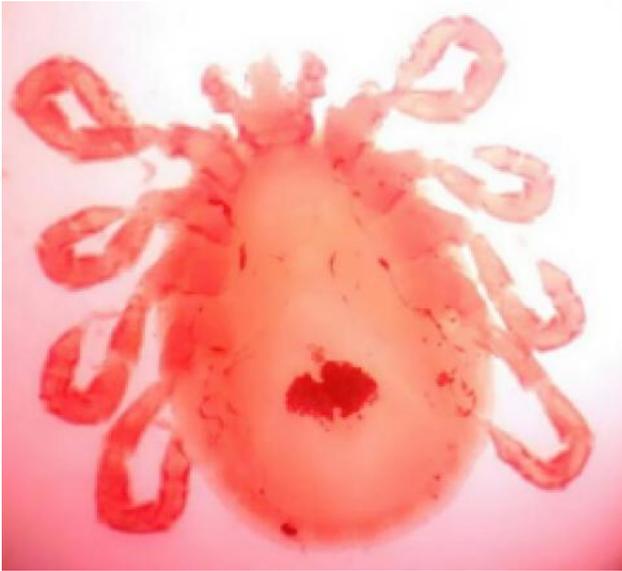
Most of the parasitic infections in the peacock go unnoticed due to the lack of investigation and moreover there are few reports on the prevalence of parasitic infection in peafowl. The correct speciation of parasites of free range birds is challenging. The description of parasites made in this literature can contribute for postgraduate teaching as well as for zoo veterinarians for treatment of peafowls.

### ACKNOWLEDGEMENT

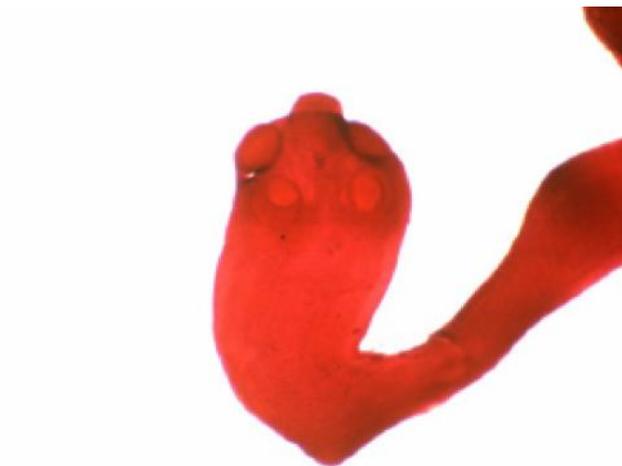
I am thankful to Dean Veterinary College, Hassan and Head of Dept of Pathology, Veterinary College Hassan for providing facilities to carry out this work .

**Table 1. Parasites, eggs and oocysts detected in peacock**

Species	Shape	Size(µm)	References
<i>E. mayurai</i>	Spherical	17.5x16mm	Ray (1966)
<i>E. pavonina</i>	Ovoid	27.5x17.5mm	Banik and Ray (1961)
Cestode eggs	Completely ovoid	51.5 x 47 mm	Sloss <i>et al.</i> (1994)
<i>Raillietina echinobothrida</i> - Scolex with round suckers			
<i>Cheilospirura hamulosa</i> - Buccal capsule leading to muscular and glandular oesophagus			
<i>Haemaphysalis</i> sp (Tick) - Second segment of pedipalp having lateral projection			



**Fig 1:** *Haemaphysalis* spp



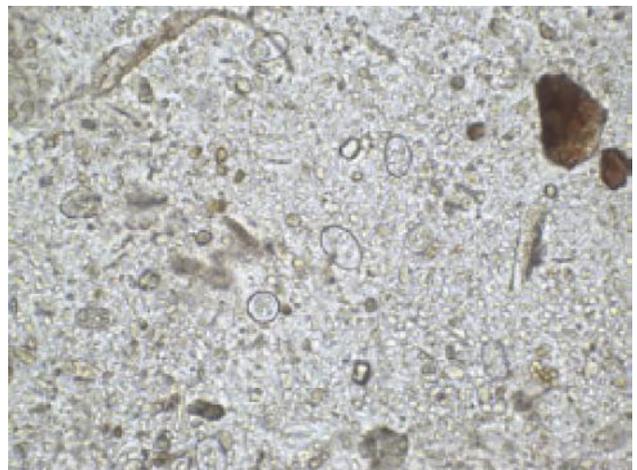
**Fig 2:** Scolex of *Raillietina ecchinobothrida* with round suckers (100X)

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**Fig 3:** Cestode egg with hexacanth



**Fig 4 and 5:** The oocysts of *Eimeria* (*E. Pavonina* embryo (400X) and *E. mayurai*) in faecal sample (400X)

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## Video Ooscopic Findings in Dogs Affected with Otitis

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### ABSTRACT

The dogs presented to Veterinary college, Hebbal, Bengaluru with clinical signs suggestive of otitis such as erythema, presence of exudate, foul odour, head shaking /tilt and evince of pain on palpation were selected as subjects for video otoscopic examination. Video otoscopic examination of 28 dogs revealed that all the 28 ( 100%) dogs showed ulceration of the ear canal, 25 (89.28%) dogs showed intact tympanic membrane, 3 dogs ( 10.71%) showed ruptured tympanic membrane, one (3.57%) dog had foreign body in the ear canal and one (3.57%) dog showed stenosis of the ear lumen. None of the dogs examined revealed neoplasms and calcification of the ear cartilage. Video otoscopic examination can be an effective tool in facilitating specific diagnosis of both external ear canal and the tympanic membrane and is generally indicated in dogs showing typical signs of otitis.

### INTRODUCTION

Otitis externa is the most common disease of the canine ear canal. The estimated incidence of otitis in dogs ranged from 4 to 20 per cent (Muller and Schmitz, 1983). Clinical signs included erythema, discharge from ear, desquamation of the epithelium, pain and pruritus. Unfortunately, many cases of the otitis remain unresolved and become progressively refractory to therapy (Radlinsky and Mason, 2006). Video otoscopy has emerged as a practical and effective tool for diagnosing and managing otitis externa and media, since it has several advantages over conventional therapy (August and Campbell, 2001). The present study was undertaken to detect the abnormalities of ear canal by video otoscopic examination.

### MATERIALS AND METHODS:

A total of 15766 dogs presented to Veterinary College Hospital, Hebbal, Bangalore during the period of six months from January 2014 to June 2014 were examined for clinical signs suggestive of otitis viz., erythema, presence of exudate, foul odour, head shaking /tilt and evince of pain on palpation. Out of the 15766 animals examined, 840 animals revealed

typical signs of otitis. Based on culture examination and invitro sensitivity test 28 dogs were selected for the otoscopic examination. The animal was positioned at right lateral recumbency, the video otoscope was positioned such that the ventral floor of the horizontal canal appears at the bottom of the screen, the left side of the screen is rostral, and the right side of the screen is caudal. When the left tympanium is visualized in this way, the dorsally positioned pars flaccida always appears at the top or top left of the screen. In the normal ear, the manubrium of the malleus is clearly seen through the translucent pars tensa. The manubrium often has a gentle hook, which points rostrally. By standardizing the position of the otoscope when examining the tympanum, photographs from the right ear or the left ear can be easily recognized.

### RESULTS AND DISCUSSION:

In the present clinical investigation the major clinical signs observed were aural pruritus, ear shaking, foul odour ear discharge, inflammation of the ear, erythema and thickening of pinna, head tilt and swelling at the base of the ear. Similar observations were made by Kale and Aher, (2004). Based on culture examination and invitro sensitivity test, 28 dogs were

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selected for the otoscopic examination. Video otoscopic examination of 28 dogs revealed that all the 28 ( 100%) dogs showed ulceration of the ear canal, 25 (89.28%) dogs showed intact tympanic membrane, three dogs (10.71%) showed ruptured tympanic membrane, one (3.57%) dog had foreign body in the ear canal and one (3.57%) dog showed stenosis of the ear lumen. None of the dogs revealed neoplasms and calcification of the ear cartilage.

Video otoscopic examination can be an effective tool in facilitating specific diagnosis of both external ear canal and the tympanic membrane and is generally indicated in dogs showing typical signs of otitis, Angus and Campbell, (2001), Cole, (2004). Griffin, (2006) opined that video otoscopy was much superior to conventional otoscopy because this technique involves inserting a very small camera into the ear, so that the ear canal appears as clear and aids in viewing the structures of the ear canal. In addition, this technique enables removal of secretion adhered to the wall of the external ear canal and an automated flushing and suction pump makes deep ear cleaning extremely efficient.

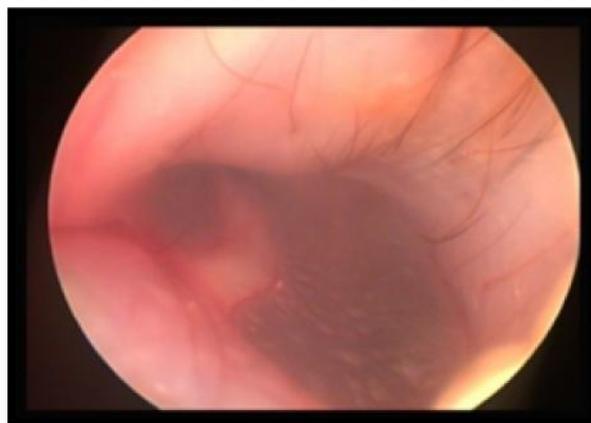
The abnormalities detected by video otoscopy included ruptured tympanic membrane, foreign body in the ear canal and stenosis of the ear lumen. None of the dogs examined in the present study revealed neoplasms and calcification of the ear cartilage. Griffin, (2006) indicated that any tear in the tympanum as revealed by video otoscopy is that otitis media is likely present. However, literature of use on video otoscopy in diagnosis of foreign body and stenosis of ear canal are lacking. The major clinical signs of dogs with otitis were aural pruritus exhibited by ear scratching, ear shaking, ear pain, foul odour ear discharge, erythema and thickening of pinna, head tilt and swelling at the base of the ear. The video otoscope is designed to enhance examination of the external ear canal and tympanic membrane while facilitating specific diagnostic and therapeutic procedures. General anesthesia is recommended to minimize head movement and the risks of injury or telescope damage.



