1. INTRODUCTION

Goat farming is one of the important agribased activities in countries where human population is very high and land availability for farming is less. Around 80% of the global goat population is in developing countries where goats are a potential source of meat, milk, fibre, hide, manure for landless rural people including small and marginal farmers. Thus, goat farming is one of the major source of revenue for thousands of small scale farmers in countries like India.

This species of animal in the country is facing diversified problems including poor management practices, underfeeding, infectious and non-infectious diseases. Numerous factors are responsible for economic losses to the goat industry, among them the health is of utmost importance. Goats are frequently exposed to ravages of infectious diseases. Among various goat diseases, mycoplasmal infections are one of the important infections which result in significant losses (DaMassa et al., 1992).

Mycoplasma is a small fastidious bacteria without cell wall which can cause disease in all major species of animals including humans that range from mild and debilitating to severe and fatal forms. The principal mycoplasma infection of small ruminants is contagious agalactia. The current importance of contagious agalactia rests in its extensive geographical distribution, economic and even genetic losses.

Contagious agalactia of goats has been known for about two centuries. The clinical disease was first described by Metaxa in Italy in 1816 and was given the name contagious agalactia in 1871 by Brusasco (Madanat et al., 2001). It was the first of the mycoplasmoses of small ruminants for which clinical descriptions and microbiological findings suggested a mycoplasmal origin (Bergonier et al., 1997). Contagious agalactia affects all types of stock breeding, both traditional and intensive, throughout the world and its preferential mammary involvement presents a major health obstacle in the development of goat production (Bergonier et al., 1997).
Contagious agalactia is an important disease in the Mediterranean countries of Europe, Asia, North Africa including India, Pakistan and in Eastern countries. The disease is caused by *Mycoplasma agalactiae*, with similar clinical and pathological features in *Mycoplasma mycoides* subsp. *mycoides*, *Mycoplasma capricolum* subsp. *capricolum* and *Mycoplasma mycoides* subsp. *capripneumoniae* infections.

Goats seem to be more susceptible to the natural disease than sheep (Kizil et al., 2007). Infection begins with intake of contaminated milk, faeces, urine, nasal and ocular discharges and excretion from affected joints. Clinical disease can be inapparent, mild, acute or chronic. In the female, acute clinical signs are first noticed at the beginning of lactation. Usually, general malaise, fever, mastitis, decreased milk yield, agalactia and keratoconjunctivitis is seen. Large number of the organisms can be shed in the milk whereas, in blood the organism remains for a short time. Often, the organism settles in the joints, causing arthritis involving one or several joints (polyarthritis). Therefore, classically the disease is characterized by the triad of mammary, joint and eye symptoms in lactating females, although other symptoms may also appear. These symptoms do not simultaneously appear in a single animal, although all three may be detected in the bulk of an affected herd.

Clinical mycoplasmoses often lacks pathognomonic characteristics and symptoms can be shared by other clinically significant infections. As a consequence, the diagnosis of an acute caprine mycoplasmal infection can be easily misinterpreted (DaMassa et al., 1992). Some animals also reduce the success of treatment as they are erroneously considered healthy based on clinical examination. Therefore, definitive diagnosis requires the isolation of the causative mycoplasmas from the affected animals, which are then identified by biochemical, serological and increasingly, molecular tests as polymerase chain reaction. Samples of preference include milk, nasal, ear swabs and joint fluid. The current therapy in countries with persistent incidence of the disease is based solely on antibiotics, namely, tetracycline, macrolide, tiamulin and fluoroquinolones.
Contagious agalactia does not show high mortality with morbidity between 30 to 60% (Madanat et al., 2001). The economic impact of the disease lies in the decrease or loss of milk production and sometimes abortions in dams. In the countries where sheep and goat dairy products are important foods as commercial commodities, contagious agalactia is a serious problem in terms of veterinary health and socio-economic impacts (Nicholas, 1998). Asymptomatically infected animals can shed mycoplasma for many years after infection, therefore, they play a very important role in the epidemiology of contagious agalactia, making unsuccessful both prophylaxis and eradication programs.

Although the significance of mycoplasmosis is well known but a meagre work regarding establishment of prevalence of mycoplasmosis in goats in Madhya Pradesh has been carried out. So in view of the above facts, this study is aimed with the following objectives-

1. To study the prevalence of contagious agalactia in goats in and around Jabalpur.
2. Detection of *Mycoplasma agalactiae* in goats using polymerase chain reaction.
3. To evolve the suitable therapy for caprine contagious agalactia.
2. REVIEW OF LITERATURE

Contagious agalactia

Contagious agalactia (CA) is an economically important disease of small ruminants caused by mycoplasmas. It is a disease predominantly of milking sheep and goats soon after lactation characterized by mastitis, arthritis and keratoconjunctivitis.

DaMassa et al. (1992) stated that the clinical form of contagious agalactia could be inapparent, mild, acute or chronic. In the females, acute clinical signs often were first noticed after freshening at the beginning of lactation. There was general malaise, fever and mastitis with decreased milk yield and agalactia.

Rana et al. (1992) experimentally induced caprine mastitis with mycoplasma ovine/caprine serogroup 11 through the teat canal. The infected glands were hot, tender, painful and swollen from the first day post infection. The mastitic milk showed characteristic physical changes and marked increase in somatic cell count. Subsequently, there was reduction in gland size and agalactia.

Smith and Sherman (1994) stated that mastitis in goats resulted from invasion of the udder by an infectious agent and California mastitis test was useful for ruling out mastitis.

Bergonier et al. (1997) reported that the disease was characterized clinically by elevated temperature, inappetence and alteration in the consistency of the milk in lactating females with decline and subsequent failure of milk production, often within 2–3 days, as a result of interstitial mastitis; lameness and keratoconjunctivitis affected about 5–10 per cent of the infected animals. Fever was common in acute cases and may be accompanied by the nervous signs but both signs were rare in the more frequently observed subacute and chronic infections. Pregnant animals may abort.

Sanchis et al. (2000) stated that in CA mastitis is generally bilateral and the udders became hot, swollen, painful with hypertrophied mammary lymph nodes and finally became atrophied with hardened nodules.
Egwu et al. (2001) conducted a study to investigate the current status of intramammary mycoplasmosis in caprine udders in Nigeria. A total of 57 and 24 milk samples were collected from udders of goats affected by mastitis and from apparently normal goats respectively. Acute and chronic mastitis were more commonly observed in goats between 1 and 3 years of age. *Mycoplasma agalactiae* and *Mycoplasma capricolum* occurred at a significantly higher rate in udders affected by mastitis than in normal healthy udders.

Madanat et al. (2001) described that CA infection frequently occurred as an enzootic disease. In lactating female animals, it was usually manifested by mastitis whereas in males, young animals and non-lactating females the disease was manifested as arthritis, keratoconjunctivitis and respiratory problems.

Kizil and Ozdemir (2006) investigated some clinical, haematological and biochemical parameters in goats with contagious agalactia caused by *Mycoplasma agalactiae*. The study was conducted in 47 goats between 2 and 3 years with mean body weights 45-55 kg and naturally infected with *Mycoplasma agalactiae* in the province of Elazig, Turkey. PCR test in the milk samples was done for the confirmatory diagnosis of *Mycoplasma agalactiae*. The results revealed that the transient fever, mastitis, arthritis and keratoconjunctivitis were the constant findings in the infected goats. The udder was filled with connective tissues but in some cases atrophy developed. The body temperature, pulse, respiration rates and the activity of enzymes like aspartate aminotransferase and lactate dehydrogenase were significantly higher in the infected goats than in the controls, while rumen contractions, plasma total protein, albumin and glucose concentrations were significantly lower. No significant difference related to white and red blood cell counts, packed cell volume, haemoglobin concentration, alkaline phosphatase, γ-glutamyl transferase activity and sodium, potassium and chloride concentrations between the groups were found.

Corrales et al. (2007) stated that contagious agalactia is an infectious syndrome caused by several species of mycoplasma and classically characterized by the triad of mammary, joint and eye symptoms, although further symptoms may also appear.
Kheirkhah et al. (2011) reported that contagious agalactia was a serious disease affecting sheep and goats which was characterized by mastitis and subsequent failure of milk production, arthritis and keratoconjunctivitis.

Pooladgar et al. (2011) stated that contagious agalactia in lactating animals is usually manifested by mastitis along with decrease in milk production.

**Prevalence of contagious agalactia**

Contagious agalactia is a disease predominantly of milking goats soon after lactation. The infection frequently occurs as an enzootic disease. It causes serious economic losses and the prevalence of infection is usually under estimated.

Borry and Entessar (1963) reported the presence of agalactia disease in sheep and goats in Iran for the first time.

Damass (1983) observed the presence of *Mycoplasma agalactiae* in udder secretion from a goat with mastitis indicating active infection.

Ghosh et al. (1989) reported 9-63 per cent of seroprevalence for mycoplasmal antibodies in goats in different districts of Tripura state.

Kinde et al. (1994) examined 322 composite milk samples of goats submitted to California Veterinary Diagnostic Laboratory System, San Bernardino and reported that 7 samples were found positive for *Mycoplasma agalactiae*.

Deniz (1996) reported a seroprevalence of 8.8 per cent and 2.35 per cent of *M. agalactiae* and *Mmm LC*, respectively in Spain using an indirect ELISA technique.

Srivastava and Singh (2000) observed a low prevalence i.e. 4.97 per cent of mycoplasma antibodies in goats of Uttar Pradesh.

Zendulkova et al. (2004) conducted a preliminary epidemiological survey to find out the presence of contagious agalactia of sheep and goats in the Jordan and reported a prevalence of 11 per cent.
De La Fe et al. (2005) reported that the best sample for analysis of CA was milk from mastitic or apparently healthy females in affected herds. He conducted a microbiological survey for Mycoplasma spp. in Spain. There was a total of 38.5 per cent positive flocks from which 37 mycoplasma isolates were obtained. Mycoplasma was isolated from 21 milk samples and Mycoplasma agalactiae was isolated from 40 per cent of the positive herds (27 per cent of all isolations).

Al-Momani et al. (2006) surveyed 104 flocks of local sheep and goats during the period of 2002–2003 for the occurrence of mycoplasma infections in Northern Jordan. The clinical signs seen in the flocks were varying degree of mastitis in sheep and goats, arthritis, mainly in kids and pneumonia in both sheep and goats of most age groups. Mycoplasmas were isolated from 26 per cent milk samples, 3.9 per cent of nasal swabs collected from goats and from 13 per cent of milk samples, 2.3 per cent of nasal swabs collected from sheep.

Azevedo et al. (2006) reported two outbreaks of contagious agalactia by Mycoplasma agalactiae in Paraíba State, Northeastern Region of Brazil. The disease was characterized by mastitis, agalactia and polyarthritis in does; polyarthritis and conjunctivitis in kids and lambs. Fever and anorexia were also observed. Morbidity was from 26.1 per cent to 100 per cent in does, 36.5 to 100 per cent in kids and 49 per cent in lambs.

Contreras et al. (2008) cultured 1068 bulk-tank goat milk samples. They showed that 84 (7.9%) were positive for the presence of mycoplasma species. Most of the species isolated were Mycoplasma agalactiae (82%).

Kizil et al. (2007) performed a study on goats naturally infected with Mycoplasma agalactia and reported that transient fever, anorexia, mastitis, arthritis and keratoconjunctivitis were observed in the infected goats.