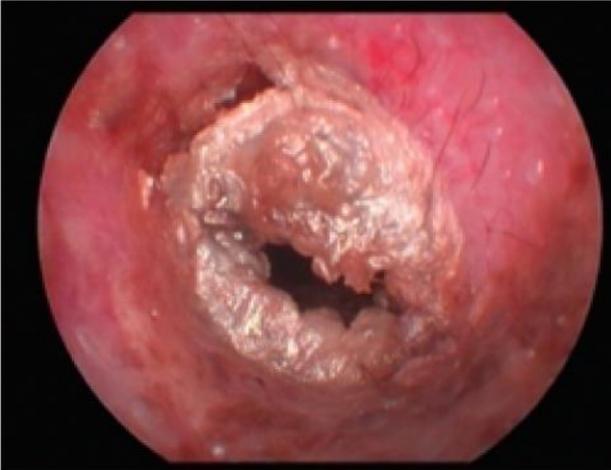
**Plate 1:** Yellowish purulent discharge from the otitic ear



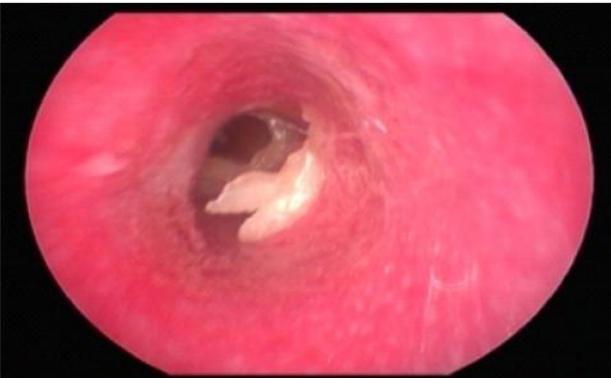
**Plate 2:** Ulcerated, erythematous and thickened ear pinnae



**Plate 3:** Intact tympanium on video otoscopy



**Plate 4:** Ruptured tympanic membrane on video otoscopy



**Plate 5:** Ulceration of ear canal on video otoscopy



**Plate 6:** Foreign body obstruction in the ear canal



**Plate 7:** Stenosis of the lumen ear canal on video otoscopy

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## Methotrexate Induced Serum Biochemical Alteration and Its Amelioration by Zinc Oxide Nanoparticles in Wistar Albino Rats\*

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### ABSTRACT

The present study was undertaken to evaluate methotrexate (MTX) induced alteration in serum biochemical parameters and its amelioration by zinc oxide nanoparticles (ZnO NP) in Wistar albino rats. Rats were randomly divided into five groups with 12 animals each. Group I was the normal control. Group II was MTX control with rats that received methotrexate at the dose rate of 5 mg/kg b.w. intraperitoneally for three consecutive days. Group III (ZnO NP control), rats were gavaged with ZnO NP at the dose rate of 50 mg/kg b.w. for 45 days. Group IV (ZnO NP pre-treatment) received ZnO NP for 14 days prior to induction of MTX toxicity and continued for 45 days. Group V rats were induced with MTX toxicity and concurrently treated with ZnO NP. Serum biochemical parameters such as AST, ALT, ALP, GGT, creatinine, TP and albumin were estimated on 7<sup>th</sup>, 21<sup>st</sup> and 45<sup>th</sup> days of experiment. Present study showed that MTX had induced hepatotoxicity as evidenced by significant ( $P < 0.05$ ) increase in the levels of ALT, AST, ALP and GGT and these levels were significantly ( $P < 0.05$ ) reduced in ZnO NP treatment groups (Group IV and V). TP and albumin levels estimated in MTX control also were found to be significant ( $P < 0.05$ ). However, ZnO NP pre-treatment (Group IV) significantly ( $P < 0.05$ ) improved the levels, lowered than those of ZnO NP treatment groups (IV and V) on all the days of observation. However, ZnO NP pre-treatment (Group IV) significantly ( $P < 0.05$ ) improved the levels of biochemical parameters than concurrent (Group V) treatment and provided better hepatoprotection against MTX induced toxicity.

**Key words :** Methotrexate, Zinc oxide nanoparticles, Wistar albino rats, biochemical parameters.

### INTRODUCTION

The concept of “one medicine” has become a popular idea in recent years, due to the realization that many diseases that occur in human patients are also recognized in nonhuman species, specifically domestic animals. Autoimmunity and neoplasias are high on the list of pathogenic etiology that is similar between humans and domestic animals. Methotrexate, a structural analogue of folic acid and a potent inhibitor of dihydrofolate reductase, is widely used as a cytotoxic drug for leukemia and other malignancies and also for treating autoimmune diseases such as psoriasis and rheumatoid arthritis (Fatimah *et al.*, 2013; Patel *et al.*,

2014). Its use is limited due to high incidence of serious dose-dependent toxicity including hepato toxicity, renal damage, bone marrow suppression and gastro intestinal mucosal inflammation (Ramadan *et al.*, 2008). These adverse MTX effects are a result of induced systemic oxidative stress during medication. Hence, there is a constant search for drugs or agents with antioxidant property to alleviate the MTX induced non target toxicities (Ozogul *et al.*, 2013)

The recent development of nanotechnology has contributed to the production and control of engineered nanoparticles, to suit various fields of science (Baek *et al.*, 2012). Zinc oxide nanoparticles are one of the most

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widely used nanoparticles in consumer products. They are extensively used in cosmetics and sunscreens because of their efficient UV absorption properties, in the food industry as additives and in packaging due to their antimicrobial properties. They are also being explored for their potential use as fungicides in agriculture and as anticancer drugs and imaging in biomedical applications (Rasmussen *et al.*, 2010). ZnO NP is also used as an adjuvant therapy to ameliorate the toxic side effects of few chemotherapeutic agents. Hence the present study was carried out to elucidate the efficacy of ZnO NP in ameliorating MTX induced hepato-toxicity.

## MATERIALS AND METHODS

### Experimental animals

Sixty male Wistar Albino rats were procured from Biogen laboratory animal facility, Attibele, Bangalore of the age 8 to 10 weeks, with an average live weight of 180- 200 g. They were housed in cages for two weeks to acclimatize under ambient temperature and standard light and dark cycle. They were given a nutritionally adequate specified rat's diet and water *ad libitum* throughout the experimental period. The experimental procedures were carried out according to the Committee for Purpose of Control and Supervision of Experiments on Animals with approval from the Institutional Animal Ethics Committee.

Methotrexate, as Filitrax-15 injection was procured from IPCA laboratories, Mumbai. Zinc oxide nanoparticles (Product number: 721077-100G, <100 nm particle size (TEM), d'40 nm average particle size, 20 weight % in H<sub>2</sub>O) were procured from Merck KGaA, Darmstadt, Germany.

### Experimental design

The rats were divided, based on the body weight, into five groups with twelve rats in each group. Group I was normal control administered with PBS intraperitoneally for three consecutive days and observed till 45 days. Group II were administered methotrexate at the dose rate of 5 mg/kg b.w. intraperitoneally with over-night fasting for three consecutive days. Group III (ZnO NP control) was

gavaged with ZnO NP at the dose rate of 50 mg/kg b.w for 45 days. Group IV was gavaged with ZnO NP at 50 mg/kg b.w. for 14 days prior to induction of MTX toxicity and continued till the end of the experiment. Group V was induced with MTX toxicity and concurrently treated with ZnONP at 50 mg/kg b.w.

### Collection of serum samples

Blood was collected from the retro-orbital plexus of the rats under light inhalant anaesthesia at different time intervals such as 7<sup>th</sup>, 21<sup>st</sup> and 45<sup>th</sup> day post induction of MTX toxicity. The separated serum samples were collected into one mL micro-centrifuge tubes and were stored at -20°C for biochemical analysis.

### Serum biochemical parameters analysed

Serum obtained from different groups at different intervals of observation were subjected for estimation of different biochemical parameters such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP),  $\alpha$ -glutamyl transferase (GGT), total proteins (TP) and albumin.

## RESULTS AND DISCUSSION

A significant ( $P < 0.05$ ) increase in the mean ALT, AST, ALP and GGT values were observed in the MTX control group, which ascertained the toxic effect of MTX on hepatocytes (Table 1-4). Serum transaminases are the indicators of hepatic cell injury and ALT and AST have been considered as markers of hepatic cell injury especially the ALT. ALT is a cytosolic enzyme and its increased activity in serum reflects a leakage in cell membrane permeability and possibility of liver damage (Rajesh and Latha, 2004; El-Azim 2014). Methotrexate, being a folic acid antagonist, blocks the synthesis of purines and pyrimidines by inhibiting several key enzymes. Inhibition of dihydrofolate reductase (DHFR) decreases tetrahydrofolate (THF) levels, which results in attenuated DNA/protein/lipid methylation, inhibition of thymidylate synthase (TS) interference with DNA synthesis, and inhibition of 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase which blocks de novo purine

synthesis. The inhibition of purine and pyrimidine is responsible for methotrexate induced toxicity (Henghe and Bruce, 2007). In addition, MTX also causes significant reduction in reduced glutathione levels leading to suppression of the antioxidant enzyme defense system sensitizing the cells to ROS (Celtikci *et al.*, 2009). Thus a rise in hepatic enzyme levels observed in the present study could be attributed to cell membrane integrity damage due to increased elaboration of ROS and decreased antioxidant levels by MTX (Kremer *et al.*, 1986; Fu *et al.*, 2008; Prey and Paul 2009, El Azim, 2014; Deepak, 2014; Vijaykumar, 2016).

It was observed that both pre-treatment and concurrent treatment with ZnO NP resulted in a progressive significant ( $P < 0.05$ ) decrease in the ALT, ALP and GGT enzyme levels. AST mean value was significantly ( $P < 0.05$ ) reduced only in the pre-treatment group (Group IV). The AST mean values remained high and comparable with that of MTX control group in concurrent treatment group (Group V) (Table 1 to 4).

The current results indicated that ZnO NP treatment provided protection against MTX induced toxicity and the pre-treatment of ZnO NPs has better hepato-protective effect than concurrent treatment.

ZnO NPs on administration are absorbed and dissociate into particulate and ionic Zn. The ionic Zn enters the cell and provides the beneficial effects of Zn. Zinc protects the cell from injury by increasing antioxidant enzymes level especially that of GPx and SOD and thus decreasing the lipid peroxidation. It also exerts its antioxidant activity by induction of metallothionines which are powerful scavengers of free radicals (Grungreiff, 2002). Zn metallothionines prevent lipid peroxidation of cell membrane and thus maintain cell membrane integrity (Dawei *et al.*, 2010 and Gao *et al.*, 2017) and prevent leakage of enzymes.

In the present study, increased GGT and ALP enzyme levels in the MTX control group indicated the possible role of MTX in induction of biliary toxicity also. Increased ALP enzyme activity in the present study also could be due to MTX induced injury to other organs such as intestine and kidney (Patel *et al.*, 2014).

A marked reduction in the protein synthetic function by liver was observed in MTX control group depicted by a significant ( $P < 0.05$ ) reduction in total protein and albumin levels (Table 5 and 6). The decreased protein values may be due to several factors such as increased intestinal protein loss, protein losing nephropathy, dietary protein deficiency, decreased feed intake including damage to liver (Patel *et al.*, 2014).

In MTX induced toxicity, blockade of tetrahydrofolate synthesis may lead to inability of cell to divide and to produce proteins contributing for reduced synthetic function (Howard *et al.*, 2016; Henghe *et al.*, 2007). ZnO NP treatment in both the groups (Group IV and V) improved the protein levels indicating the protective effect of ZnO NPs like stabilization of cytostructure of hepatocytes through their antioxidant property.

ZnO NP control (Group III) showed the levels of ALP, GGT, TP and albumin comparable with those of normal. However, ALT and AST were marginally higher than normal and significantly ( $P < 0.05$ ) lower than MTX treated groups (II, IV and V) which could be attributed to the mild cellular degenerative effect of ZnO NP on an account of bioaccumulation with longer period of treatment (Choi and Choy, 2014).

**Conclusion:** Methotrexate induces toxicity at 5 mg/kg b.w. intraperitoneally for three consecutive days and zinc oxide nanoparticles partially ameliorate methotrexate induced toxicity and pre-treatment with zinc oxide nanoparticles provides prophylactic relief from the immediate toxic effects of methotrexate.

**Table 1. Mean±SE values of serum alanine aminotransferase (U/L) of different groups at different time intervals**

Groups	7 <sup>th</sup> day	21 <sup>st</sup> day	45 <sup>th</sup> day
Group I (Control)	21.577±0.246 <sup>ax</sup>	23.082±0.229 <sup>ax</sup>	24.834±0.411 <sup>ax</sup>
Group II (MTX)	137.200±10.61 <sup>bx</sup>	83.408±0.787 <sup>by</sup>	67.959±1.676 <sup>bz</sup>
Group III(ZnONP)	35.334±1.08 <sup>cx</sup>	37.234±1.09 <sup>cx</sup>	39.878±0.95 <sup>cx</sup>
Group IV (ZnONP+MTX)	76.883±0.874 <sup>dx</sup>	43.853±0.495 <sup>cy</sup>	39.138±1.107 <sup>cy</sup>
Group V (MTX+ZnONP)	87.197±1.464 <sup>dx</sup>	53.945±0.382 <sup>dcy</sup>	44.210±1.340 <sup>cz</sup>

Values with different superscripts vary significantly at P<0.05

**Table 2. Mean±SE values of serum aspartate aminotransferase (U/L) of different groups at different time intervals**

Groups	7 <sup>th</sup> day	21 <sup>st</sup> day	45 <sup>th</sup> day
Group I (Control)	83.750 ± 0.284 <sup>ax</sup>	84.480 ± 0.351 <sup>ax</sup>	85.603 ± 1.321 <sup>ax</sup>
Group II (MTX)	186.500±1.310 <sup>bx</sup>	176.873±2.671 <sup>by</sup>	103.381±0.250 <sup>bz</sup>
Group III(ZnONP)	94.151±2.092 <sup>cx</sup>	104.647±1.242 <sup>cx</sup>	97.146±2.044 <sup>cx</sup>
Group IV (ZnONP+MTX)	151.797±0.055 <sup>dx</sup>	137.729±729 <sup>dy</sup>	101.047±1.067 <sup>bcz</sup>
Group V (MTX+ZnONP)	191.404±1.262 <sup>bx</sup>	165.302±0.497 <sup>ey</sup>	113.055±0.481 <sup>dz</sup>

Values with different superscripts vary significantly at P<0.05

**Table 3. Mean±SE values of serum alkaline phosphatase (U/L) of different groups at different time intervals**

Groups	7 <sup>th</sup> day	21 <sup>st</sup> day	45 <sup>th</sup> day
Group I (Control)	91.167±0.091 <sup>ax</sup>	91.190±0.534 <sup>ax</sup>	92.373±0.688 <sup>ax</sup>
Group II (MTX)	593.00±2.543 <sup>bx</sup>	316.072±3.593 <sup>by</sup>	282.950±1.525 <sup>bz</sup>
Group III(ZnONP)	95.088±0.898 <sup>ax</sup>	94.560±1.823 <sup>ax</sup>	100.742±2.115 <sup>ax</sup>
Group IV (ZnONP+MTX)	367.851±1.648 <sup>cx</sup>	250.883±0.754 <sup>cy</sup>	131.211±5.734 <sup>cz</sup>
Group V (MTX+ZnONP)	555.049±9.992 <sup>dx</sup>	317.351±3.197 <sup>by</sup>	181.326±0.727 <sup>dz</sup>

Values with different superscripts vary significantly at P<0.05

**Table 4 : Mean ± SE values of gamma glutamyl transferase (U/L) of different groups at different time intervals**

Groups	7 <sup>th</sup> day	21 <sup>st</sup> day	45 <sup>th</sup> day
Group I (Control)	2.490±0.052 <sup>ax</sup>	2.545±0.021 <sup>ax</sup>	2.664±0.188 <sup>ax</sup>
Group II (MTX)	10.285±0.327 <sup>bx</sup>	8.395±0.212 <sup>by</sup>	4.006±0.001 <sup>bz</sup>
Group III(ZnONP)	2.986±0.115 <sup>ax</sup>	3.103±0.064 <sup>cx</sup>	2.943±0.152 <sup>ax</sup>
Group IV (ZnONP+MTX)	4.617±0.061 <sup>cx</sup>	3.374±0.019 <sup>cy</sup>	2.751±0.135 <sup>az</sup>
Group V (MTX+ZnONP)	5.660±0.158 <sup>dx</sup>	4.273±0.009 <sup>dy</sup>	3.450±0.058 <sup>caz</sup>

Values with different superscripts in rows and column vary significantly at P<0.05

**Table 5 : Mean ± SE values of serum total protein (g%) of different groups at different time intervals**

Groups	7 <sup>th</sup> day	21 <sup>st</sup> day	45 <sup>th</sup> day
Group I (Control)	6.359 ± 0.036 <sup>ax</sup>	6.419 ± 0.120 <sup>ax</sup>	6.706 ± 0.302 <sup>ax</sup>
Group II (MTX)	4.545 ± 0.040 <sup>bx</sup>	5.246 ± 0.047 <sup>by</sup>	5.903 ± 0.023 <sup>bz</sup>
Group III(ZnONP)	6.578 ± 0.002 <sup>ax</sup>	6.329 ± 0.089 <sup>ax</sup>	6.426 ± 0.073 <sup>abx</sup>
Group IV (ZnONP+MTX)	5.286 ± 0.011 <sup>cx</sup>	6.481 ± 0.046 <sup>ay</sup>	6.757 ± 0.140 <sup>ay</sup>
Group V (MTX+ZnONP)	4.920 ± 0.113 <sup>bex</sup>	5.585 ± 0.085 <sup>by</sup>	6.378 ± 0.045 <sup>abz</sup>

Values with different superscripts vary significantly at P<0.0

**Table 6 : Mean ± SE values of serum albumin (g%) of different groups at different time intervals**

Groups	7 <sup>th</sup> day	21 <sup>st</sup> day	45 <sup>th</sup> day
Group I (Control)	3.843 ± 0.224 <sup>ax</sup>	4.430 ± 0.142 <sup>ay</sup>	4.266 ± 0.271 <sup>ay</sup>
Group II (MTX)	2.415 ± 0.025 <sup>bx</sup>	2.865 ± 0.047 <sup>by</sup>	3.551 ± 0.073 <sup>bz</sup>
Group III(ZnONP)	4.728 ± 0.014 <sup>cx</sup>	3.958 ± 0.087 <sup>cy</sup>	4.018 ± 0.078 <sup>ay</sup>
Group IV (ZnONP+MTX)	2.760 ± 0.013 <sup>bx</sup>	3.731 ± 0.035 <sup>cdy</sup>	3.973 ± 0.072 <sup>aby</sup>
Group V (MTX+ZnONP)	2.245 ± 0.116 <sup>bex</sup>	3.174 ± 0.036 <sup>bey</sup>	3.112 ± 0.029 <sup>by</sup>

Values with different superscripts vary significantly at P<0.05

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## ***In Vivo* Anti-Inflammatory and Analgesic Activity of Lablab Purpureus Leaf Extract in Rats<sup>#</sup>**

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### **ABSTRACT**

The present study was undertaken to evaluate the *in vivo* anti-inflammatory and analgesic activity of the methanolic extract of leaves of *Lablab purpureus* plant using carrageenan induced paw oedema and acetic acid induced writhing, respectively, in Wistar rats. The leaf extract at the doses of 400 and 600 mg/kg is having anti-inflammatory as well as analgesic property *in vivo*. The methanolic plant leaf extract showed anti-inflammatory and analgesic property that can be utilized to explore the therapeutic efficacy.

**Key words :** *Lablab purpureus*, anti-inflammatory, analgesic, rat

Many plants are traditional sources for food and pharmaceutical products. Since the difference of a therapeutic and a toxic effect depends on the dose, there are fluent transitions between consumption, therapy and toxicity of nutraceuticals in food, medicines or plants (Helena and Leila, 2016). Over a very long time period, medicinal plants are considered as repository of numerous types of bioactive compounds possessing varied potential therapeutic properties. The vast array of therapeutic effects associated with medicinal plants includes anti-inflammatory, antiviral, antitumor, anti-malarial, analgesic and many other properties (Raina *et al.*, 2014).

*L. purpureus* is commonly referred to as 'Field bean' or 'Hyacinth bean', is a legume widespread throughout the tropics. In India, it is an important legume used as a pulse and vegetable for human consumption and forage (Maheshu *et al.*, 2013). It is a versatile multipurpose plant that offers food, fodder, green manure and some traditional home remedies (Devaraj, 2016). Various parts of this plant are having many pharmacological properties such as hepatoprotective property (Ramamani *et al.* 1979), antidiabetic property (Kante and Reddy, 2013; Singh and Sankar 2012),

anti-inflammatory and analgesic property (Momin *et al.*, 2012; Proma *et al.* 2014), antimicrobial activity (Priya and Jenifer, 2014) and antioxidant property (Momin *et al.*, 2012).

### **MATERIAL AND METHODS**

#### **Collection and identification of plant material**

The fresh aerial parts of the plant were collected in the month of October and November 2017 from Bailahongal Taluk of Belagavi District, Karnataka State, which was botanically identified. The collected plant materials were washed under running tap water and were allowed to drain before air drying under shade for two weeks. The leaves of the plant were separated and ground mechanically using the household mixer and the obtained powder was sieved to get the coarse powder which was kept in air tight containers for further use.

#### **Experimental animals**

Wistar albino rats used in the present study were procured from Adita Biosys Private Limited, Plot No: SPL – 26, II Phase, Kssidc Insudtrial Estate,

<sup>#</sup> Part of thesis submitted by the first author to Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar

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Antharasanhalli, Tumkuru – 567 106, Karnataka with CPCSEA approval No: 1868/PO/Bt/S/16/CPCSEA. The animals were maintained in standard management conditions as per the CPCSEA guidelines. Food and water were provided *ad libitum*. The IAEC approval has been taken prior to start of the experiment and the approval No. is: VCS/IAEC/14/2017-18, dt. 10.06.2017.

### Selection and preparation of doses

Three doses (200, 400 and 600 mg/kg) were selected based on the previous pharmacological study of same plant, in which the plant extract at the dose levels of 50, 100, 200 and 400 mg/kg showed efficacy (Ahmed *et al.*, 2015).

### Experimental design

After one week of acclimatization, 30 rats (of either sex) were randomly divided into five groups (n=6). The grouping of animals along with the respective treatment is shown in Table 1.

### Anti-inflammatory activity

The anti-inflammatory activity of *L. purpureus* leaf extract was carried out using carrageenan induced paw edema as the acute inflammatory model (Winter *et al.*, 1962). The food was withdrawn (but not water) 12 hours before the start of experiment. Acute inflammation was produced by sub planter administration of 0.1 ml of 1% w/v carrageenan in normal saline in the right hind paw of the rats. The paw volume was measured at 0h, 0.5, 1, 2 and 5h after carrageenan injection by using plethysmometer (Kavimani *et al.*, 1996; Oyemitan *et al.*, 2008). The animals of Group I received distilled water (1 ml/100g) and served as control. The Groups II, III and IV received (*per oral*) methanol extract of *L. purpureus* leaves at the dose of 200, 400 and 600 mg/kg b.wt., respectively. Group V received reference drug meloxicam at dose of 2 mg/kg (subcutaneously). The respective treatments were given to all the groups 1 hour before the treatment with carrageenan. The increase in paw volume at respective time was calculated by subtracting the paw volume at 0 h from the paw volume during that time.

Increase in paw volume at time (t) = Actual paw volume at time (t) – Paw volume at 0 h

$$\text{Inhibition (\%)} = \frac{V_c - V_t}{V_c} \times 100$$

The *per cent* inhibition was calculated using the following formula;

Where,  $V_c$  = Mean increase in paw volume of control animals at time (t)

$V_t$  = Mean increase in paw volume of treated animals at time (t)

### Analgesic activity

The analgesic activity of methanolic extract of *L. purpureus* leaves was studied against acetic acid-induced writhings according to the method given by Dey *et al.* (2010). This method was used preferentially to evaluate the peripheral action of extract. The animals were divided into 5 groups comprising six rats (of either sex) in each group. Group I served as control. Group II, III and IV received 200, 400 and 600 mg/kg methanolic extract of *L. purpureus* leaves respectively. Groups V received meloxicam (2 mg/kg, subcutaneously). Sixty minutes after the treatment of respective groups, 10 ml/kg of 1.0 *per cent* acetic acid was injected (intraperitoneal) for the induction of writhing. The writhing effect was indicated by stretching of at least one hind limb. This response was observed from 5 min after the acetic acid administration and up to 20 min and reduction in number of writhings in the treated groups and standard were compared with animals in the control group. The percentage protection of abdominal constrictions was calculated by the formula;

$$\text{Protection (\%)} = \frac{W_c - W_t \text{ or } W_r}{W_c} \times 100$$

Where,  $W_c$  = Mean no. of writhes (control),  $W_t$  or  $W_r$  = Mean no. of writhes (test or reference drug).