Ramdev et al. (2008) recorded the seroprevalence of mycoplasma in sheep and goats of Himachal Pradesh based on agglutination test using Mycoplasma mycoides subsp. capri colored antigen. Out of the 314 serum samples screened, 15 (4.77%) were found positive for mycoplasmosis.
The prevalence was found to be slightly higher in goats (5.02%) as compared to sheep (4.44%). The seroprevalence of mycoplasmosis was highest in Kangra district (11.59%) followed by Mandi district (9.67%) and Hamirpur district (2.17%).

Hadush et al. (2009) recorded 32.68 per cent seroprevalence in goats positive for mycoplasma antibodies using complement fixation test in Ethiopia.

Bidhendi et al. (2011) reported a prevalence of 5.5 per cent of *Mycoplasma agalactiae* in sheep and goat milk samples in Kordestan province, Iran.

Kashoo et al. (2011) conducted a study to find out the field applicability of three serological tests viz. SAT with coloured antigen, Indirect Haemagglutination test (IHA) and Enzyme linked immunosorbent assay (ELISA) in screening of contagious agalactia. For this study, a total of 493 serum samples from goats of various parts of Uttar Pradesh and Uttarakhand were collected and results showed that ELISA based on whole cell sonicated antigen detected 13.74 per cent field samples as seropositive as compared with IHA (6%) and SAT (10.14%).

Zahid et al. (2012) conducted a serological survey of contagious agalactia between February and December 2009 in different parts of Jammu and Kashmir and reported a prevalence of 32.03 per cent by slide agglutination test (SAT) and 20.3 per cent by enzyme linked immunosorbent assay (ELISA).

**Identification of *mycoplasma agalactiae* using polymerase chain reaction**

Contagious agalactia may be caused by four different mycoplasma species: *Mycoplasma agalactiae (Ma)*, *Mycoplasma mycoides* subsp. *mycoides* LC (*Mmm LC*), *Mycoplasma capricolum* subsp. *capricolum* (*Mcc*) and *Mycoplasma putrefaciens* (*Mp*). All these species contribute significantly to economic losses. However, *Mycoplasma agalactiae* is the main causal organism of contagious agalactia of goats (Nicholas, 1995).
Recently, genomic detection of the organism has been made possible by the development of gene probes. The probes are usually complementary to segments of 16S ribosomal RNA or chromosomal DNA (rRNA) (Mattson et al., 1991; Dedieu et al., 1992 and Tola et al., 1994). Since their specificity is high and they have facilitated great progress in the diagnosis of mycoplasma infections, so efforts of research have been focused on the development of polymerase chain reaction (PCR) techniques that seem to be even more sensitive.

Razin (1994) stated that PCR is a valuable method for diagnosis of mycoplasma infections using 16srRNA and also *Mycoplasma agalactiae* PG2, P80 predicted lipoprotein.

Laurence et al. (1995) reported the application of PCR for detection of mycoplasmas causing contagious agalactia i.e. *M. agalactiae*, *M. capricolum* subsp. *capricolum* and *M. mycoides spp. mycoides*. It was based on two polymerase chain reaction assays: the Ma-PCR for the detection of *M. agalactiae* and the MYC-PCR for ‘mycoides cluster’, including *M. capricolum* subsp. *capricolum* and *M. mycoides spp. mycoides*.

Tola et al. (1997) extracted DNA from sheep milk to use for polymerase chain reaction diagnosis of *Mycoplasma agalactiae*. A total of 357 samples from 21 newly infected flocks (group 1) and 87 samples from 8 flocks infected in the past (group 2) were tested and the PCR results were compared with those of conventional culture. By PCR, 175 positives were detected in group 1, while by culture only 153 were detected. Milk samples from group 2 were detected negative, both by PCR assay and by culture. So, the PCR was reported to be much faster than culture, it also reduces the time required for diagnosis from several days to 5 hours.

Greco et al. (2001) used multiplex polymerase chain reaction (m-PCR) for the simultaneous detection of several species of small ruminant mycoplasmas. Two sets of oligonucleotide primers specific for *Mycoplasma agalactiae* and *Mycoplasma mycoides* cluster (*M.m. cluster*) were used in the test. Fifty six samples (44 milk samples, two nasal swabs, six ocular swabs, three vaginal swabs and one sample of fibrinous exudate from carpal joint)
from sheep and goats with clinical signs of contagious agalactia were examined. The m-PCR confirmed the identification of 35 Ma strains, 12 M. mycoides cluster strains while, in 4 samples both Ma and M. mycoides cluster were revealed.

Bashiruddin et al. (2005) evaluated PCR systems for the identification and differentiation of *Mycoplasma agalactiae* and *Mycoplasma bovis*.

Azevedo et al. (2006) identified the isolates of *Mycoplasma agalactiae* in Paraiba State, Northeastern Region of Brazil, with the help of culture characteristics, immunoperoxidase test and PCR. Results suggested that PCR was faster than the traditional tests and it can be used as confirmatory test in the diagnosis of contagious agalactiae.

Kizil et al. (2007) used PCR for identification of *M. agalactiae* from the milk samples collected from suspected goats. The specific 176 bp band were obtained from the DNA amplification of *M. agalactiae* using the specific primers.

Zendulkova et al. (2007) conducted a study to identify the *Mycoplasma agalactiae* using polymerase chain reaction (PCR) in the sheep and goats showing clinical signs of contagious agalactia. The results revealed that out of 35 animals, 21 (4 sheep and 17 goats) tested positive for *Mycoplasma agalactiae*.

Amores et al. (2010) evaluated the validity of PCR for the direct detection of *Mycoplasma agalactiae* and reported that PCR proved to be a rapid and sensitive method for the detection of mycoplasmas.

Bidhendi et al. (2011) isolated and identified *M. agalactiae* with culture and PCR technique in milk samples in Kordestan province, Iran. A total of 367 milk samples were collected from sheep and goat. Specific published primers amplify a 375 bp gene of *M. agalactiae* were used for PCR. Twenty (5.5%) out of 367 were positive in PPLO agar and 5 (25%) out of these isolates were positive with *Mycoplasma agalactiae* primers. Four (75%)
out of 5 isolates was from sheep and 1 (25%) from goat. Result of PCR with 367 milk samples showed that 11 (3%) of them were positive with these primers.

Kheirkhah et al. (2011) reported that PCR can be used as trusty and supersede test in the detection of Mycoplasma agalactiae from affected goats and among different collecting sites, milk secretion samples are suitable for PCR detection.

Kumar et al. (2011) reported that PCR based detection is a rapid and simple method for identification of the mycoplasmal organism and can be an effective tool for epidemiological surveys.

Khezri et al. (2012) tested sixty nine samples (including milk, conjuctival swabs, synovial fluid and ear canal swabs) by PCR assay during 2011-2012 in Kurdish sheep in Kurdistan, Iran and reported that Mycoplasma agalactiae was involved in 32.6 per cent of the cases of CA.

Kumar et al. (2013) studied association of Mycoplasma mycoides subsp. capri (Mmc) with natural goat mastitis detecting the Mmc DNA using molecular methods. In this study, Mmc was isolated from milk samples of 171 goats with or without clinical signs of mastitis. Mmc isolates were further characterized by biochemical and species-specific PCR methods. Intra species strain variation was also studied by 16S amplified rDNA restriction analysis (16S ARDRA).The study recovered a total of 6 Mmc isolates (3.5%).Three types of intra species variants among the recovered Mmc isolates were found by 16S ARDRA.

Mohammadpour et al. (2013) conducted a study on isolation and identification of Mycoplasma agalactiae by PCR on suspected sheep samples in Kerman Province, Iran and reported the presence of one isolate of Mycoplasma agalactiae in the milk samples.

Paterna et al. (2014) reported that the detection of Mycoplasma spp. by PCR is unaffected by sample freezing i.e. frozen samples may be used for widespread surveillance program for mastitis and CA in goats.
Therapy for caprine contagious agalactia

Mycoplasma the causative agent of contagious agalactia, is inherently refractory to certain groups of antibiotics owing to its lack of cell wall. Antibiotics showing efficacy for Mycoplasma should possess the characteristics like activity against bacteria without cell wall, long persistence in the plasma efficient diffusion into tissues, passage of high concentrations from the blood to milk (even becoming concentrated in the mammary gland) and very low minimum inhibitory concentrations (MIC).

Ball et al. (1987) conducted therapeutic trial in ewes experimentally infected with Mycoplasma californicum and reported that three days treatment with both oxytetracycline and tylosin successfully eliminated the infection.

Bergonier et al. (1997) reported that the initial medicinal treatments of contagious agalactia were based on arsenical compounds, notably the sodium or zinc salts of acetarsol. The main antibiotics that could be used to treat contagious agalactia are the tetracyclines, macrolides, florfenicol, tiamulin and the fluoroquinolones.

Ayling et al. (2000) stated that antibiotics which are ineffective in vitro are unlikely to be effective in vivo, those with strong activities invitro will not necessarily perform well in the field.

Mackie et al. (2000) recorded mastitis outbreak from a dairy herd in Ireland in March 1999 and Mycoplasma californicum, Mycoplasma canadense was diagnosed as the organisms responsible for mastitis in freshly calved cows. Treatment with a combination of intramammary chlortetracycline (426 mg/dose) and intramuscular tylosin (10 mg/kg) after each milking for three consecutive days rapidly eliminated the implicated organisms and prevented the development of chronic infection.

Madanat et al. (2001) suggested that the results of treatment may be poor or ineffective against mycoplasmas if the therapeutic dose is not well defined and the antibiotic is not administered for a sufficiently long period.
Loria et al. (2003) mentioned the use of macrolides, such as tylosin, erythromycin and spiramycin, lincosamides, such as lincomycin and quinolones, such as enrofloxacin in the treatment of CA.

Azevedo et al. (2006) reported that in an outbreak of mycoplasmosis when the affected goats were treated with 20 mg/kg tylosin daily for five days, most of the animals recovered although seven were euthanized and seven died spontaneously.

Kizil et al. (2007) conducted a study to measure the concentrations of vitamin A, E, C, β-carotene, glutathione (GSH), malondialdehyde (MDA), activities of catalase (CAT), glutathione peroxidases (GSHPx) in blood samples of goats naturally infected with *Mycoplasma agalactiae*. A significant increase in MDA concentrations, significant reductions of GSH, β-carotene, vitamin C, vitamin E concentrations and of GSHPx activity compared to the controls were evidenced in *Mycoplasma agalactiae* infected goats. The results clearly demonstrated the occurrence of an oxidative stress leading to deficiency of enzymatic and non-enzymatic antioxidant systems during this infection.

Li et al. (2012) tested the antimycoplasmal activity of triclosan alone as well as the *in-vitro* interaction of triclosan and the fluoroquinolones viz. gatifloxacin (GAT), moxifloxacin (MXF), levofloxacin (LVX), sparfloxacin (SPX), ciprofloxacin (CIP), enrofloxacin (EFX) and norfloxacin (NOR) against five mycoplasma species. This study demonstrated that triclosan had antimycoplasmal activity against both fluoroquinolones sensitive species and fluoroquinolones resistant species isolated from clinical cases. A synergistic antimycoplasmal effect between triclosan and GAT, MFX, EFX against the five mycoplasma species was observed. The combination of triclosan with LVX, SPX, CIP, NOR displayed either synergistic activity or indifference against the same mycoplasma species. No antagonism was observed for any drug combination against any of the species tested.
3. MATERIAL AND METHODS

Place of work

The work was conducted in the Department of Veterinary Medicine, College of Veterinary Science & A.H., N.D.V.S.U., Jabalpur (M.P).