## RESULTS AND DISCUSSION

In the present study, leaf extract at the dose of 400 and 600 mg/kg showed significant ( $p < 0.05$ ) anti-

inflammatory property on comparison of paw volume of control rats at different time intervals. After 5 h of carrageenan administration the *per cent* reduction of paw oedema volume by the extract at the dose levels of 200, 400 and 600 mg/kg were 2.33, 15.11 and 18.60, respectively. The reference drug (meloxicam) reduced the paw oedema volume at 5 h of carrageenan administration by 27.90 *per cent*. There was no significant difference between the group I and II (Table 2 and Figure 1).

In acetic acid induced writhing model the extract of *L. purpureus* leaves at the dose of 400 and 600 mg/kg. produced 30.62 and 39.18 *per cent* reduction of writhing response, respectively. The results were found to be significant ( $p < 0.05$ ) when compared with the control group. The *per cent* reduction of writhings by reference drug (meloxicam) was 42.78. There was no significant ( $p < 0.05$ ) difference between the group I and II (Table 3 and Figure 2).

The results from present study confirmed the results of *in vitro* study conducted by Momin *et al.* (2012) using two Bangladeshi bean pods *Lablab purpureus* (Sweet ‘white’ and ‘purple’) by protease

inhibition method and he reported that there was a linear relation of % inhibition for the white bean pods which indicated positive anti-inflammatory property.

This is supported by the study of analgesic activity of aerial part of the plant in mice model by Proma *et al.* (2014) who reported that the extract at doses of 50, 100, 200 and 400 mg/kg reduced the number of abdominal constrictions by 23.1, 34.6, 42.3 and 61.5%, respectively.

The anti-inflammatory and analgesic activity might be attributed to the various phytochemical constituents present in the plant methanol extracts like alkaloids, steroids and saponins (Onasanwo *et al.*, 2012).

### CONCLUSION

The current study revealed that the methanolic leaf extract of *L. purpureus* is having significant anti-inflammatory and analgesic activity at optimum doses in rats. Thus, there exists a vast scope to explore the role of individual phytochemical constituents responsible for anti-inflammatory and analgesic activity exerted by the extract in rats.

**Table 1: The grouping of animals along with the respective treatments**

Groups	Treatment and dose
Group I	Vehicle control-distilled water (1ml/100g, p.o.)
Group II	Methanol extract of <i>L. purpureus</i> (200 mg/kg, p.o.)
Group III	Methanol extract of <i>L. purpureus</i> (400 mg/kg, p.o.)
Group IV	Methanol extract of <i>L. purpureus</i> (600 mg/kg, p.o.)
Group V	Meloxicam (2 mg/kg, SC)

**Table 2: *In vivo* anti-inflammatory activity of *L. purpureus* leaves in carrageenan induced paw oedema model**

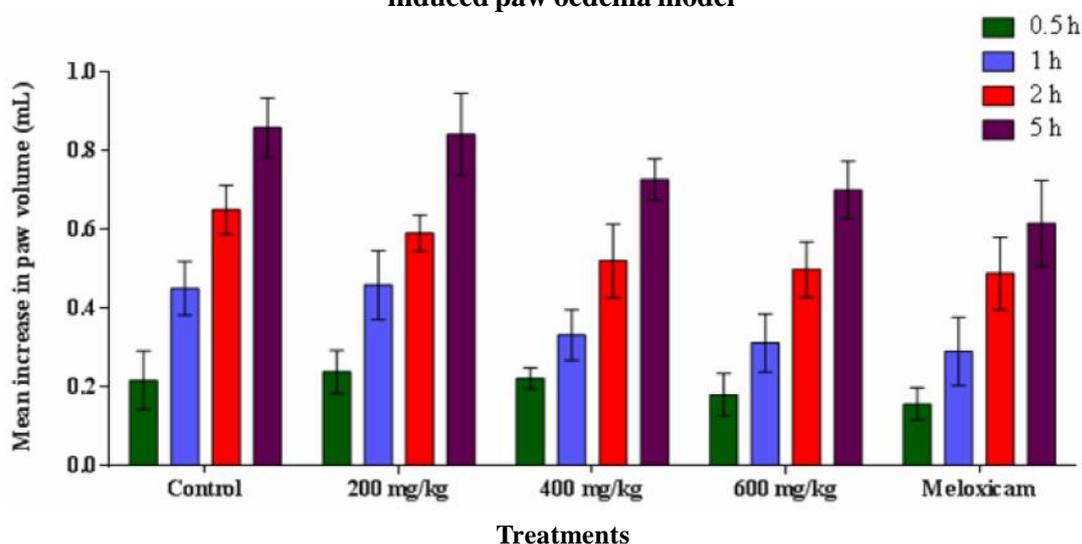
Groups and treatment	Mean increase in paw volume (ml)				% inhibition of paw oedema at 5 h
	0.5 h	1 h	2 h	5 h	
Group I Control	0.22 ± 0.03	0.45 ± 0.03	0.65 ± 0.03	0.86 ± 0.03	—
Group II (200 mg/kg)	0.24 ± 0.02	0.46 ± 0.04	0.59 ± 0.02	0.84 ± 0.04	2.33
Group III (400 mg/kg)	0.22 ± 0.01	0.33 ± 0.03*	0.52 ± 0.04*	0.73 ± 0.02**	15.11
Group IV (600 mg/kg)	0.18 ± 0.02	0.31 ± 0.03**	0.50 ± 0.03**	0.70 ± 0.03**	18.60
Group V Meloxicam	0.16 ± 0.02	0.29 ± 0.04**	0.49 ± 0.04***	0.62 ± 0.04***	27.90

**Table 3: *In vivo* analgesic activity of *L. purpureus* leaves in acetic acid induced writhing model**

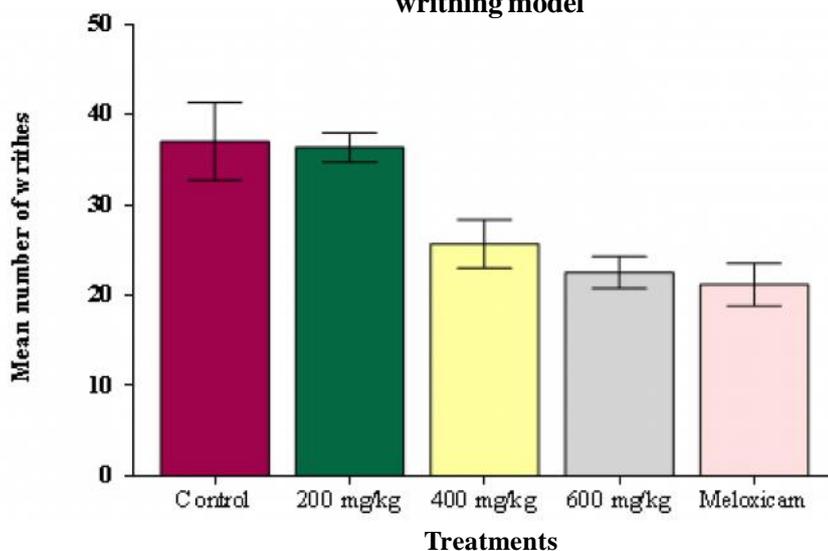
Sl. No.	Groups	Treatment and dose	Mean number of writhes	% protection
1	Group I	Control (distilled water)	37.00 ± 4.28	—
2	Group II	200 mg/kg	36.33 ± 1.61	1.81
3	Group III	400 mg/kg	25.67 ± 2.67*	30.62
4	Group IV	600 mg/kg	22.50 ± 1.77**	39.18
5	Group V	Meloxicam (2 mg/kg)	21.17 ± 2.36**	42.78

**Note :** Data were analyzed by one way ANOVA followed by Dunnett's multiple comparisons test and compared with control group. Data are represented in mean ± SEM (n=6), \**p*< 0.05, \*\**p*< 0.01

**Figure 1 : *In vivo* anti-inflammatory activity of *L. purpureus* leaves in carrageenan induced paw oedema model**



**Figure 2 : *In vivo* analgesic activity of *L. purpureus* leaves in acetic acid induced writhing model**



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## Association of AA-Genotype of *MspI*-RFLP in *INHA* (exon 1) with Cystic Ovarian Disease in HF Crossbred Cattle

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### ABSTRACT

The present study was carried out to determine the polymorphism of *INHA* (exon 1) gene and its possible influence on cystic ovarian disease in HF crossbred cows. The genomic DNA samples were drawn from 155 Holstein Friesian (HF) crossbred cows across southern districts of Karnataka, India and subjected to PCR amplification and detection of *MspI*-RFLP in *INHA* (Exon 1) gene segment. Results revealed three genotypes viz. AA (47%), AG (42%), and GG (11%) in the studied population of HF crossbred cows. The gene frequency of A and G alleles were 0.68 and 0.32, respectively. The studied population was in Hardy Weinberg Equilibrium. The observed and expected heterozygosities were 0.0709 and 0.0680, respectively. This study revealed for presence of *MspI*-RFLP in *INHA* (EXON 1), where AA genotype of *INHA* gene was associated with higher incidence of cystic ovarian disease in HF crossbred cattle.

**Key words:** *INHA*, Cystic Ovarian Disease, HF, Crossbred Cattle, PCR-RFLP, Polymorphism

### INTRODUCTION

Inhibin is a peptide hormone produced by granulosa cells in the ovary that regulates the circulatory levels of *FSH* by exerting inhibitory feedback mechanism. The cystic follicles release higher amount of inhibin into circulation, which leads to down regulation of *FSH* synthesis and secretion from the pituitary gland, thereby affecting folliculogenesis (Bhardwaj *et al.*, 2012). Li *et al.* (2015a) reported that the c.-42G>A and c.3222G>A polymorphisms of *INHA* gene were significantly ( $P<0.05$ ) associated with follicular cysts and that sows with c.-42GG and c.3222GG genotypes had lower risk of developing cysts. Li *et al.* (2015b) elucidated a new insertion/deletion fragment polymorphism of inhibin- $\alpha$  gene associated with follicular cysts in Large White Yorkshire sows. In view of the above, a study was carried out to identify and characterize the genetic polymorphism in exon 1 region of *INHA* gene by RFLP analysis and to elucidate the relationship between polymorphism in *INHA* gene and COD in HF crossbred cattle.

### MATERIALS AND METHODS

The present study was undertaken on 155 Holstein Friesian (HF) crossbred cows from villages of Ramanagara and Bengaluru Rural districts and cows maintained at the Department of Livestock Farm Complex (LFC), Veterinary College, Bengaluru. The experimental animals were divided into two groups viz., COD affected and COD unaffected/ apparently healthy animals as control group. Identification of COD affected HF crossbred cattle was done based on the history, clinical symptoms, per rectal palpation and ultrasound scanning of ovarian structures. Genomic DNA was isolated from venous blood by following high salt method as described by Miller *et al.* (1988). Exon 1 region of *INHA* gene was amplified by using primer set (Forward primer-52 CTCTCGATGAGACAGGCTCC32 Reverse primer-52 ACAATGCTGGGTCTGGACT32) proposed by Tang *et al.* (2011).

Each PCR reaction was performed with a total volume of 25  $\mu$ l consisting of a. 2x Red PCR Master Mix, b. Forward primer - 1  $\mu$ l (2 p mol), c. Reverse

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primer - 1  $\mu$ l (2 p mol), d. DNA template -1  $\mu$ l (50 -100 ng), and e. Nuclease free water - 9.5  $\mu$ l. The cycle conditions included an initial denaturation at 95 °C for 5 min, followed by 34 cycles of denaturation at 94 °C for 45 sec, annealing at 59 °C for 45 sec and extension at 72°C for 45 sec and final extension at 72°C for 10 min.

The amplified 249 bp PCR products of the *INHA* gene were resolved on 1.5 per cent agarose gel in parallel with 100 bp DNA ladder at a constant voltage of 100 V for 45 to 60 min. The gels were visualized under a Gel documentation system (Biorad Molecular imager Gel Doc XR+, USA).

A restriction enzyme RE, *MspI* having recognition template 5'... C<sup>-</sup> CGG ...3' and 3'... GGC- C ...5' digestion mixture was prepared with a total volume of 31  $\mu$ l consisting of a. Autoclaved triple distilled water 18.0  $\mu$ l, b. 10x assay buffer for RE - 2  $\mu$ l, c) RE (10U/ $\mu$ l) -1  $\mu$ l, and d) PCR product 10  $\mu$ l. The *MspI* digested product of *INHA* (exon 1) was resolved on 3.5 per cent agarose gel in parallel with 100 bp ladder at a constant voltage of 100 V for 90 min. The details of RE used and recognition site are presented in table 1.

PCR products of representative samples of resultant patterns were subjected for sequencing at Eurofins Genomics India Pvt. Ltd., Bengaluru. The sequences obtained were analyzed, consensus was created, annotated and multiple sequence analysis was performed by using CLC Main Work Bench Software (CLC BIO 2011, USA).

## RESULTS AND DISCUSSION

The amplified PCR products were resolved on 1.5 per cent agarose gel. The size of the amplified products for *INHA* (exon 1) gene in the studied population was 249 bp (Figure.1).

The PCR amplicons of *INHA* gene were digested with *MspI* restriction enzyme, which showed three allelic patterns. The first pattern had three bands of size 123, 95 and 31 bp, second pattern had four bands of size 95, 79, 44 and 31 bp and third pattern had five bands of size 123, 95, 79, 44 and 31 bp. They were

designated as AA, GG and AG genotypes, respectively (Figure 2). This is in agreement with the genotypic patterns reported by Tang *et al.* (2011), Sang *et al.* (2011) and Madrid *et al.* (2015) in Chinese Holstein cows, Chinese Holstein bulls and Antioquia Holstein cows, respectively.

PCR-RFLP analysis of *INHA* gene in HF crossbred cows revealed three genotypes *viz.*, AA, GG and AG with a respective frequency of 0.47, 0.42 and 0.11. The frequency of AA genotype was highest among in HF crossbred cows. The higher frequency of AA genotype was in agreement with reports of Sang *et al.* (2011) in Chinese Holstein cattle. Contrary to this, Tang *et al.* (2011) and Madrid *et al.* (2015) have reported higher frequency of heterozygotes in Chinese Holstein cows and Antioquia Holstein cattle, respectively. The frequencies obtained in the present study were 0.68 and 0.32 for A and B alleles, respectively. Higher frequency observed for A allele in the present study was supported by Sang *et al.*, 2011; Tang *et al.*, 2011; and Madrid *et al.*, 2015 in Chinese Holstein cows, Chinese Holstein bulls and Antioquia Holstein cattle, respectively.

Further, the observed and expected heterozygosities were 0.4193 and 0.4350 in HF crossbred cows. The  $\chi^2$  test showed that the studied population was in compliance with Hardy–Weinberg equilibrium. The BLAST search of sequence of *INHA* gene for possible match yielded around 38 hits in the NCBI nucleotide data base. Among these 100 per cent identity was observed with accession number KU255187.1 of *Bos indicus*. Alignment of A and G allele sequences of *INHA* (Exon 1) gene using CLC Main Work bench showed an SNP A > G transition at position 78 and the same was confirmed in chromatogram (Figure. 3).

The present study revealed significant association of *INHA* genotypes with COD in HF crossbred cows. Chi square test revealed significant association ( $P < 0.05$ ) between the genotypes and COD in HF crossbred cows, where in majority of animals (82.1 %) with AA genotype were cystic. In AG genotype 60 per cent animals were unaffected. In GG genotype 64.70 per cent animals were unaffected (Table 2).

However, Sang *et al.* (2011) have reported lower artificial insemination rates (AIR) in bulls with AA genotype than those with GG genotype which indicates the detrimental effect of ‘G’ allele on AIR. Similarly, Madrid *et al.* (2015) have revealed the detrimental effect of G allele on female fertility, calving interval and the number of services per conception, which in turn decrease the reproductive efficiency of the herd. Further, Tang *et al.* (2011) have observed that Chinese Holstein cows with GG genotype led to a significant increase in the number of ova and in turn more transferable embryos (TNO), than AG and AA genotypes. Li *et al.* (2015a) have reported that the c.-42G>A and c.3222G>A polymorphisms of INHA gene were significantly ( $P<0.05$ ) associated with follicular cysts and that sows with c.-42GG and c.3222GG genotypes had lower risk of developing cysts. Li *et al.* (2015b) have elucidated a new insertion/ deletion fragment polymorphism of inhibin- $\alpha$  gene associated with follicular cysts in Large White sows. Sequence analysis revealed a 283 bp fragment insertion/ deletion (I/D) polymorphism in INHA subunit gene. The distribution of insertion/ deletion was significantly ( $P<0.05$ ) different between cystic and normal sows. Sows with I allele had a higher risk of developing follicular cysts. No such evidence was observed in this study perhaps due to species specificity.

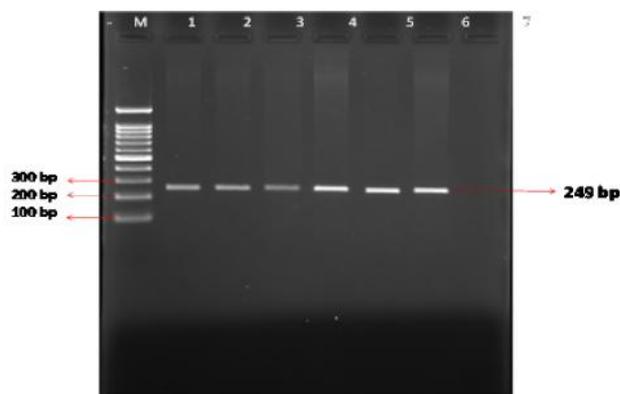
### CONCLUSION

Substantial evidence existed for the presence of genetic variability in *INHA* gene in HF crossbred cows,

where in, ‘AA’ genotype/ ‘A’ allele of *INHA* gene was associated with higher incidence of COD. Hence *INHA* gene in HF crossbred cows may be considered as candidate genes for selection of COD risk free animals, but suitable validation and confirmation in larger populations is necessary.

### ACKNOWLEDGMENTS

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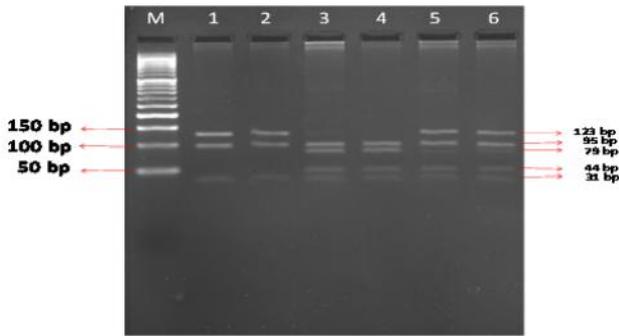


**Figure 1 :** Agarose gel (1.5 %) showing PCR amplified product of *INHA* (exon 1) gene. Lane M: Molecular marker (100 bp DNA ladder), Lanes 1, 2, 3, 4, 5, 6: PCR amplified product 249 bp (HF crossbred) and Lane 7: No Template Control.

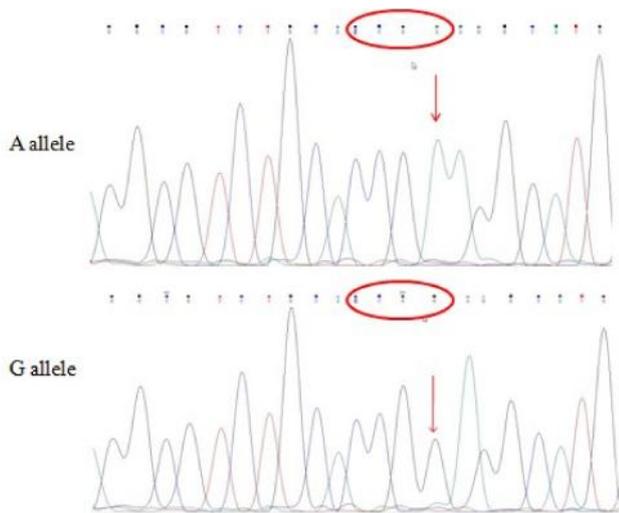
**Table 2 :** Observed genotypes of *INHA* gene and their association with COD in HF crossbred cows

Groups	Total number of animals	Genotypic frequency			χ <sup>2</sup> Value 16.95** ( p < 0 .05)
		AA	AG	GG	
COD affected	105	60	39	06	
COD unaffected	50	13	26	11	
Total	155	73	65	17	

**Note:** \*\*- Significant at P d” 0.01



**Figure 2:** Agarose gel (3.5 %) showing band pattern of RE (*MspI*) digested product of *INHA* (exon 1) in HF crossbred cows. Lane M: Molecular Marker (50 bp DNA ladder), Lane 1 & 2: Homozygous genotype AA in HF crossbred (123, 95 & 31bp), Lane 3 & 4: Homozygous genotype GG in HF crossbred (95, 79, 44 and 31 bp), Lane 5 & 6: Heterozygous genotype AG in HF crossbred (123, 95, 79, 44 & 31 bp).



**Figure 3:** Chromatogram showing A>G transition for *INHA* (exon 1) gene at position 78 of the PCR amplified product (restriction site).

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## Effect of Whey Protein Concentrate (WPC) on the Physico Chemical and Sensory Characteristics of Khoa \*

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### ABSTRACT

In this study, Khoa was developed by replacing milk fat with WPC at 25, 50, 75 and 100 per cent levels. As the milk fat replacement with WPC increased the percent moisture, protein, ash and yield were found to be significantly increased when compared with control. Simultaneously, the per cent fat and lactose, in all WPC added samples, were decreased. The addition of WPC did not show any significant impact on the colour and appearance scores, but the body and texture and overall acceptability scores were significantly better with respect to the product prepared by replacing 25 per cent fat with WPC. The addition of WPC in products at appropriate level contributed better body and texture score (8.58) and yield (20.16) compared to control Khoa .

**Key words** : Khoa, WPC, Physico-chemical, Sensory

### INTRODUCTION

Khoa is one of the most popular traditional dairy products in India. It is also known as khoya, khava, kava, Khawa, palghoa or mawa. According to (FSSR 2011), it is sold as Pindi, Danedar, Dhap, Mawa or Kava, means the product is obtained from cow, buffalo, goat or sheep milk or milk solids or a combination thereof by rapid drying. The milk fat content shall not be less than 30 percent on dry weight basis of finished product. Khoa contains concentrated quantities of proteins, minerals, fat and lactose in addition to fat soluble vitamins. With the changing lifestyle in affluent and technologically developed societies, diseases like obesity, diabetes and cardiovascular diseases have become major health problems. More than 425 million people have diabetes in the world. There were over 72.946.400 cases of diabetes in India in 2017 (IDF, 2017). It's the need of the hour to create low calorie food and dairy products for the target population like diabetic and CVD patients without much affecting the sensory qualities.

Whey contains approximately 20 per cent of the original proteins of milk. In addition, it contains other

proteins such as Lactoferrin, Immunoglobulin, Ceruloplasmin and milk enzymes such as lysozyme, lipase and xanthin oxidase, which are present in low concentrations. Whey proteins are one of the highest quality natural proteins and contain essential amino acids such as tryptophan, leucine, isoleucine, threonine and lysine. Being aware of the impact of the fat on health, today health conscious consumers are looking for the low fat dairy products with added functionality. In this regard, attempt was made to utilize WPC in preparation of Khoa.