Duration of work

The study was conducted for a period of one year i.e. from April 2014 to March 2015.

Animals

For this study, a total of 705 lactating goats were screened over a period of 12 months i.e. from April 2014 to March 2015. The goats belonged to the areas in and around Jabalpur viz. Goat farm, Amanala, Goat farm, Adhartal, nearby villages of Jabalpur like Ganiyari (Patan), Nuniakala (Panagar), Noni (Shahpura), Yadav goat farm (Choti Pipariya) and the clinical cases brought to TVCC, Veterinary College, Jabalpur. The details are presented in table 01, plate 01 and 02.

Table 01: Details of goats screened and samples collected

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Place</th>
<th>Goats screened</th>
<th>Samples collected (blood and milk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TVCC, Co.V.Sc. &amp; AH, Jabalpur</td>
<td>278</td>
<td>94</td>
</tr>
<tr>
<td>2</td>
<td>Goat Farm Amanala</td>
<td>151</td>
<td>72</td>
</tr>
<tr>
<td>3</td>
<td>Live Stock Farm Adhartal</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>Ganiyari (Patan)</td>
<td>42</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>Nuniakala (Panagar)</td>
<td>83</td>
<td>37</td>
</tr>
<tr>
<td>6</td>
<td>Noni (Shahpura)</td>
<td>73</td>
<td>38</td>
</tr>
<tr>
<td>7</td>
<td>Yadav goat farm, Chhoti Pipariya (Mandala Road)</td>
<td>53</td>
<td>11</td>
</tr>
</tbody>
</table>

| Total  | 705    | 282    |
Out of 705 lactating goats, a total of 282 goats showing signs of clinical mastitis or suspected for mastitis were selected for further study. Details of milk samples collected are outlined in table 02 and suspected cases of contagious agalactia are shown in plate 03. Presence of clinical mastitis was done on the basis of typical clinical symptoms like swelling of udder, abnormal secretion, enlargement of supramammary lymph nodes and/or raised clinical parameters. The confirmation of mastitis in suspected cases was done by California Mastitis test (CMT). Seroprevalence of Mycoplasma spp. causing contagious agalactia was done using slide agglutination test whereas the presence of *Mycoplasma agalactiae* was confirmed by polymerase chain reaction.

**Table 02: Details of milk samples collected**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Health status of goats</th>
<th>Goats selected for study</th>
<th>Milk samples collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Clinical Mastitis</td>
<td>137</td>
<td>137</td>
</tr>
<tr>
<td>2</td>
<td>Suspected for mastitis</td>
<td>145</td>
<td>145</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>282</td>
<td>282</td>
</tr>
</tbody>
</table>

After confirmation of the presence of *Mycoplasma agalactiae*, animals were selected for the therapeutic trial.

**Collection of samples**

**a. Collection of blood samples**

For prevalence study, about 3 ml of blood was collected aseptically from the jugular vein of the animal in clot activator vaccutainer and allowed to stand for at least 2 hrs to harvest the serum, as shown in Plate 04. The serum was collected and frozen at -20°C until further use.

**b. Collection of milk samples**

Milk for CMT and molecular diagnosis was collected on day ‘0’ i.e pre-treatment and on days ‘7’ and ‘14’ post treatment. The teats were cleaned with 70 % alcohol and about 5 ml of pooled milk from both the udder halves was collected from the suspected cases in sterile tubes, as shown in plate 05. The samples were embedded in ice, brought to the laboratory and then stored at -20°C till further processing.
Parameters of the study

1. **History**

   Complete history of the cases was recorded including age, breed, drop in milk yield and duration of illness.

2. **Clinical examination**

   All the goats were clinically examined for the presence of symptoms like swelling of udder, abnormal secretion and/or enlargement of supramammary lymph nodes. The body temperature, pulse rate and respiration rate was measured prior to and post treatment on days 0, 7 and 14.

**Body temperature (°F)**

   The body temperature of the ailing goats was recorded by inserting the clinical thermometer in the rectum of the animal in such a way that its bulb remained in direct contact with rectal mucosa for two minutes and reading was recorded in °F.

**Pulse rate (per minute)**

   Pulse rate was recorded by palpating the femoral artery for a period of one minute.

**Respiration rate (per minute)**

   Respiration rate was recorded by feeling the air movements at nostrils. For this, back of the hand was placed in front of the nostrils and the air current was felt and counted for a period of one minute.

3. **Processing of serum samples**

   Serum samples were used for slide agglutination test to identify the presence of Mycoplasma spp. (Roy *et al.*, 2010).

**Slide agglutination test**

   Slide agglutination test was performed by using coloured antigen and the harvested serum from the suspected cases i.e test serum. The coloured antigen was procured from the Department of Bacteriology and
Mycology, Division of Indian Veterinary Research Institute (I.V.R.I.), Izatnagar (U.P). Details are shown in plate 06.

Test procedure

One drop (0.03 ml) of test serum was taken on a clean grease free glass slide by micropipette. The antigen bottle was shaken well to ensure homogenous suspension and one drop (0.03 ml) of whole cell coloured antigen was added to the drop of test serum. The antigen and serum were mixed thoroughly with a toothpick and the slide was rotated for 1 to 2 minutes. The result was read after 2 to 3 minutes. Positive result was indicated by definite clumping while in case of negative reaction, mixture remained homogeneous without formation of any clumps.

4. Processing of milk samples

Milk samples were used for performing the California mastitis Test and Polymerase chain reaction.

California mastitis test

The CMT was carried out in milk samples as described by Schalm et al. (1971). The details of the test are as follows:

<table>
<thead>
<tr>
<th>CMT Score</th>
<th>Reaction</th>
<th>Mean no. neutrophils per ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>No Reaction</td>
<td>68,000</td>
</tr>
<tr>
<td>Trace</td>
<td>Slight slime, tends to disappear with continued swirling</td>
<td>268,000</td>
</tr>
<tr>
<td>1</td>
<td>Distinct slime but without gel</td>
<td>800,000</td>
</tr>
<tr>
<td>2</td>
<td>Immediate gel formation; moves as a mass during swirling</td>
<td>2,560,000</td>
</tr>
<tr>
<td>3</td>
<td>Gel develops a convex surface and adheres to the bottom of the cup</td>
<td>≥ 10,000,000</td>
</tr>
</tbody>
</table>

Interpretation of result was done according to Shearer and Harris (1992) i.e. milk from non-infected glands yielded a “negative”, “trace” or “1” scores and scores of “2” or “3” indicated mastitis. Processing of milk sample is shown in plate 07.
Polymerase chain reaction

Polymerase chain reaction (PCR) was performed to identify the *Mycoplasma agalactiae* and thus for confirmatory diagnosis of contagious agalactia. The DNA required for PCR was extracted from the milk samples of suspected animals. The following media was required to process the milk samples for DNA extraction.

Media

PPLO (Pleuro Pneumonia Like Organisms) broth

Composition

| PPLO broth base (powder) | 21 g |
| Distilled water         | 700 ml |
| Mycoplasma supplement   | 300 ml |

Procedure

The PPLO broth was prepared according to the method described by Morton and Lecce (1953). For this, 21 g of PPLO broth base (powder) was dissolved in 700 ml of distilled water and mixed thoroughly. The medium was autoclaved at 15 lbs (121°C) for 15 minutes and cooled upto approximately 45°C. 300 ml of mycoplasma supplement was added aseptically to the medium and mixed well. Pictorial presentation of procedure of broth preparation is shown in plate 08.

Preparation of mycoplasma broth culture using milk samples

Approximately 0.1 ml of milk samples were inoculated into mycoplasma broth base containing mycoplasma supplement and incubated in a humid atmosphere containing 5% carbon dioxide at 37°C for 7 days. The broths were checked daily for growth. Positive broth cultures were stored at 4°C till further use (Kizil et al., 2007).

Extraction of DNA

DNA extraction was performed by chelex based extraction of DNA using Insta Gene Matrix (Bio-Rad laboratories, India Pvt. Ltd.) as described by Giraffa et al. (2000) and method described by Liu et al. (2001).
Chelex based extraction of DNA using Insta Gene Matrix

For extraction of DNA, 1 ml of mycoplasma broth culture was vortexed for 10 sec and centrifuged for 1 minute at 10,000–12,000 rpm. The supernatant was removed and 200 µl of Insta Gene matrix was added to the pellet. The suspension was incubated at 56°C for 15–30 minutes and again vortexed at high speed for 10 seconds. The cell suspension was heated in a boiling water bath for 8 minutes and again vortexed at high speed for 10 seconds and spun at 10,000–12,000 rpm for 2–3 minutes. Then 20 µl of the resulting supernatant was used per 50 µl PCR reaction and the remainder supernatant was stored at -20°C for further use.

DNA extraction as per Liu et al. (2001)

1 ml of mycoplasma broth culture was centrifuged at 13,000 rpm for 10 min. The cell pellet was then washed twice with 1 ml of PBS and resuspended in a final volume of 20 µl of PBS. The cell suspension was heated in a boiling water bath for 10 min and immediately placed on ice for at least 10 min. After cooling, the lysate was centrifuged at 13,000 rpm for 2 min. The supernatant containing DNA was collected and stored at -20°C until further use.

Reconstitution of oligonucleotide primers

The oligonucleotide primers were synthesized and supplied in lyophilized form by Integrated DNA Technologies, Avantor Performance Materials India Limited, Faridabad. They were reconstituted to 100 µM/µl stocks in sterile nuclease free water (NFW). Primers were used at a working dilution of 50 pmol/µl in sterile NFW (Kizil et al., 2007).

Primers for Mycoplasma agalactiae

The specific primers were used in the study to identify Mycoplasma agalactiae as per Kizil et al. (2007).

ma-mp 1 5’-AGCAGCACAATACTCGAGA-3’ (forward)
ma-mp 1 5’-AACACCTGGATTGTTTGGAT-3’ (reverse)
PCR Conditions for detection of *Mycoplasma agalactiae*

The PCR was carried out in 50µl of reaction mixture which was composed of:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR mastermix</td>
<td>25 µl</td>
</tr>
<tr>
<td>Forward primer</td>
<td>1 µl</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>1 µl</td>
</tr>
<tr>
<td>Nuclease free water</td>
<td>3 µl</td>
</tr>
<tr>
<td>Sample DNA</td>
<td>20 µl</td>
</tr>
</tbody>
</table>

The cycling conditions used for amplifying the ma-mp 81 DNA gene of *Mycoplasma agalactiae* were as follows:

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Temperature/TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30 cycles of denaturation</td>
<td>94°C for 1 min</td>
</tr>
<tr>
<td>2</td>
<td>annealing</td>
<td>54°C for 1 min</td>
</tr>
<tr>
<td>3</td>
<td>extension</td>
<td>72°C for 1 min</td>
</tr>
<tr>
<td>4</td>
<td>final extension</td>
<td>72°C for 10 min</td>
</tr>
</tbody>
</table>

The amplification of specific PCR product was checked by electrophoresis of the PCR product in 1.5% agarose gel and viewed in UV transilluminator system.

**Detection of PCR product by gel electrophoresis**

**Preparation and casting of gel**

Agarose of 1.5 per cent concentration in 1X TBE buffer was prepared. The boiled agarose was allowed to cool down to about 50°C and ethidium bromide 0.5 µg/ml was added. The molten agarose was poured in casting tray of the electrophoresis unit fitted with comb and allowed to set. After solidification of agarose, the comb was removed and the gel tray was immersed in the main electrophoresis unit containing 500 ml of 1X TBE buffer.
Electrophoresis of PCR product

10 μl of PCR product was loaded in the wells and in one well 100bp DNA ladder was loaded. Electrophoresis was performed at 80 V and the mobility was monitored by the migration of dye.