### MATERIALS AND METHODS

Fat, protein, ash and moisture contents of the Khoa samples were estimated (IS: SP 18 (Part X1), 1981). Poly ethylene terephthalate (PET) cups were used for packaging of Khoa samples. Whey protein concentrate (WPC instant 80) was procured from KP Manish pvt co. Bengaluru. Fresh milk (4.5% fat and 8.5% SNF) was standardized and heat treated with vigorous stirring, when the product attained doughy stage, WPC was added at the rate of 25, 50, 75 and 100% level to milk to replace fat, then cooled, moulded and Khoa was obtained.

## RESULTS AND DISCUSSION

**Effect of various levels of WPC on the physico chemical characteristics of Khoa :** It's evident from Table 1 that incorporation of various levels of WPC had significant effect on the physico chemical characteristics of Khoa. The higher moisture per cent (40.92%) resulted in 100 per cent milk fat replaced Khoa by WPC, whereas, control Khoa had lowest moisture values of 33.19%. As the addition of WPC level increased the moisture per cent also significantly increased from 35.38 to 40.92. Similarly, fat per cent was found to be decreased in treated Khoa sample from 17.02 to 1.03 when compared to control Khoa. It was observed from the Table 1 that the protein content in the treated Khoa samples significantly increased from 21.32 to 32.03% compared to control Khoa (17.34%). This is due to the fact that the added WPC contains higher protein (80 per cent). Lactose per cent of treated samples with WPC decreased from 22.60 to 21.62. The water activity of control Khoa resulted in lowest value of 0.84 whereas, the water activity of fat replaced Khoa with WPC at 50, 75 and 100 per cent levels resulted in constant higher value of 0.87. The control Khoa sample had lesser yield per cent of 19.60 whereas, treated Khoa samples by WPC at 25 to 100 per cent resulted in increased yield per cent from 20.16 to 21.85. The results clearly indicates that addition of WPC had significant effect on the yield. The results are in agreement with the findings of Somayeh *et al.* (2008) and Busra *et al.* (2014) who observed that the addition of WPC and buttermilk powder improved yield in terms of dough stability.

**Effect of replacement of milk fat with WPC on the sensory characteristics of khoa:** The Khoa was prepared by replacing milk fat with WPC at 25, 50, 75 and 100 per cent levels and its effect on the sensory characteristics of Khoa are presented in Table 2. The highest colour and appearance score was secured by control (8.26) and the lowest score was secured by

treated Khoa sample having added WPC at 100 per cent (8.15). Among the treated samples, 25 per cent WPC added Khoa secured highest colour and appearance score (8.24). There was significant ( $p < 0.05$ ) increase in body and texture score (8.58) for 25 per cent fat replaced Khoa when compared to control (8.40). Hence, incorporation of WPC up to 25 per cent level was found to be acceptable. At higher levels of WPC incorporation, the product had very soft body, which could be due to the retention of more moisture in WPC incorporated products. The maximum flavour score was secured by the control Khoa (8.26) whereas, minimum flavour score was given to 100 per cent milk fat replaced Khoa with WPC (6.41). There was ( $p < 0.05$ ) significant decrease of flavour scores with increase in levels of addition of WPC. The treated Khoa sample with 25 fat replacement secured maximum mean score for overall acceptability of 8.50 when compared with control with the score of 8.31.

Devaraj (2005) prepared Gulabjamun with 10 per cent WPC incorporation and showed better body and texture and Vani, (2000) used blends of WPC and SMP in the ratio of 40:60 which resulted in good sensory quality of Gulabjamun and was comparable with the control sample. The Fruit Yoghurt containing inulin and WPC was found acceptable (Harmeet *et al.*, 2007). Yashashiwini and Arunkumar (2017) depicted that the WPC incorporation in date syrup yoghurt conferred better sensory qualities.

## CONCLUSION

The partial replacement of milk fat with WPC could be the reason for significant increase in moisture, yield and protein content of the product with reduction in fat. Hence, WPC could be used as a functional ingredient in Khoa due to its high functional property and adds value to the food and products prepared by such Khoa can create consumers interest and market potentiality.

**Table 1: Effect of replacement of milk fat with WPC on the physico chemical characteristics of khoa**

Fat replacement levels (%)	Moisture %	Fat %	Protein %	Lactose %	Ash %	a <sub>w</sub>	Yield %
Control	33.19 <sup>a</sup>	22.95 <sup>a</sup>	17.34 <sup>a</sup>	22.95 <sup>a</sup>	3.57 <sup>a</sup>	0.84 <sup>a</sup>	19.60 <sup>a</sup>
25	35.38 <sup>b</sup>	17.02 <sup>b</sup>	21.32 <sup>b</sup>	22.60 <sup>b</sup>	3.68 <sup>b</sup>	0.86 <sup>b</sup>	20.16 <sup>a</sup>
50	37.23 <sup>c</sup>	11.40 <sup>c</sup>	25.09 <sup>b</sup>	22.26 <sup>c</sup>	4.02 <sup>c</sup>	0.87 <sup>a</sup>	20.72 <sup>c</sup>
75	39.14 <sup>d</sup>	6.07 <sup>d</sup>	28.66 <sup>c</sup>	21.93 <sup>d</sup>	4.20 <sup>d</sup>	0.87 <sup>a</sup>	21.28 <sup>d</sup>
100	40.92 <sup>e</sup>	1.03 <sup>e</sup>	32.03 <sup>c</sup>	21.62 <sup>e</sup>	4.40 <sup>e</sup>	0.87 <sup>a</sup>	21.85 <sup>e</sup>
CD (pd <sup>**</sup> 0.05)	1.18	3.54	0.241	0.22	0.15	0.01	0.36

**Table 2: Effect of replacement of milk fat with WPC on the sensory characteristics of khoa**

Fat replacement levels (%)	Colour and appearance	Body and texture	Flavour	Overall acceptability
Control	8.26	8.40 <sup>a</sup>	8.26 <sup>a</sup>	8.31 <sup>a</sup>
25	8.24	8.58 <sup>b</sup>	8.20 <sup>a</sup>	8.50 <sup>b</sup>
50	8.21	8.43 <sup>a</sup>	7.48 <sup>b</sup>	8.10 <sup>c</sup>
75	8.18	7.50 <sup>c</sup>	7.00 <sup>c</sup>	7.50 <sup>d</sup>
100	8.15	7.15 <sup>d</sup>	6.41 <sup>d</sup>	7.15 <sup>e</sup>
CD (pd <sup>**</sup> 0.05)	NS	0.12	0.36	0.15

**Note :** All values are average of three trials

NS : Non significant

Figures with the same superscripts within a column indicates non significant difference

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## Radical Mastectomy in a Jamnapari Goat: A Case Report

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### ABSTRACT

An adult pregnant goat suffering from bilateral chronic suppurative fibrotic mastitis since 6 months was presented to the clinic. Animal was previously treated with antibiotics and anti-inflammatory drugs without any response. On clinical examination, the mammary gland found pendulous almost touching ground with fibrotic and pus filled nodules throughout the udder. Bilateral radical mastectomy was performed under diazepam and Ketamine anesthesia.

**Key words :** *Jamunapari doe, chronic Mastitis, Radical Mastectomy.*

Mastitis is the inflammation of the mammary gland, which may occur due to any bacterial infection secondary to teat or udder injury or poor management (Marogna *et al.*, 2010). Chronic mastitis develops from improperly treated case of acute mastitis which is manifested as formation of abscess within the mammary parenchyma (Scott, 2007). Early recognition and prompt treatment are important for limiting tissue damage and production losses. Udder is supplied with external pudendal artery separately to each half of udder and drained by a circular venous plexus derived from the external pudendal vein, subcutaneous abdominal vein and the perineal vein separately from each half of udder. The affected udder is enlarged and highly vascularized, which makes haemostasis problematic during partial mastectomy. Radical mastectomy (unilateral or bilateral) is a salvage procedure and indicated in cases of chronic suppurative mastitis, gangrenous mastitis and neoplastic or hyperplastic conditions of the udder (Andreasen *et al.* 1993, Cable *et al.* 2004). The present case report describes surgical management of bilateral chronic suppurative mastitis in a pregnant goat.

### CASE HISTORY AND OBSERVATIONS

A four and half years old 4-month pregnant goat weighing 35 kg was presented to Department of Veterinary Surgery and Radiology, Veterinary College, Hebbal, Bengaluru with the history of chronic mastitis

since 6 months which was extensively enlarged and contained pus filled nodules which was non-responsive to medical treatment. Both the mammary glands were dry since the onset of the disease and the goat was healthy as far as feed and water intake were concerned. Clinical examination and palpation of the udder revealed that pendulous mammary gland was affected with numerous soft and hard nodules in the udder parenchyma (Fig. 1). Aspiration of soft nodules revealed liquid whitish pus.

### DIAGNOSIS AND TREATMENT

Based on the history, clinical examination, and non-responsiveness to medical treatment it was diagnosed as fibrotic and infected udder and decided to perform bilateral mastectomy. Animal was fasted for 24 hours for food and water for 12 hours. Meloxicam (0.2 mg/kg) and Ceftriaxone sodium (20 mg/kg) were intravenously administered approximately 1 hour before surgery. The animal was restrained in right lateral recumbency with left hind limb elevated slightly. The surgical site was prepared aseptically (Fig 2). General anaesthesia was induced by using diazepam @ 2 mg/kg BW and Ketamine hydrochloride @ 4 mg/kg intra muscularly.

A circular skin incision around the base of udder was made and then tissue was separated from the skin by careful blunt dissection in order to avoid damage to

blood vessels and minimizing bleeding at surgical at surgical site (Fig 3). The external pudic artery, vein, perineal artery and large subcutaneous vein were isolated and ligated separately using chromic catgut no.1 to avoid hemorrhage. During surgery, bleeders were controlled by ligation with chromic catgut no.0. After careful resection of the mammary gland, the subcutaneous tissue was sutured with chromic catgut no.1 in simple continuous suture pattern and the skin with polyamide black no. 0 in horizontal mattress pattern. Post-operative care with Ceftriaxone 20 mg/kg, IM, BID for seven days and Meloxicam 0.2 mg/kg BW, IM, OD) for 3 days was advised. Wound dressing was done with povidone iodine solution and fly repellent D-Mag spray. Skin sutures were removed on 15<sup>th</sup> postoperative day and animal recovered uneventfully.

#### DISCUSSION AND CONCLUSION

The doe had a history of chronic mastitis with discrete abscesses and fibrosis of udder which was non responsive to medical treatment. Chronically infected does in herd system are recommended for culling to prevent transmission of infection. Radical mastectomy (unilateral or bilateral) was indicated as a salvage procedure in such cases of chronic suppurative mastitis, gangrenous mastitis and neoplastic or hyperplastic conditions of the udder (Andreasen *et al.*, 1993, Cable *et al.*, 2004). Such cases of udder abscessation (single or multiple) associated with chronic mastitis has been reported in sheep (Scott, 2007). The goat presented was pregnant and the udder was pendulous, causing pain and discomfort. So we have decided to do radical mastectomy to alleviate the pain and discomfort to the doe as indicated by Cable *et al.* (2004). The general anesthesia induced by the combination of Ketamine and diazepam which was safe during pregnancy and provided sufficient surgical plane of anesthesia. Similar combination of drugs was used during unilateral mastectomy in a goat by Kumar *et al.* (2012). With good post operative care and antibiotic treatment, the doe recovered uneventfully.