Visualization of PCR product

After sufficient migration the gel was examined in the UV transilluminator gel documentation system and print was obtained. Steps of processing milk samples for molecular diagnosis are shown in plate 09.

Experimental design

To study the efficacy of different drugs for the treatment of contagious agalactia, a total of 24 lactating goats positive for Mycoplasma agalactiae were placed into four groups i.e. T1 – T4, each group comprised of 6 animals. Six clinically healthy goats and negative for CMT as well as mycoplasmosis were selected to serve as healthy control (Group C). The details of therapeutic trial are presented in Table 03.

Table 03: Experimental design for therapeutic trial against contagious agalactia in goats

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of animals</th>
<th>Drugs and Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>06</td>
<td>Oxytetracycline* @ 15 mg/kg b.wt., I/M × 5 days</td>
</tr>
<tr>
<td>T2</td>
<td>06</td>
<td>Tylosin @ 20 mg/kg b.wt., I/M × 5 days</td>
</tr>
<tr>
<td>T3</td>
<td>06</td>
<td>Oxytetracycline* @ 15 mg/kg b.wt., I/M × 5 days + Tocopherol @ 2 mg/kg b.wt &amp; Sodium selenite @ 0.06 mg/kg b.wt , I/M once</td>
</tr>
<tr>
<td>T4</td>
<td>06</td>
<td>Tylosin @ 20 mg/kg b.wt., I/M × 5 days + Tocopherol @ 2 mg/kg b.wt &amp; Sodium selenite @ 0.06 mg/kg b.wt , I/M once</td>
</tr>
<tr>
<td>C</td>
<td>06</td>
<td>-</td>
</tr>
</tbody>
</table>

* 2% lignocain was used along with the drug

In addition to antimicrobial drugs, symptomatic and supportive therapy was done by administration of fluids and electrolytes (Inj. Normal saline @ 10-20 ml/kg body wt. IV, anti-inflammatory drugs (Inj. Meloxicam @ 0.2 – 0.3 mg/kg body wt. IM), antipyretic drugs (Injection Paracetamol @ 10 mg/kg body wt. IM) were given according to the clinical condition.
**Therapeutic response evaluation**

The response of therapeutic study was evaluated on the basis of:

1. Improvement in clinical condition of the goats.
2. California mastitis test and polymerase chain reactions after the completion of treatment i.e. on days 7 and 14 post treatment.

**Score Card**

The efficacy of treatment in clinical mastitis can be judged by improvement in three categories viz. abnormal milk, abnormal gland and abnormal animal behavior (Radostits *et al.*, 2010). As mastitis is the outward clinical manifestation of contagious agalactia. Hence, keeping this view in mind, a score card was prepared on the basis of recovery in various parameters to assess the efficacy of various treatments in different groups. The parameters were selected as CMT for abnormal milk, inspection and palpation of udder for abnormal gland and behavior for abnormal animal.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td><strong>CMT</strong></td>
<td>No reaction</td>
</tr>
<tr>
<td><strong>Udder inflammation</strong></td>
<td>None</td>
</tr>
<tr>
<td><strong>Behavior</strong></td>
<td>Alert</td>
</tr>
</tbody>
</table>

Each goat was observed for CMT reactions, udder inflammation and behavior. Score was given in each row representing the marks allotted to each parameter. Total score was calculated by adding the score of individual parameter and assessment was done as per the following score card.

<table>
<thead>
<tr>
<th>Score</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Healthy</td>
</tr>
<tr>
<td>1 – 3</td>
<td>Mild illness</td>
</tr>
<tr>
<td>4 – 6</td>
<td>Moderate illness</td>
</tr>
<tr>
<td>7 – 9</td>
<td>Severe illness</td>
</tr>
</tbody>
</table>
Data analysis

Overall prevalence of mycoplasmosis and contagious agalactia were calculated by dividing the number of positive samples by the total number of samples. Age wise and breed wise prevalence were calculated by category wise dividing the number of positive samples by the total number of samples (Thrusfield, 2004).

Statistical analysis

Analysis of data of prevalence studies was done by using Chi square test. The alterations in clinical parameters in different treatment groups at different intervals were analyzed using hierarchical design of ANOVA and means were compared using Duncan’s multiple range test (Snedecor and Cochran, 1994).
4. RESULTS

Prevalence of contagious agalactia

The study on prevalence of contagious agalactia in goats in and around Jabalpur was based on screening the lactating goats for the presence of clinical signs pertaining to contagious agalactia, performing slide agglutination test and molecular identification of *Mycoplasma agalactiae* in the suspected animals.

A total of 705 lactating goats belonging to organised goat farms (Goat farm, Amanala, Livestock farm, Adhartal, Goat farm, Chhoti Pipariya) as well as unorganised sector of goatry in and around Jabalpur viz. villages like Ganiyari (Patan), Nuniakala (Panagar), Noni (Shahpura) and clinical cases brought to TVCC, Veterinary College, Jabalpur were screened for the presence of clinical symptoms of contagious agalactia. On the basis of clinical symptoms of contagious agalactia like mastitis, history of abortion, respiratory, ocular and mixed signs, 282 lactating goats were suspected for the disease.

Out of 282 lactating goats, 137 goats (48.58%) were found positive for clinical mastitis, 52 goats (18.44%) showed respiratory signs like mucoid nasal discharge, dyspnoea, etc., 33 goats (11.70%) showed ocular signs, 09 goats (3.19%) revealed history of abortion and 51 goats (18.08%) showed mixed signs of mastitis, respiratory signs, ocular signs and/or history of abortion. Results are outlined in table 04 and figure 01.

Table 04: Details of clinical picture in the goats suspected for contagious agalactia

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Clinical picture</th>
<th>No. of goats showing signs (n=282)</th>
<th>Per cent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mastitis</td>
<td>137</td>
<td>48.58</td>
</tr>
<tr>
<td>2</td>
<td>Respiratory signs</td>
<td>52</td>
<td>18.44</td>
</tr>
<tr>
<td>3</td>
<td>Ocular signs</td>
<td>33</td>
<td>11.7</td>
</tr>
<tr>
<td>4</td>
<td>Abortion</td>
<td>09</td>
<td>3.19</td>
</tr>
<tr>
<td>5</td>
<td>Mixed signs</td>
<td>51</td>
<td>18.08</td>
</tr>
</tbody>
</table>
CMT Score

Out of 145 suspected goats, 46.21 per cent goats (67 out of 145 goats) showed CMT score of +1, 32.41 per cent goats (47 out of 145 goats) showed CMT score of +2 and 21.38 per cent goats (31 out of 145 goats) showed +3 score of CMT. Thus, animals having +2 and +3 scores i.e. 78 out of 145 goats were suffering from sub clinical mastitis. The details of CMT scores are outlined in table 05 and figure 02. Therefore, a total of 215 goats (137 goats suffering from clinical mastitis and 78 goats suffering from sub clinical mastitis) were found positive for mastitis.

Table 05: CMT score of milk samples of suspected goats

<table>
<thead>
<tr>
<th>S.No.</th>
<th>CMT score</th>
<th>Goats positive (n=145)</th>
<th>Per cent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+ 1</td>
<td>67</td>
<td>46.21</td>
</tr>
<tr>
<td>2</td>
<td>+ 2</td>
<td>47</td>
<td>32.41</td>
</tr>
<tr>
<td>3</td>
<td>+ 3</td>
<td>31</td>
<td>21.38</td>
</tr>
</tbody>
</table>

Seroprevalence of mycoplasmosis in lactating goats

The overall seroprevalence of mycoplasmosis during April 2014 to March 2015 was 9.50 per cent (67 out of 705 goats) in lactating goats. However, the seroprevalence among the goats suffering from mastitis was reported to be 31.16 per cent i.e. 67 out of 215 mastitic goats. The results are shown in table 06 and figure 03.

Table 06: Seroprevalence of mycoplasmosis in lactating goats

<table>
<thead>
<tr>
<th>Goats</th>
<th>Number examined</th>
<th>Number positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Screened</td>
<td>705</td>
<td>67</td>
<td>9.50</td>
</tr>
<tr>
<td>Mastitic goats</td>
<td>215</td>
<td>67</td>
<td>31.16</td>
</tr>
</tbody>
</table>

Seroprevalence of mycoplasmosis in organised and unorganised goatry

Seroprevalence of mycoplasmosis in organised goat farms was observed higher i.e. 19.65 per cent (45 out of 229 goats) than the
seroprevalence in unorganised sector of goatry i.e. 4.62 per cent (22 out of 476 goats). Significant variation was noticed in the seroprevalence with respect to rearing pattern of goatry. Results are represented in table 07 and figure 04.

Table 07: Seroprevalence of mycoplasmosis in organized and unorganized goatry

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Sector/ Rearing Pattern</th>
<th>Number screened</th>
<th>Number positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Organized goatry</td>
<td>229</td>
<td>45</td>
<td>19.65</td>
</tr>
<tr>
<td>2</td>
<td>Unorganized goatry</td>
<td>476</td>
<td>22</td>
<td>4.62</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 32.144 \quad df = 1 \quad p = 0 \]

Age wise seroprevalence of mycoplasmosis

The age wise seroprevalence of mycoplasmosis in lactating goats revealed highest prevalence i.e. 13.12 per cent (21 out of 160 goats) in the goats of above 4 years of age followed by 9.34 per cent (27 out of 289 goats) prevalence in goats of 3 to 4 years of age and lowest prevalence i.e. 7.42 per cent in goats of 2 to 3 years of age (19 out of 256 goats). The age wise seroprevalence revealed a non-significant variation among various groups. The details are outlined in table 08 and figure 05.

Table 08: Age wise seroprevalence of mycoplasmosis in lactating goats

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Age group</th>
<th>Number screened</th>
<th>Number positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2-3 years</td>
<td>256</td>
<td>19</td>
<td>7.42</td>
</tr>
<tr>
<td>2</td>
<td>3-4 years</td>
<td>289</td>
<td>27</td>
<td>9.34</td>
</tr>
<tr>
<td>3</td>
<td>Above 4 years</td>
<td>160</td>
<td>21</td>
<td>13.12</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 3.0457 \quad df = 2 \quad p = 0.218092 \]
Breed wise seroprevalence of mycoplasmosis

The breed wise seroprevalence study of mycoplasmosis in lactating goats revealed a highest prevalence of 34.69 per cent (17 out of 49 goats) in Barbari breed followed by prevalence of 21.43 per cent (6 out of 28 goats) in Black Bengal breed, 17.48 per cent in Sirohi breed (25 out of 143 goats), 9.09 per cent in Jamunapari breed (8 out of 88 goats) and lowest prevalence of 2.77 per cent (11 out of 397 goats) in non-descript breed of goats. The breed wise seroprevalence of mycoplasmosis showed significant variation (p<0.05) among various breeds of goats. Results are outlined in table 09 and figure 06.

Table 09: Breed wise seroprevalence of mycoplasmosis in lactating goats

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Breed</th>
<th>Number screened</th>
<th>Number positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Barbari</td>
<td>49</td>
<td>17</td>
<td>34.69</td>
</tr>
<tr>
<td>2</td>
<td>Black Bengal</td>
<td>28</td>
<td>06</td>
<td>21.43</td>
</tr>
<tr>
<td>3</td>
<td>Jamunapari</td>
<td>88</td>
<td>08</td>
<td>09.09</td>
</tr>
<tr>
<td>4</td>
<td>Sirohi</td>
<td>143</td>
<td>25</td>
<td>17.48</td>
</tr>
<tr>
<td>5</td>
<td>Non descript</td>
<td>397</td>
<td>11</td>
<td>02.77</td>
</tr>
</tbody>
</table>

$\chi^2 = 54.3346 \text{ df } = 4 \text{ p } \leq 0.00001$

Association of mycoplasmosis with status of mastitis in lactating goats

Out of 137 lactating goats suffering from clinical mastitis, 32.12 per cent goats (44 out of 137 goats) were suffering from mycoplasmosis whereas out of 78 goats suffering from sub clinical mastitis, 29.49 per cent goats (23 out of 78 goats) were suffering from mycoplasmosis. The association of mycoplasmosis with status of mastitis in lactating goats was found to be non significant. The details are shown in table 10 and figure 07.
Table 10: Association of mycoplasmosis with status of mastitis in lactating goats

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Status of mastitis</th>
<th>Number screened</th>
<th>Number positive for mycoplasmosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>1</td>
<td>Clinical mastitis</td>
<td>137</td>
<td>44</td>
</tr>
<tr>
<td>2</td>
<td>Sub clinical mastitis</td>
<td>78</td>
<td>23</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 0.0846 \quad df = 1 \quad p = 0.771215 \]

Detection of *Mycoplasma agalactiae* in goats using polymerase chain reaction

The growth of mycoplasmas in PPLO broth media was demonstrated by changes in colour or turbidity due to the bacterial biochemical activity and metabolism. When milk samples collected from lactating goats suspected of contagious agalactia were subjected to PCR test, a specific 176 bp bands obtained from the DNA amplification of *Mycoplasma agalactiae* using primers ma-mp 1F and ma-mp 1R were observed (Plate 10).

Prevalence of contagious agalactia in lactating goats

The overall prevalence of contagious agalactia during April 2014 to March 2015 was 4.39 per cent (31 out of 705 goats) in lactating goats. However, the prevalence among the goats suffering from mastitis was observed 14.42 per cent i.e. 31 out of 215 mastitic goats. The results are shown in table 11 and figure 08.

Table 11: Prevalence of contagious agalactia in lactating goats

<table>
<thead>
<tr>
<th>Goats</th>
<th>Number screened</th>
<th>Number positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Screened</td>
<td>705</td>
<td>31</td>
<td>4.39</td>
</tr>
<tr>
<td>Mastitic goats</td>
<td>215</td>
<td>31</td>
<td>14.42</td>
</tr>
</tbody>
</table>
Prevalence of contagious agalactia in organised and unorganised goatry

Prevalence of contagious agalactia in organised goat farms was higher i.e. 10.92 per cent (25 out of 229 goats) than the unorganised sector of goatry i.e. 1.26 per cent (6 out of 476 goats). Significant variation was noticed in the prevalence with respect to rearing pattern of goatry (Table 12, Figure 09).

Table 12: Prevalence of contagious agalactia in organized and unorganized goatry

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sector/ Rearing Pattern</th>
<th>Number screened</th>
<th>Number positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Organized goatry</td>
<td>229</td>
<td>25</td>
<td>10.92</td>
</tr>
<tr>
<td>2</td>
<td>Unorganized goatry</td>
<td>476</td>
<td>6</td>
<td>1.26</td>
</tr>
</tbody>
</table>

$\chi^2 = 30.4771 \ df = 1 \ p = 0$

Age wise prevalence of contagious agalactia

The age wise prevalence of contagious agalactia in lactating goats revealed highest prevalence i.e. 6.87 per cent (11 out of 160 goats) in above 4 years age group followed by 4.49 per cent prevalence (13 out of 289 goats) in goats of 3 to 4 years of age group and lowest prevalence i.e. 2.73 per cent in goats of 2 to 3 years of age group (7 out of 256 goats). The age wise prevalence revealed a non-significant variation among various groups. Results are depicted in table 13 and figure 10.

Table 13: Age wise prevalence of contagious agalactia in lactating goats

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Age group</th>
<th>Number screened</th>
<th>Number positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2-3 years</td>
<td>256</td>
<td>07</td>
<td>2.73</td>
</tr>
<tr>
<td>2</td>
<td>3-4 years</td>
<td>289</td>
<td>13</td>
<td>4.49</td>
</tr>
<tr>
<td>3</td>
<td>Above 4 years</td>
<td>160</td>
<td>11</td>
<td>6.87</td>
</tr>
</tbody>
</table>

$\chi^2 = 3.6636 \ df = 2 \ p = 0.160124$
Breed wise prevalence of contagious agalactia

The breed wise prevalence study of contagious agalactia in lactating goats revealed a highest prevalence of 12.24 per cent (6 out of 49 goats) in Barbari breed followed by prevalence of 11.19 per cent (16 out of 143 goats) in Sirohi breed, 7.14 per cent in Black Bengal breed (2 out of 28 goats), 3.41 per cent in Jamunapari breed (3 out of 88 goats) and lowest prevalence of 1.01 per cent (4 out of 397 goats) in non-descript breed of goats. The breed wise prevalence of contagious agalactia showed significant variation (p<0.05) among various breeds of goats. Results are outlined in table 14 and figure 11.

Table 14: Breed wise prevalence of contagious agalactia in lactating goats

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Breed</th>
<th>Number screened</th>
<th>Number positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Barbari</td>
<td>49</td>
<td>06</td>
<td>12.24</td>
</tr>
<tr>
<td>2</td>
<td>Black Bengal</td>
<td>28</td>
<td>02</td>
<td>07.14</td>
</tr>
<tr>
<td>3</td>
<td>Jamunapari</td>
<td>88</td>
<td>03</td>
<td>03.41</td>
</tr>
<tr>
<td>4</td>
<td>Sirohi</td>
<td>143</td>
<td>16</td>
<td>11.19</td>
</tr>
<tr>
<td>5</td>
<td>Non descript</td>
<td>397</td>
<td>04</td>
<td>01.01</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 30.5124 \text{ df } 4 \text{ p } <0.00001 \]

Association of contagious agalactia with status of mastitis in lactating goats

Out of 137 lactating goats suffering from clinical mastitis, 15.33 per cent goats (21 out of 137 goats) were reported to be suffering from contagious agalactia whereas, out of 78 goats suffering from sub clinical mastitis, 12.82 per cent goats (10 out of 78 goats) were reported to be suffering from contagious agalactia. The association of contagious agalactia with mastitis status of lactating goats was found to be non significant. The details are shown in table 15 and figure 12.
Table 15: Association of contagious agalactia with mastitis status of lactating goats

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Mastitis Status</th>
<th>Animals screened</th>
<th>Animal positive for contagious agalactia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>1</td>
<td>Clinical mastitis</td>
<td>137</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>Sub clinical mastitis</td>
<td>78</td>
<td>10</td>
</tr>
</tbody>
</table>

$\chi^2 = 0.1907$  df = 1  p = 0.662366

Evolving the suitable therapy for caprine contagious agalactia

Clinical parameters

Body temperature

Body temperature of all the 24 goats under therapeutic trial was recorded on days 0 (pre-treatment), 7 and 14 post treatment and compared with the healthy goats.

The results revealed that body temperature of goats on day 0 pre-treatment was significantly higher in all treatment groups than healthy control group i.e. goats of group T1, T2, T3 and T4 showed mean body temperatures 104.0±0.18°F, 103.5±0.28°F, 103.7±0.23°F, 103.5±0.25°F, respectively and 101.3±0.24°F in goats of healthy control group. After treatment, the mean body temperature on day 7 in goats of group T1, T2, T3 and T4 was reduced to 102.3±0.46°F, 102.5±0.29°F, 102.3±0.36°F and 101.5 ±0.25°F, respectively. On day 14 post treatment, the mean body temperatures of groups T1, T2, T3 and T4 were 102.0±0.4°F, 101.8±0.29°F, 101.7±0.40°F, 101.2±0.35°F, respectively.

A significant improvement in the body temperature of goats was noticed in all treatment groups on days 7 and 14 post treatment. However, the mean body temperatures on day 7 post treatment in group T4 and the body temperature on day 14 post treatment in groups T1, T2 and T3 were essentially similar to healthy goats. The detailed variation in temperature in different treatment groups at different intervals are outlined in table 16 and figure 13.
Table 16: Mean body temperature (°F) in different treatment groups at different intervals

<table>
<thead>
<tr>
<th>Group</th>
<th>Temperature (°F)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>T1</td>
<td>104.0±0.18</td>
</tr>
<tr>
<td>T2</td>
<td>103.5±0.28</td>
</tr>
<tr>
<td>T3</td>
<td>103.7a±0.23</td>
</tr>
<tr>
<td>T4</td>
<td>103.5a±0.25</td>
</tr>
<tr>
<td>C</td>
<td>101.3d±0.24</td>
</tr>
</tbody>
</table>

Different superscripts indicate a significant difference (p≤0.05)

Pulse rate

Pulse rate of all the goats under therapeutic trial was recorded on days 0 pre-treatment, 7 and 14 post treatment and compared with healthy goats.

The results revealed that pulse rate of goats on day 0 before treatment was significantly higher in all the treatment groups than the healthy control group i.e. animals of group T1, T2, T3 and T4 showed mean pulse rate of 94.7±0.88/min., 93.5±0.76/min., 94.0±1.0/min., 93.0±1.09/min., respectively and 71.5±0.72/min. in goats of healthy control group. After being treated, the mean pulse rate on day ‘7’ in goats of group T1, T2, T3 and T4 were 75.8±1.87/min., 73.0±1.67/min., 73.3±2.25/min. and 69.8±0.75/min., respectively. On day 14 post treatment, the mean pulse rates in groups T1, T2, T3 and T4 were 71.8±1.60/min., 70.6±0.88/min., 69.6±2.01/min. and 70.3±0.76/min., respectively.

A significant improvement in the pulse rate of goats was noticed in all treatment groups on days 7 and 14 post treatment. However, the mean pulse rate of day 7 post treatment in goats of group T4 and the pulse rate on day 14 post treatment in groups T1, T2 and T3 were essentially similar to healthy goats. The detailed variation in pulse rate in different treatment groups at different intervals are presented in table 17 and figure 14.
Table 17: Mean pulse rate (per minute) in different treatment groups at different intervals

<table>
<thead>
<tr>
<th>Group</th>
<th>pulse rate (per minute)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 7</td>
<td>Day 14</td>
</tr>
<tr>
<td>T1</td>
<td>94.7±0.88</td>
<td>75.8±1.87</td>
<td>71.8±1.60</td>
</tr>
<tr>
<td>T2</td>
<td>93.5±0.76</td>
<td>73.0±1.67</td>
<td>70.6±0.88</td>
</tr>
<tr>
<td>T3</td>
<td>94.0±1.0</td>
<td>73.3±2.25</td>
<td>69.6±2.01</td>
</tr>
<tr>
<td>T4</td>
<td>93.0±1.09</td>
<td>69.8±0.75</td>
<td>70.3±0.76</td>
</tr>
<tr>
<td>C</td>
<td>71.5±0.72</td>
<td>71.0±0.85</td>
<td>71.3±0.56</td>
</tr>
</tbody>
</table>

Different superscripts indicate a significant difference (p≤0.05)

Respiration rate

Respiration rate of all the goats under therapeutic trial was recorded on days 0 pre-treatment, 7 and 14 post treatment and was compared with healthy goats.