After 15 days, the goat was presented to clinic with complete healing of the surgical wound. The present case report describes the successful surgical

management of bilateral chronic suppurative mastitis in a pregnant goat under local anesthesia.



**Figure 1 : Pendulous mammary gland with numerous fluctuating and hard nodules.**



**Figure 2 : Preparation of site for surgery.**



**Figure 3 : Ligation of artery and vein.**

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## Modified Thomas Splint-Cast Combination for the Management of Tibial Fracture in a Pony-A Case Study

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### ABSTRACT

A two years old male pony presented to the Department of Surgery and Radiology, Veterinary College, Hebbal with the history of acute lameness on right hindleg. On clinical examination, animal was recumbent with painful swelling and crepitation at mid shaft of right tibia. Mediolateral radiograph of tibia revealed complete, splintered, mid diaphyseal fracture. Animal was anesthetized using Xylazine and Ketamine intravenously at 1.1 mg/kg body weight and 2.2 mg/kg body weight respectively. Animal was positioned on lateral recumbency with affected limb facing upward. Fiber glass cast applied on the affected bone including both the adjacent joints and the limb was supported by thomas splint. Post operatively Streptopenicillin 2.5g intramuscular and Flunixin meglumine 10 ml were administered for 5 days. Animal started to bear weight on affected limb 15<sup>th</sup> day onwards and recovered uneventfully in 3 months.

**Key words:** Pony, Fracture, MTSCC.

Open reduction and internal fixation is the treatment of choice for most long bone fractures in horses, enabling early return to function through correct anatomic reduction and stable internal fixation. The cost-to-outcome ratio may prevent some owners from choosing surgical intervention; however, external coaptations (splints or casts) are rarely used as sole fixation in horses since they do not provide adequate fracture reduction and immobilization, and thereby increase the risk of excessive callus formation or delayed or non-union. Furthermore, the prolonged duration of external coaptation is associated with complications such as pressure sores, tendon laxity, and contralateral limb laminitis, which can prevent a functional outcome and return to expected use (Ladefoged, 2016). The thomas splint was developed more than 100 years ago for the treatment of femoral fractures in people, and has since been modified for use in domestic animals (Robinson and O'Meara, 2009). External coaptation using modified Thomas splint-cast combinations (MTSCC) has been used as an economically feasible treatment option for the management of tibial and radial-ulnar fractures in cattle. Return of the animal to production soundness is

reported between 45% and 93% of cases, depending on population, fracture type and the criteria of success (Baird and Adams, 2014). MTSCC is less expensive than open reduction and internal fixation and is potentially feasible in the field since it does not require aseptic technique or specialized equipment. The objective of this report was to describe the management of tibial fracture by MTSCC.

### CASE HISTORY AND OBSERVATION

A two years old male pony was presented to the Department of Veterinary Surgery and Radiology, Veterinary College, Hebbal, with a history of acute lameness on right hindleg. On clinical examination, animal was recumbent, abrasions, painful swelling and crepitation at right tibial region was present. The radiograph of ML view of right tibia revealed complete, splintered, mid diaphyseal fracture of right tibia.

### TREATMENT AND DISCUSSION

Animal anesthetized intravenously with Xylazine and Ketamine combination at 1.1 mg/kg body weight and 2.2 mg/kg body weight respectively. Animal

positioned on lateral recumbency with affected limb facing upward. Long strip of roller gauze applied around the hoof and secured to the hoof to allow traction of the limb and reduction of the fracture prior to application of the fiberglass. Erosion area on the skin was dried using Boric acid antiseptic powder. A layer of cotton wool bandage was then applied and secured with a tight layer of bandage gauze. Fiberglass cast applied and allowed to harden for 30 minutes. Thomas splint was fabricated using reinforcing 6mm diameter aluminum rods to the normal standing angle and limb length. The thomas splint was applied and secured to the limb using adhesive tape.

The use of MTSCC is primarily reported for use in cattle with gastrocnemius and superficial digital flexor muscle rupture (Lescun *et. al*, 1998). The use of MTSCC may be considered a salvage option and an alternate to euthanasia when owners cannot afford surgical treatment, especially when there are no athletic expectations for the animal. External coaptation does not allow for meticulous anatomic reduction, and therefore may be associated with more discomfort in the early phase of fracture healing. Body weight of over 320 kg is a major risk factor for an unsuccessful outcome in horses with fractures of the third metacarpal or metatarsal bone treated by open reduction and internal fixation (Bischofberger *et al*, 2009), since external coaptation cannot achieve comparable fracture immobilization to internal fixation. Studies in cattle treated with MTSCC or transfixation pin casting have advised against attempting treatment in animals of over 300-400 kg (Gangl *et al*, 2006). The MTSCC supports axial alignment of the limb and distributes weight-bearing to the axilla through the proximal ring, thereby alleviating compressive stresses exerted on the fracture fragments.

The most frequent complication of external coaptation with casts is development of pressure sores or abrasion (Janicek *et al*, 2013). Sores associated with MTSCC are generally in the axilla or inguinal region, caused by pressure as weight-bearing is transferred to the axilla and inguinal region through the proximal ring.

In the present case tibial fracture was at risk of becoming open, causing soft-tissue laceration and further fragment displacement as a result of distal limb abduction caused by proximal muscle contraction. Full limb casts would not provide sufficient immobilization of the stifle. Hence, fiberglass was applied in conjugation with thomas splint. Animal recovered uneventfully in 3 months.



Figure 1 - Site of fracture in pony



Figure 2 – Radiograph revealed Splintered fracture of mid shaft diaphysis of right tibia



Figure 3- Thomas splint prepared for external coaptation



Figure 4 – Pony after Modified thomus splint cast combination application.

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## Intramedullary Pinning in Bird –A Case Report

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### ABSTRACT

Avian bones are thin and brittle because of their high calcium content, hence they are more prone for fractures. Routinely recommended methods of fracture stabilization for avian patients are external coaptation methods. A bird, with green plumage, which was unable to fly, was presented to the Department of Veterinary Surgery and Radiology, Veterinary College, Hebbal, Bengaluru with a history of the bird grounded in the street with injured wing. The physical examination revealed crepitus at radius and ulna of left wing and on radiograph revealed transverse fracture at distal 1/3<sup>rd</sup> of radius and ulna. The fractures of both radius and ulna were stabilized with 23G hypodermic sterile needle by Normograde method of intramedullary pinning. Postoperatively, the wing was immobilized with supportive bandage and bird got recovered uneventfully.

**KEY WORDS:** Bird, Fracture of radius and ulna, Intramedullary pinning

Intramedullary pinning has gained importance and it is challenging to veterinary surgeons in the repair of fractured bones in birds. Wing and leg fractures in birds are most common problems (Forbes & Kubiak, 2011). External coaptation methods are essentially the only methods of fracture stabilization routinely recommended for avian patients. With recent technical advancements, surgeons are now attempting to repair more challenging and difficult avian fractures and have a more predictably successful outcome (Bennett and Kuzma, 1992). Internal fixation refers to the surgical implantation of stabilization devices such as intramedullary (IM) pins, circlage wires, and bone plates.

### CASE HISTORY AND OBSERVATIONS

A bird, which was unable to fly was presented to the Department of Veterinary Surgery and Radiology, Veterinary College, Hebbal, Bengaluru with the history of bird being grounded on the street with injured wing. On physical examination crepitus was felt on palpation of left wing at radius and ulna. Mediolateral radiographic examination of the left wing revealed transverse fracture at distal 1/3<sup>rd</sup> of radius and ulna (Figure 1).

### TREATMENT AND DISCUSSION

The bird was subjected for the intramedullary pinning of both radius and ulna by using 23G hypodermic needle. Surgical site was prepared by plucking the feathers and scrubbed. The bird was anaesthetized with Isoflurane and oxygen mixture. The fracture of both radius and ulna were stabilized with 23G hypodermic sterile needle by Normograde method of intramedullary pinning (Figure 2) and wing was immobilized with supportive bandage (Figure 3). Postoperatively, bird was on medication with Proviboost drops, 2 drops b.i.d for 15 days orally and Cephalexin drops (125mg/5ml), one drop b.i.d for 7 days orally. Bird got recovered without any complication.

Fracture management is a major clinical problem in Birds. Thus, successful fracture treatment needs proper diagnosis and effective management in birds (Jalila *et al.*, 2014). Fractures of radius and ulna in the wings of birds are associated with faithlessness of birds. The normograde method of Intramedullary pinning was nothing but the pin was inserted at one end of the long bone and driven it through the medullary cavity and continue till it reached the metaphyseal part at other end of bone. The supremacy of normograde method

of intramedullary pinning was that the pin can be placed more precisely and there is less manipulation at the fracture site (Fossum, 2013). The other immobilization methods may include splints, casts, circlage wires or hybrid combination of these methods, depending upon the type of fracture, condition of bone and animal health status. After few days, on palpation callus formation was felt at the fractured site indicating clinical union after which needles were removed. Later the bird was able to fly.

### CONCLUSION

Successful intramedullary pinning was done by hypodermic needle for the transverse fracture at distal 1/3<sup>rd</sup> of radius and ulna of left wing for a non-descriptive green plumage bird, which enabled the bird to fly normally.



Figure 1 : C-Arm image of left wing revealing complete fracture of both radius and ulna.

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Figure 2 : C-Arm image showing Intramedullary pins *in situ*



Figure 3 : Post- operative bandaging done to left wing

## Surgical Retrieval of Foreign Body (Wooden Stick) from the Sinus Tract in a Labrador Retriever Dog – A Case Study

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### ABSTRACT

A male Labrador Retriever dog aged about 1 ½ year was presented to the Department of Veterinary Surgery and Radiology, Veterinary College, Hebbal, Bengaluru, having the history of swelling on ventral aspect of neck, discharging pus since 9-10 months with no improvement on local treatment. On physical examination, the clinical features were suggestive of the chronic sinus tract at left ventrolateral aspect of neck. Exploration of chronic sinus tract under general anaesthesia revealed a foreign body (wooden stick) and it was removed. The dog recovered uneventfully without any complication.

**Key words :** Cervical region, Sinus tract, Wooden stick.

Presence of foreign bodies in the subcutaneous tissue is problematic because they are not easily recognized on initial physical examination or radiographic evaluation and often they are incompletely removed. This may lead to recurrent abscess formation and subsequently draining tracts. These patients return with complications such as swelling or draining tracts weeks to months (Young *et al.*, 2004). Foreign bodies in the subcutaneous tissue at cervical region may be due to penetration of oesophagus by sharp objects (Hunt *et al.*, 2004). Prolonged entrapment of these foreign materials within the oesophagus increases the risk of developing moderate to severe esophagitis (Thompson *et al.*, 2012) leading to oesophageal perforations, fistulae and oesophageal stricture. The majority (88.6%) of foreign bodies are bones or bone fragments (Juvet *et al.*, 2010).

### CASE HISTORY AND OBSERVATION

A 1½ year old Labrador Retriever dog with the history of swelling at neck region and discharging pus since 9-10 months was presented to the Department of Surgery and Radiology, Veterinary College, Hebbal, Bengaluru.

Lateral cervical radiograph taken 10 months prior to presentation of animal revealed no lesions suggestive of foreign body in the oesophagus except some soft tissue swelling (Fig 1). Hence it was treated locally for 10 months, with no improvement in the condition. On physical examination, purulent sinus tract discharging pus on left ventrolateral aspect of neck was noticed and on palpation of ventral aspect of neck region did not reveal any foreign body (Fig 2). Exploration of sinus tract was decided under general anaesthesia.