The results revealed that respiration rate of goats on day 0 before treatment was significantly higher in all the treatment groups than the healthy control group i.e. goats of group T1, T2, T3 and T4 showed mean respiration rate of 47.8±1.14/min., 47.8±1.08/min., 45.5±1.30/min., 45.3±1.75/min., respectively and 24.1±0.47/min. in goats of healthy control group. After treatment the mean respiration rate on day 7, in goats of group T1, T2, T3 and T4 were 34.0±2.20/min., 35.3±2.47/min., 35.5±2.80/min. and 27.1±0.75/min., respectively. On day 14 post treatment, the mean respiration rates in groups T1, T2, T3 and T4 were 30.5±1.86/min., 31.0±1.98/min., 32.0±2.36/min. and 26.8±1.37/min., respectively.

A significant improvement in the respiration rate of goats was noticed in all treatment groups on days 7 and 14 post treatment. However, the mean respiration rate of days 7 and 14 post treatment in goats of group T4 was essentially similar to healthy goats. The detailed variation in respiration rate in different treatment groups at different intervals are presented in table 18 and figure 15.
Table 18: Mean respiration rate (per minute) in different treatment groups at different intervals

<table>
<thead>
<tr>
<th>Group</th>
<th>Respiration rate (per minute)</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td>47.8\textsuperscript{a}±1.14</td>
<td>34.0\textsuperscript{b}±2.20</td>
<td>30.5\textsuperscript{bc}±1.86</td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td>47.8\textsuperscript{a}±1.08</td>
<td>35.3\textsuperscript{b}±2.47</td>
<td>31.0\textsuperscript{bc}±1.98</td>
</tr>
<tr>
<td>T3</td>
<td></td>
<td>45.5\textsuperscript{a}±1.30</td>
<td>35.5\textsuperscript{b}±2.80</td>
<td>32.0\textsuperscript{bc}±2.36</td>
</tr>
<tr>
<td>T4</td>
<td></td>
<td>45.3\textsuperscript{a}±1.75</td>
<td>27.1\textsuperscript{cd}±1.42</td>
<td>26.8\textsuperscript{cd}±1.37</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>24.1\textsuperscript{d}±0.47</td>
<td>24.0\textsuperscript{d}±0.45</td>
<td>24.5\textsuperscript{d}±0.43</td>
</tr>
</tbody>
</table>

Different superscripts indicate a significant difference (p≤0.05)

**Clinical score**

To evaluate the severity of illness before treatment and improvement in clinical condition in terms of response to therapy, a clinical score card was prepared for the first time. On the basis of clinical score card, the animals were grouped as healthy (score 0), mild illness (score 1-3), moderate illness (score 4-6) and severe illness (score 7-9). All the 24 goats were observed daily and clinical score was recorded during the defined period of treatment. The details of clinical scores of individual goats under therapeutic trial in different groups are represented in table 19.

**Group T1 (Oxytetracycline)**

In group T1, on the basis of clinical score all the six goats showed severe illness on day 0 before treatment. During five days of treatment, there was gradual improvement in all the goats, however, the recovery was more marked in two goats which recovered from severe to moderate illness on second day of treatment. Remaining four goats recovered upto moderate illness on fourth day of treatment. On day 7 post treatment, three goats recovered completely and remaining three showed only mild illness. On day 14 post treatment, five goats showed complete recovery but one goat showed mild illness.
Group T2 (Tylosin)

In group T2, on the basis of clinical score, all the six goats showed severe illness on day 0 before treatment. During the course of treatment, there was gradual improvement in all goats. Three goats showed recovery from severe to moderate illness on second day of treatment. On third day of treatment, five goats recovered upto moderate illness and one showed only mild illness. On fourth day of treatment, one goat recovered completely, two goats showed moderate illness. On fifth day of treatment, only two goats showed mild illness and remaining four goats recovered completely. On days 7 and 14 post treatment, all goats showed complete recovery.

Group T3 (Oxytetracycline, Tocopherol and Sodium selenite)

In group T3, on the basis of clinical score, five goats showed severe illness and one was moderately ill on day 0 before treatment. During treatment, there was gradual improvement in all goats. On third day of treatment, five goats recovered to moderate illness and one showed only mild illness. On fifth day of treatment, four goats showed mild illness and remaining two goats recovered completely. On day 7 post treatment, five goats showed complete recovery but one still showed mild illness. However, on day 14 post treatment, all the six goats recovered completely.

Group T4 (Tylosin, Tocopherol and Sodium selenite)

In group T4, on the basis of clinical score, five goats showed severe illness and one showed moderate illness on day 0 before treatment. During treatment, there was gradual improvement in all goats. On second day of treatment, four goats recovered from severe to moderate illness. On third day of treatment, two goats showed only mild illness and recovered completely on fourth day of treatment. On fifth day of treatment, all goats recovered completely.
### Table 19: Clinical score in goats at different intervals in different treatment groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Goats</th>
<th>Pre treatment</th>
<th>Post treatment (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0</td>
<td>1</td>
</tr>
<tr>
<td>T1</td>
<td>i</td>
<td>08</td>
<td>08</td>
</tr>
<tr>
<td></td>
<td>ii</td>
<td>09</td>
<td>09</td>
</tr>
<tr>
<td></td>
<td>iii</td>
<td>09</td>
<td>09</td>
</tr>
<tr>
<td></td>
<td>iv</td>
<td>07</td>
<td>07</td>
</tr>
<tr>
<td></td>
<td>v</td>
<td>08</td>
<td>07</td>
</tr>
<tr>
<td></td>
<td>vi</td>
<td>09</td>
<td>09</td>
</tr>
<tr>
<td>T2</td>
<td>i</td>
<td>08</td>
<td>08</td>
</tr>
<tr>
<td></td>
<td>ii</td>
<td>09</td>
<td>09</td>
</tr>
<tr>
<td></td>
<td>iii</td>
<td>08</td>
<td>08</td>
</tr>
<tr>
<td></td>
<td>iv</td>
<td>08</td>
<td>07</td>
</tr>
<tr>
<td></td>
<td>v</td>
<td>09</td>
<td>08</td>
</tr>
<tr>
<td></td>
<td>vi</td>
<td>07</td>
<td>06</td>
</tr>
<tr>
<td>T3</td>
<td>i</td>
<td>09</td>
<td>09</td>
</tr>
<tr>
<td></td>
<td>ii</td>
<td>09</td>
<td>09</td>
</tr>
<tr>
<td></td>
<td>iii</td>
<td>06</td>
<td>06</td>
</tr>
<tr>
<td></td>
<td>iv</td>
<td>08</td>
<td>08</td>
</tr>
<tr>
<td></td>
<td>v</td>
<td>08</td>
<td>08</td>
</tr>
<tr>
<td></td>
<td>vi</td>
<td>09</td>
<td>08</td>
</tr>
<tr>
<td>T4</td>
<td>i</td>
<td>08</td>
<td>08</td>
</tr>
<tr>
<td></td>
<td>ii</td>
<td>09</td>
<td>08</td>
</tr>
<tr>
<td></td>
<td>iii</td>
<td>06</td>
<td>06</td>
</tr>
<tr>
<td></td>
<td>iv</td>
<td>07</td>
<td>07</td>
</tr>
<tr>
<td></td>
<td>v</td>
<td>08</td>
<td>08</td>
</tr>
<tr>
<td></td>
<td>vi</td>
<td>09</td>
<td>08</td>
</tr>
</tbody>
</table>
Polymerase chain reaction

Polymerase chain reaction was performed for confirmation of *Mycoplasma agalactiae* in milk samples of all the 24 goats under different therapeutic regimen on day 0 pre-treatment. Evaluation of different therapies was done by performing PCR on days 7 and 14 post treatment. The agarose gel electrophoresis is depicted in plates 11 to 18.

**Group T1 (Oxytetracycline)**

Milk samples of all the six goats of group T1 were subjected to PCR test on day 0 pre-treatment. A specific 176 bp band was obtained from DNA amplification of *Mycoplasma agalactiae* using specific primers. After being treated with oxytetracycline, milk samples of these goats were again subjected to PCR test on days 7 and 14 post treatment and results revealed that two of the goats showed specific 176 bp band on day 7 post treatment. On day 14 post treatment, no samples revealed the presence of specific band for *Mycoplasma agalactiae*. It indicated that shedding of organism was observed in two goats on day 7 post treatment but no organism was identified on day 14 post treatment (Plate 11 and Plate 12).

**Group T2 (Tylosin)**

In goats of group T2, PCR results on day 0 pre-treatment revealed 176 bp band specific for primers ma-mp 1F and ma-mp 1R. After being treated with tylosin, on days 7 and 14 post treatment no specific bands were observed on agar gel electrophoresis of the amplification of 176 bp fragment specific for *Mycoplasma agalactiae*. Results showed that no shedding of causative organism was observed in the milk samples of goats indicating complete cure of goats (Plate 13 and Plate 14).

**Group T3 (Oxytetracycline, Tocopherol and Sodium selenite)**

In group T3, when milk samples of all six goats were subjected to PCR test on day 0 pre treatment, results showed band of 176 bp on agar gel electrophoresis of the amplified fragment specific for *Mycoplasma agalactiae*. After treatment on days 7 and 14 post treatment, no bands were
observed which indicated complete absence of causative organism in the milk samples of goats after treatment (Plate 15 and Plate 16).

**Group T4 (Tylosin, Tocopherol and Sodium selenite)**

In goats of group T4, PCR results on day 0 pre-treatment showed 176 bp band specific for primers ma-mp 1F and ma-mp 1R of *Mycoplasma agalactiae*. However, no such bands were observed on days 7 and 14 post treatment which indicated that shedding of organisms in milk samples had stopped and goats were completely cured (Plate 17 and Plate 18).
5. DISCUSSION

An investigation about a disease helps to understand the distribution and epidemiology of the disease status. Contagious agalactia is an economically important disease of small ruminants caused by mycoplasmas. It is a disease predominantly of milking sheep and goats. Many workers had pointed out the foci of mycoplasmal infections in India but a meagre work regarding establishment of prevalence of mycoplasmosis in goats in Madhya Pradesh has been carried out. Hence, results of prevalence of mycoplasmosis as well as contagious agalactia in goats, detection of *Mycoplasma agalactiae* in milk samples of goats using polymerase chain reaction and a suitable therapy for contagious agalactia have been discussed here.

Clinical picture in contagious agalactia

In the present study, 705 lactating goats belonging to organised and unorganised sector of goatry were screened. On the basis of clinical symptoms of contagious agalactia like mastitis, history of abortion, respiratory, ocular and mixed signs, 282 lactating goats were suspected for the disease. Precisely, 48.58 per cent showed clinical mastitis, 18.44 per cent showed respiratory signs, 11.70 per cent showed ocular signs, 3.19 per cent revealed history of abortion and 18.08 per cent showed mixed signs. The clinical symptoms observed during the disease correlates well with the previous studies (DaMassa *et al*., 1992; Rana *et al*., 1992; Bergonier *et al*., 1997; Sanchis *et al*., 2000; Kheirkhah *et al*., 2011 and Pooladgar *et al*., 2011). However, Madanat *et al.* (2001) and Corrales *et al.* (2007) observed additional symptoms of arthritis in goats affected with contagious agalactia.