### TREATMENT AND DISCUSSION

The dog was administered with Atropine sulphate @ 0.04mg/kg b wt, SC and Triflupromazine @ 1 mg/kg b wt IV as sedative. General anaesthesia was induced by Thiopentone sodium (2.5%) @ 12.5 mg /kg b wt IV. Animal was placed on right lateral recumbency with neck extended and surgical site was prepared aseptically. A linear cutaneous incision was made over the sinus tract and widened bluntly (Fig 1). On deep dissection and exploration, a wooden piece (Fig 4) was noticed in the sinus tract and removed with the help of artery forceps. The cavity was flushed thoroughly with normal saline and muscle and subcutaneous tissues were sutured using chromic catgut (1-0) in simple interrupted pattern. Skin was closed using polyamide

black (1-0), in simple interrupted pattern. Povidine iodine 5% ointment was applied on surgical wound and allowed to heal without dressing. Postoperatively, Amoxicillin @ 20mg/kg b wt, b.i.d, was administered for 10 days.

A sinus wound is a discharging blind-ended tract that extends from the surface of an organ to an underlying area or abscess cavity (Everett, 1985). Treatment of sinus should involve removal of root cause which will remove irritation, in turn facilitates granulation tissue growth. The majority of reported cases of oesophageal foreign bodies involve bone or raw hide chews (Thompson *et al.*, 2012), but can consume indiscriminately any number of foreign bodies, such as balls, needles and wood sticks. At thoracic inlet the oesophagus takes turn from left side and run dorsal to trachea, which is the common site of obstruction although it can lodge at cranial oesophagus, caudal oesophagus near the diaphragm and at the level of bifurcation of trachea (Juvet *et al.*, (2010). Temporary distension and esophagitis is followed by oesophageal ischemia, and after one week, oesophageal necrosis is evident (Glazer and Walters, 2008), which will leads to localised abscessation, In some of the cases, perforation of oesophagus caused by sharp foreign body and lodgement of the same in the subcutaneous tissue may leads to sinus tracts if left without removing the root cause. As in the present case.. Clinical signs vary depending on location of obstruction but include ptyalism, gagging, dysphagia, regurgitation, vomiting, haemoptysis, and repeated attempts to swallow (Leib and Sartor, 2008). In chronic

cases, anorexia and weight loss may also be noticed. In the present case, the sinus tract was treated for long period of time without any improvement. Exploration of tract revealed a foreign body in the sinus tract and removal of it facilitated recovery of animal within 15 days.

## CONCLUSION

Chronic sinus tract in a Labrador Retriever dog with recurrent symptoms for 10 months was treated by removing a foreign body (wooden piece) by exploratory surgery.



Fig 1. Radiograph showing soft tissue swelling of ventral aspect of neck



Fig 2. Sinus tract discharging pus before exploration



Fig 3. Intra operative exploration of sinus



Fig 4. Retrieved foreign body (Wooden stick)

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## Non-surgical Retrieval of Oesophageal Foreign Body in a Dog – A Case Report

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### ABSTRACT

An one and half year old female Labrador Retriever dog was presented to the department of Surgery and Radiology, Veterinary college, Bengaluru with a history of retching, vomiting and anorexia.. On physical examination, a palpable mass was appreciated at the cranial cervical region but on oral examination no abnormality was detected. Lateral cervical radiography revealed presence of a radiodense mass in cranial oesophagus. Under general anaesthesia the foreign body was retrieved per orally with the aid of C-arm image intensifier without performing esophagotomy. Animal was kept on antibiotic and analgesic for 3 days, and it recovered uneventfully.

**Key words:** Dog, Oesophageal obstruction, Bone, C-arm.

Oesophageal foreign body obstructions are one of the commonly encountered emergency presentations with an acute signs of gagging, retching, coughing, vomiting, dysphagia or odynophagia. The common place of obstruction is the caudal oesophagus between heart and diaphragm (Thompson *et al.*, 2012). It can also occur in the cranial oesophagus just caudal to pharynx, the thoracic inlet and the heart base. The common oesophageal foreign bodies in canines are bones, balls, toys, fish hooks and wooden sticks (Thompson *et al.*, 2012). The obstruction at caudal oesophagus accounts for 60 to 66 per cent of all canine oesophageal foreign bodies. About 50 to 60 per cent of oesophageal foreign body obstruction was found to be caused by bones or bone fragments (Aertsens *et al.*, 2016). Immediate intervention should be made to remove the foreign body per orally using grasping forceps. But forceful retrieval of firmly lodged foreign body may induce or enlarge perforations (Kyles, 2012). This paper describes the non-surgical retrieval of oesophageal foreign body which helped in early relief and avoided surgery.

### CASE HISTORY AND OBSERVATIONS

An one and half year old Labrador Retriever bitch was presented to the department of Surgery and

Radiology, Veterinary College, Bengaluru with a history of retching, vomiting and anorexia since two days. On physical examination palpable mass appreciated at the cranial cervical region. Upon per oral examination no foreign body noticed in the oral cavity. A survey radiograph of lateral cervical region revealed a presence of radiodense mass in the cranial oesophagus (Fig 1). It was decided to retrieve the foreign body per orally with the aid of C-arm image intensifier under general anaesthesia.

### TREATMENT AND DISCUSSION

The dog was premedicated with Atropine sulphate @ 0.04 mg/kg B.W. I/M, preemptive analgesia - Tramadol @ 1mg/kg B.W. I/M and a preanesthetic - Xylazine @ 1 mg/kg B.W. I/M. After 10 minutes, 2.5% Thiopentone sodium was administered to effect for induction and maintained with the same. With the aid of C-arm image intensifier, sequential images of the foreign body were taken and by using long bent grasping forceps, the foreign body was retrieved from the oesophagus per orally with utmost care (Fig 2) and it was a bone piece (Fig 3). Post removal, animal was injected with Inj. Amoxicillin @ 20 mg/kg B.W. and Meloxicam @ 0.3 mg/kg B.W. I/M for three days and animal recovered uneventfully.

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In dogs with oesophageal foreign body obstruction, vomiting or regurgitation is seen frequently. In case of partial obstruction, only liquids can pass through and enter stomach, but both the partial and complete obstruction has to be attended as early as possible. Upon survey radiography, radiopaque objects such as bones can be easily identified as the present case but it would be difficult in case of radiolucent objects. So, positive contrast studies will be helpful in detecting the location of foreign body and also perforations if any (Luthi, 1998). Sometimes accumulation of gas or fluid cranial to obstruction was recorded (Guilford and Strombeck, 1996). Thompson *et al.*, (2012) conducted a study on a series of cases of oesophageal foreign body obstructions in canines and described that oesophagitis, oesophageal tear, aspiratory pneumonia and oesophageal stricture formation were commonly reported complications. The less-common and more-severe complications were pneumothorax, pneumomediastinum, pleural effusion, pyothorax, hemothorax, pneumonitis, bronchoesophageal fistula, aorto-esophageal fistula, cardiopulmonary arrest, and death whereas, no such complications were recorded from the present case. They also concluded that the bones were the most commonly reported oesophageal foreign bodies and others included threads, balls, toys,

fish hooks and wooden sticks. The non-surgical technique of oesophageal foreign body retrieval was guided by C-Arm image intensifier which helped in avoiding the postoperative complications of oesophagotomy. Other non-surgical techniques include flexible endoscopy (Zimmer, 1984) and forceps retrieval under fluoroscopic guidance (Moore, 2001). If the foreign body could not be retrieved per orally, alternatively it can be pushed to the stomach (Pearson, 1966) and removed through gastrotomy which has excellent prognosis and minimal complications and dehiscence (Cornell, 2012) as compared to oesophagotomy.



Figure 1: C-arm Image intensifier image showing radiodense foreign body in the cranial cervical oesophagus.



Figure 2: C-arm image showing grasping of foreign body in the oesophagus by forceps



Figure 3: Retrieved foreign body (bone)

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## Therapeutic Management of Sarcoptic Mange in Rabbits with Selamectin Spot On – A case Report

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### ABSTRACT

Four rabbits were presented to the department of TVCC with a complaint of partial, anorexia, weakness, itching, hair fall and dandruff especially on the ears and on the legs. Physical examination revealed, rabbits were weak and debilitated, scratching, dandruff, white indurated dry crust like lesions on ears, tip of the nose and hind limbs. Microscopic examination revealed the presence of sarcoptic mites in skin scrapings. Based on the above investigation present case was diagnosed as sarcoptic mange infestation. All the four rabbits were treated with Selamectin @ 6 mg/kg BW (**Selamec™**) as spot on and all rabbits recovered uneventfully by three weeks .

**Key words:** Rabbits, Sarcoptic mange, Selamectin

In domestic pets and fur bearing animals, dermatological problems are one of the most common clinical entities (Deshmuk *et al.*, 2010). Rabbits are affected with wide variety of parasitic infestations among which the incidence of mange is high. Mange is a most obstinate, persistent and contagious disease with zoonotic importance. It is characterized by pruritis, alopecia and in prolonged illness animal become emaciated, and may even die due to cachexia. It is the most common parasitic infestation in many commercial rabbitaries and is a major constraint in rabbit production in India. Overcrowded living conditions and poor hygiene are significant factors for infection (McCarthy *et al.*, 2004). The avermectin group of drugs such as ivermectin, abamectin, doramectin, eprinomectin and Selamectin can be used to treat rabbits that are naturally infected with scabies (Kachhawa *et al.*, 2013).

### CASE HISTORY, DIAGNOSIS AND TREATMENT

Four non descriptive rabbits of less than one year age were presented to department of TVCC with a history of partial anorexia, intense itching, scratching, loss of fur and white indurated dry crust like lesions on the ears, legs and neck region (Fig.1). Clinical examination revealed that the animals were emaciated

and appeared dull. Rectal temperature was 99<sup>o</sup>F and the conjunctival mucous membranes were normal. Based on the physical examination the cases were tentatively diagnosed as mange infection. For confirmation, skin scrapings were collected from the lesions and were subjected to direct and indirect methods of examination as per the standard methods and were confirmed to be sarcoptic scabies var caniculi. All four rabbits were treated with Selamectin (**Selamec™** @ 6mg/kg body weight as a spot on once. All four rabbits were recovered uneventfully within three weeks of application

### DISCUSSION

The epidemiological study sighted that *Sarcoptes scabiei* var *cuniculi* is mostly found in Indian rabbits, (Darzi *et al.* 2007; Ravindran and Subramanian 2000). *Sarcoptes* are burrowing fur mites; produce their pathogenic effects by burrowing activity and mechanical damage caused by the parasites during excavation, secretions and excretions causes irritation, allergic reactions to some of their extracellular products and especially the release of IL-1 (Henry 1996; Wall and Shearer 1997). Among various species of mites, *Sarcoptes scabiei* is a deep burrowing mite in epidermis causing intense itching, pruritis, pyoderma, crust

formation, scale production, thickening and wrinkling on skin of affected areas. Being a contagious parasitic skin disease, mites are generally spread from rabbit to rabbit by direct skin contact between infected and non-infected animals or, through contact with the environment.

Though many treatments are being tried against mange infestation in rabbits, organophosphorous compounds are largely employed for the treatment of the same. Indiscriminate use of these compounds may prove hazardous in terms of residual effect. As an alternative to organophosphorous compounds, the avermectin group of drugs such as ivermectin, selamectin, abamectin, doramectin, and, eprinomectin can be used to treat rabbits that are naturally infected with scabies (Kachhawa *et al.*, 2013). Selamectin (**Selamec™**) at a dose of 6mg/ kg body weight as a spot on was also found effective and safe in the treatment of sarcoptic mange in rabbits.

Kurtdede, *et al.*, (2007) suggested the administration of selamectin topically to the skin in a single spot which was found to be safe, easy, practical and less time-consuming. In the present study, selamectin was used as a drug of choice to avoid the traditional routes of administration like injection, bathing, or instillation of acaricide into the ear canal. The use of selamectin applied as a spot-on formulation on a single occasion for ectoparasite control provides a safe and effective means of treating animals with minimum stress.



(Fig.1)



(Fig.2)

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