Contagious agalactia, therefore, should be regarded as a syndrome which share mammary, ocular, articular and respiratory symptoms with occasional abortions. Although these symptoms are not generally present in an individual animal but the complete syndrome may be observed with in a flock (Bergonier *et al*., 1997).
CMT Score

Mastitis is a general term which refers to inflammation of the mammary gland, regardless of cause. Clinical mastitis is characterized by visible abnormalities in the udder or milk. On the contrary, subclinical mastitis may only be detectable by measures of the milk’s cellular content (somatic cells). The predominant cells in milk are epithelial and white blood cells, the latter of which increase to tremendous numbers (millions/ml) whenever injury or infection of the gland occurs. Thus, by determining the number of cells present in a sample of milk from the mammary gland one can determine the likelihood of mastitis even though all other visible signs of inflammation are absent. Moreover, somatic cell counts on milk samples from individual goats can be performed with reasonable accuracy using the CMT (Shearer and Harris, 1992).

In the present investigation, since study was conducted on female lactating goats showing clinical mastitis and other signs of contagious agalactia. So, milk samples of 145 goats other than showing signs of clinical mastitis were subjected to CMT. Results revealed that out of 145, 67 goats showed CMT score of +1, 47 goats showed CMT score of +2 and 31 goats showed +3 score of CMT. Thus, animals having +2 and +3 scores i.e. 78 out of 145 goats were suffering from sub clinical mastitis and further included in the study. The score of CMT depicts the reaction of reagent with milk in terms of gel formation. The results of present study was in accordance with Shearer and Harris (1992) who stated that CMT reagent reacts with genetic material of somatic cells present in milk to form a gel and in general, milk from non-infected glands yield a negative (0), trace or >1 reaction. Scores of >2 or >3 are indicative of mastitis. Contreras et al. (1996) also stated that CMT score of 2 and 3 discriminated well between infected and uninfected gland in goats.

Relatively higher CMT score of goat milk for subclinical mastitis as compared to that of cow may be due to the reason that normal goat milk has a higher cell count than normal milk from cows. The probable reason is that the white blood cells in milk, together with a relatively small number of epithelial cells from milk-secreting tissues, are known as somatic cells. These cells are an important part of the goat’s natural defense mechanism. When
udder tissue is injured or becomes infected, significant numbers of white blood cells accumulate in the milk. The higher cell count of goat milk is in part caused by an increase in rate of sloughing of epithelial cells which synthesize and secrete the milk components and the presence of cytoplasmic masses which occur as a consequence of the apocrine secretory process (Shearer and Harris, 1992).

**Seroprevalence of mycoplasmosis**

In the present investigation, the overall seroprevalence of mycoplasmosis during the study period i.e. from April 2014 to March 2015 was found to be 9.50 per cent (67 out of 705 goats) in lactating goats and 31.16 per cent among mastitic goats (67 out of 215 mastitic goats) by using SAT. Ghosh *et al.* (1989) reported 9-63 per cent of seroprevalence for mycoplasmal antibodies in goats in different districts of Tripura state of India whereas, Srivastava and Singh (2000) observed a lower prevalence of 4.97 per cent of mycoplasma antibodies in goats of Uttar Pradesh. Similarly, Ramdev *et al.* (2008) also recorded a lower seroprevalence i.e. 4.44 per cent in sheep and 5.02 per cent in goats of Himachal Pradesh by using agglutination test. However, Hadush *et al.* (2009) recorded comparatively higher seroprevalence of 32.68 per cent in goats positive for mycoplasma antibodies using complement fixation test in Ethiopia.

The results of present study indicated the presence of mycoplasmosis in the goats in and around areas of Jabalpur, although there is variation with the findings of previous workers. In the present study, preliminary screening for mycoplasmosis was done with the help of serological method as they are very useful in supporting the diagnosis based on laboratory examination and are of value particularly in epidemiological investigation (Madanat *et al.*, 2001). The serological test employed in this study was slide agglutination test which is simple, easy to perform, less time consuming and require less technical knowledge. Therefore, SAT can be used effectively for diagnosis and monitoring of large number of animals for mycoplasmosis (Kashoo *et al.*, 2011). However, the variation in the prevalence rates of mycoplasmosis might be attributed to the fact that the diagnostic tests varied between the different studies previously conducted. Moreover, there may be difference in managerial conditions, climate, study design and screening methods used.
Prevalence of contagious agalactia

In the present study, the overall prevalence of contagious agalactia was reported as 4.39 per cent in lactating goats. However, the prevalence among the goats suffering from mastitis was 14.42 per cent i.e. 31 out of 215 mastitic goats. The prevalence during the study period was recorded by identifying the presence of Mycoplasma agalactiae in the milk samples. Similarly, Damass (1983) observed the presence of Mycoplasma agalactiae in udder secretion from a goat with mastitis indicating active infection. Although a higher prevalence i.e. 8.8 per cent was reported by Deniz (1996) in Spain using an indirect ELISA technique, however, 11 per cent prevalence was reported in sheep and goats in the Jordan by Zendulkova et al. (2004) and 7.9 per cent in bulk-tank goat milk samples by Contreras et al. (2008). However, Bidhendi et al. (2011) reported a prevalence of 5.5 per cent of Mycoplasma agalactiae in sheep and goat milk samples in Kordestan province, Iran.

The results of present study can be supported by the previous studies conducted throughout the world, although prior to this investigation no such study was conducted in lactating goats in Madhya Pradesh. The possible reason is that the isolation and identification of mycoplasma is difficult and culture of mycoplasmas is not routinely carried out in most of the diagnostic laboratories. As PCR has recently been accepted as a valuable diagnostic method for mycoplasma infections, therefore in the present work, PCR was used for confirmatory diagnosis of contagious agalactia. Thus, the present study indicated the prevalence of contagious agalactia in and around areas of Jabalpur.

Prevalence in organised and unorganised goatry

In the present study, significant variation was noticed in the prevalence with respect to rearing pattern of goats. Seroprevalence of mycoplasmosis in organised goat farms was reported to be higher (19.65%) than in unorganised sector (4.62%). Similarly, the prevalence of contagious agalactia in organised goat farms was reported to be significantly higher (10.92%) than the unorganised sector of goatry (1.26%). Similar findings were reported by Perreau (1984) and Kinde (1994) who reported that intensive
rearing system of goats resulted in hyperacute and acute forms of contagious agalactia. On the other hand, Nicholas et al. (1982) investigated higher prevalence of contagious agalactia in Central Western region of France where intensive husbandry practices of goatry was followed as compared to Alps, where traditional system of rearing was followed.

The results are in accordance with the previous studies indicating a higher prevalence in organized sector as compared to unorganized sector of goatry. It might be due to the reason that contagious agalactia is a highly contagious disease which spreads by ingestion of feed, water or milk contaminated with infected milk, urine, faeces, nasal, ocular and genital discharges. So, when animals are reared under intensive system, they come in close contact with each other resulting in development of clinical form of infection while, traditional extensive system of rearing resulted only in sporadic cases of the disease (Bergonier et al., 1997).

Age wise prevalence

The age wise seroprevalence of mycoplasmosis revealed a non-significant variation among various groups. Although, highest prevalence (13.12%) was observed in the goats of above 4 years of age followed by 9.34 per cent prevalence in goats of 3 to 4 years of age and lowest prevalence (7.42%) was observed in goats of 2 to 3 years of age. Similarly, the age wise prevalence of contagious agalactia also revealed a non-significant variation among various age groups with highest prevalence of 6.87 per cent in goats of above 4 years age group followed by 4.49 per cent in goats of 3 to 4 years of age group and lowest prevalence i.e. 2.73 per cent in goats of 2 to 3 years of age group.

Although scanty literature is available in regard to age wise prevalence of contagious agalactia but Egwu et al. (2001) conducted a study to investigate the current status of intramammary mycoplasmosis in caprine udders in Nigeria and reported that acute and chronic mastitis were more commonly observed in goats between 1 and 3 years of age. On the contrary, Bergonier et al., (1997) reported that experimental inoculation resulted in more severe disease in young animals than adult goats.
Direct association of age with the prevalence of contagious agalactia in the present study might be attributed to the fact that female goats in the age group of 2 to 3 years are not routinely exposed to causal agent, since most of them are in their first lactation (Egwu et al., 2001). Lactation may facilitate multiplication of mycoplasmas and their clinical manifestation in the udder. Hence, subsequent kidding and lactation has been shown to increase the chances of infection (Gross et al., 1978).

**Breed wise prevalence**

Analysis of data of prevalence with respect to breed showed significant variation among various breeds of goats. Seroprevalence study of mycoplasmosis in lactating goats revealed a highest prevalence (34.69%) in Barbari breed followed by 21.43 per cent in Black Bengal breed, 17.48 per cent in Sirohi breed, 9.09 per cent in Jamunapari breed and lowest (2.77%) prevalence in non-descript breed of goats.

The breed wise prevalence study of contagious agalactia in lactating goats revealed a highest prevalence of 12.24 per cent in Barbari breed followed by prevalence of 11.19 per cent in Sirohi breed, 7.14 per cent in Black Bengal breed, 3.41 per cent in Jamunapari breed and lowest prevalence of 1.01 per cent in non-descript breed of goats.

As *Mycoplasma agalactia* is considered as highly virulent agent of contagious agalactia for which the variability between individuals or breeds is of little significance. Moreover, Bergonier et al., (1997) have reported that firm conclusions cannot be drawn regarding the variations in susceptibility attributable to breed. The variability in breed wise prevalence might be due to the variability in the number of samples examined in each category. However, comparatively lower prevalence was observed in the breeds of goats i.e. Jamunapari and non-descript breeds which were reared in unorganized sector. So, whenever there is an outbreak, it often spreads between flocks or herds of different breeds (Perreau, 1984).

**Association with mastitis status**

The results of present study revealed presence of contagious agalactia in mastitic goats. However, the association of mycoplasmosis and contagious agalactia with mastitis status i.e. clinical as well as subclinical
mastitis in lactating goats was found to be non significant. The results of present study revealed that among the goats suffering from clinical mastitis, 32.12 per cent were suffering from mycoplasmosis whereas, among sub clinical mastitic cases, 29.49 per cent goats were reported to be suffering from mycoplasmosis. Similar observations were noticed in contagious agalactia where among cases of clinical mastitis, 15.33 per cent goats have suffered from contagious agalactia and among cases of sub clinical mastitis, 12.82 per cent goats were reported to be suffered from the disease.

A few scientists have studied the association of intramammary mycoplasmosis and contagious agalactia with mastitis. DaMassa et al. (1992) stated that clinical form of contagious agalactia result in general malaise, fever and mastitis that lead to decreased milk yield and agalactia. Similarly, Egwu et al. (2001) isolated five mycoplasma spp. and acholeplasma spp. from mastitic cases in goats. They further reported that significantly higher prevalence was observed in udders with mastitis than in apparently normal healthy goats. Similar findings were reported by Madanat et al. (2001) who observed that 45 per cent of the CA affected goats had mastitis.

In the present study, a non-significant difference in the prevalence was observed in cases of clinical mastitis as compared to sub clinical mastitis in goats. Previously no such study was conducted to know the prevalence of mycoplasmas in mastitic and sub-clinical mastitic caprine udders in this region. Therefore, it is likely that some of the previously diagnosed cases of caprine mastitis may have been associated with mycoplasmas. Thus present study indicated *Mycoplasma agalactiae* as one of the prevalent cause of caprine mastitis.

**Detection of *Mycoplasma agalactiae* in goats using polymerase chain reaction**

Contagious agalactia may be caused by four different mycoplasma species. However, *Mycoplasma agalactiae* is the main causal organism of contagious agalactia of goats (Nicholas, 1995). In the present work, milk samples collected from female lactating goats suspected of contagious agalactia were subjected to PCR test, a specific 176 bp bands obtained from the DNA amplification of *Mycoplasma agalactiae* using primers ma-mp 1F and ma-mp 1R were observed.
Use of PCR in confirmatory diagnosis of contagious agalactia is supported by work of many scientists (Razin, 1994 and Laurence et al., 1995) who stated that PCR is a valuable method for diagnosis of mycoplasma infections. Similarly, Tola et al. (1997) extracted DNA from sheep milk to use for polymerase chain reaction (PCR) for diagnosis of *Mycoplasma agalactiae* and compared it with traditional culture techniques. The results revealed that by PCR 175 positives were detected while, by culture only 153 were detected positive. So, the PCR was reported to be much faster than culture, it has also reduced the time required for diagnosis. However, a comparative study between culture characteristics, immunoperoxidase test and PCR was conducted by Azevedo et al. (2006) to isolate *Mycoplasma agalactiae* in Brazil. Results suggested that PCR was faster than the traditional tests and it can be used as confirmatory test in the diagnosis of contagious agalactia.

Other scientists (Kizil et al., 2007; Zendulkova et al., 2007; Amores et al., 2010; Bidhendi et al., 2011 and Kheirkhah et al. 2011) also used PCR for identification of *M. agalactiae* from the milk samples collected from suspected goats and reported that PCR can be used as trusty and supersede test in detection of *Mycoplasma agalactiae* from affected goats and among different collecting sites, milk samples are suitable for PCR detection.

The results of present study of detection of *Mycoplasma agalactiae* by using PCR were promising and correlate well with the results of previous studies. In the present investigation, only PCR was adopted for identification of *Mycoplasma agalactiae* in milk samples because it has provided a rapid and early diagnosis when performed in clinical samples. Thus, it enabled to carry out early therapeutic measures when results were positive. Moreover, mycoplasmas are very fastidious pathogens (Srivastava et al., 2010) and isolation of mycoplasmas is considered to be one of the most difficult tasks for diagnostic laboratories due to their inability to grow easily in laboratory medium in spite of the great improvement in medium formulations (Al- Momani et al., 2006). Additionally, animals with mycoplasma mastitis may shed the pathogen intermittently and shedding of Mycoplasma spp. into milk
may be below the threshold of detection by standard culture methods (Biddle et al., 2003). PCR is a newer technique based on molecular biology. McAuliffe et al. (2003) stated that in near future molecular technology will be used not only to identify mycoplasma species, but also to detect them without the need of culture. PCR has the advantage of easy use, rapid availability of results and is more suited for processing large number of specimens (Kizil et al., 2007).

Evolving the suitable therapy for caprine contagious agalactia

Clinical parameters

In the present study, among the clinical parameters significantly higher temperature, pulse rate and respiration rate were observed in the goats as compared to healthy ones on day 0 before treatment. Following treatment with different drugs in different groups, all these parameters approached to normal and were essentially similar to that of healthy goats on days 7 and 14 post treatment. However, the improvement in pulse rate and respiration rate were significantly higher in animals of group T4 whereas, improvement in temperature in all the groups was essentially similar.

The literature on the study of clinical parameters in contagious agalactia is meager. Although, Madanat et al. (2001) stated the presence of brief febrile syndrome due to mycoplasmaemia in infected animals. However, few scientists (Kizil et al., 2007 and Macun et al., 2010) reported transient fever, rise in pulse and respiration rate along with other symptoms in goats infected with Mycoplasma agalactiae and observed that after treatment these parameters were reduced to normal values.

The results of present study are in correlation with that of previous workers. Raised clinical parameters in ailing animals are an indication of ongoing infection with other signs specific for contagious agalactia (Macun et al., 2010) and improvement after treatment showed the efficacy of drugs for that disease. However, treatment with tylosin along with tocopherol and sodium selenite in the animals of group T4 proved to be most efficacious in terms of improvement in clinical parameters.
Clinical score

Clinical score can be used as indicators of health. However, such systems are based on subjective judgement. Some of the scoring systems have been developed for other diseases but no traceable literature is available in regard to caprine contagious agalactia. Although, according to Radostits et al. (2010), the efficacy of treatment in clinical mastitis can be judged by improvement in three categories viz. abnormal milk, abnormal gland and abnormal animal behavior. As in the present work, the therapeutic study was conducted in confirmed cases of contagious agalactia in female lactating goats with mastitis as one of the clinical sign. So, for numerical assessment of the response of therapy against contagious agalactia in goats and on the basis of three categories stated by Radostits et al. (2010), a clinical score card was formulated for the first time. On the basis of clinical score card, the animals were grouped as healthy (score 0), mild illness (score 1-3), moderate illness (score 4-6) and severe illness (score 7-9).

In the present study, in group T1, clinical scores of all the six goats showed severe illness on day 0 before treatment. On day 7 post treatment, three goats recovered completely and remaining three showed only mild illness. On day 14 post treatment, five goats showed complete recovery but one goat showed mild illness. In group T2, all six goats showed severe illness on day 0 before treatment. On fourth day of treatment, one goat recovered completely, two goats showed moderate illness. On fifth day of treatment, only two goats showed mild illness and remaining four goats recovered completely. On days 7 and 14 post treatment, all goats showed complete recovery. In group T3, five goats showed severe illness and one was moderately ill on day 0 before treatment. On fifth day of treatment, four goats showed mild illness and remaining two goats recovered completely. On day 7 post treatment, five goats showed complete recovery but one still showed mild illness. However, on day 14 post treatment, all the six goats recovered completely. In group T4, five goats showed severe illness and one showed moderate illness on day 0 before treatment. On third day of treatment, two goats showed only mild illness and recovered completely on fourth day of treatment. On fifth day of treatment, all goats recovered completely.
A gradual recovery was noticed in all the goats but fastest being in the goats of group T4 i.e. receiving treatment with tylosin along with tocopherol and sodium selenite. The second most efficacious treatment group in terms of clinical recovery was of goats receiving treatment with tylosin alone followed by goats receiving treatment with combination of oxytetracycline, tocopherol and sodium selenite. The treatment group T1 i.e. oxytetracycline alone was comparatively least efficacious.

The results of present study were in accordance with that of previous studies (Bergonier et al., 1997 and Azevedo et al., 2006). There was a gradual improvement in terms of clinical picture of the disease and no death was reported in goats during the study period. Evidently it indicated strong antimicrobial activity of the drugs and the therapeutic regimen was effective in alleviating the clinical signs.

**Polymerase chain reaction**

PCR proved to be a rapid and sensitive method for the detection of mycoplasmas (Amores et al., 2010). In the present study, PCR was performed for confirmation of *Mycoplasma agalactiae* in milk samples of all the 24 goats on day 0 pre-treatment and evaluation of different therapies was done by performing PCR on days 7 and 14 post treatment.

In group T1, milk samples of all six goats were subjected to PCR test on day 0 pre-treatment. A specific 176 bp band was obtained from DNA amplification of *Mycoplasma agalactiae* using specific primers confirming the presence of causative organism in the milk samples. After receiving treatment when the milk samples were again subjected to PCR on day 7 post treatment, two goats showed the presence of organism but on day 14 post treatment no organism was detected in PCR test performed in the milk samples of goats. This showed complete recovery on day 14 post treatment. However, in goats of group T2, T3 and T4, PCR results on day 0 pre-treatment revealed 176 bp bands specific for primers ma-mp 1F and ma-mp 1R of *Mycoplasma agalactiae*. After being subjected to treatments as per the therapeutic regimen, PCR tests on days 7 and 14 post treatment revealed no specific bands indicating complete recovery from seventh day onwards.
In the present study, the results of clinical parameters, clinical score and PCR indicated that among four different therapeutic regimens, treatment with combination of tylosin, tocopherol and sodium selenite was found to be most efficacious in field cases of CA in goats followed by the treatment with tylosin alone, combination of oxytetracycline, tocopherol and sodium selenite and least effective being oxytetracycline alone.

Similar drugs were used by Ball et al. (1987) for therapeutic trial in ewes experimentally infected with Mycoplasma californicum and reported that three days treatment with both oxytetracycline and tylosin successfully eliminated the infection. However, Mackie et al. (2000) recorded mastitis outbreak due to mycoplasma like Mycoplasma californicum and Mycoplasma canadense in Ireland in cows. They also reported that treatment with combination of intramammary chlortetracycline (426 mg/dose) and intramuscular tylosin (10 mg/kg) after each milking for three consecutive days rapidly eliminated the implicated organisms and prevented the development of chronic infection. Moreover, some more drugs i.e. erythromycin, spiramycin, lincomycin and enrofloxacin along with tylosin were used by Loria et al. (2003) in the treatment of CA and favourable results were obtained.

Similar to the present study, Azevedo et al. (2006) used 20 mg/kg tylosin daily for five days for the treatment in an outbreak of mycoplasmosis in goats and reported that most of the animals recovered although some were euthanized and few died spontaneously. Kizil et al. (2007) conducted a study in goats naturally infected with Mycoplasma agalactiae and observed a significant increase of MDA concentrations, significant reductions of GSH, β-carotene, vitamin C, vitamin E concentrations and of GSHPx activity as compared to the controls. The results clearly demonstrated the occurrence of an oxidative stress leading to deficiency of enzymatic and non-enzymatic antioxidant systems during this infection.

The results of present investigation were in accordance with the previous studies. The present therapeutic regimen included antibiotic like oxytetracycline and tylosin. Both proved to be efficacious in alleviating the mycoplasma infection. This might be due to the fact that mycoplasma the causative agent of contagious agalactia, lacks cell wall and thus is inherently
refractory to certain groups of antibiotics. However, the mechanism of action of both tylosin and oxytetracycline is by inhibiting protein synthesis, so they work well for mycoplasmas. The higher dose rate of drugs and five day period of treatment in the present study was selected because of the previous lack of success of two days treatment (Mackie et al., 1982) and also due to the fact that results of treatment may be poor or ineffective against mycoplasmas if the therapeutic dose is not well defined and the antibiotics is not administered for a sufficiently long period (Madanat et al. 2001).

In the present investigation, tylosin with the combination of tocopherol and sodium selenite was found to be most efficacious. Addition of antioxidants in the therapy might have increased the efficacy. The probable reason is that, many potentially toxic reactive oxygen species are generated through normal oxidative metabolism and the body has adapted by developing a complex system of protective antioxidants. Oxidative stress is defined as an alteration in the steady-state balance between oxidant and antioxidant agents in the cells. Under more stressful conditions such as mastitis which is one of the most principal clinical symptoms in agalactia, hydroxy radicals are released by infiltrated neutrophils causing mammary cell injury and abnormal radical production leads to an oxidative stress. Hence, it can be useful to add the antioxidant vitamins to the classical treatment procedures to get rid of the disease (Kizil et al., 2007).
6. SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

6.1 Summary

Contagious agalactia (CA) of goats has been known for about two centuries. It affects all types of stock breeding, both traditional and intensive, throughout the world. Its preferential mammary involvement presents a major health obstacle in the development of goat production. Clinical mycoplasmoses often lacks pathognomonic characteristics and symptoms can be shared by other clinically significant infections. As a consequence, the diagnosis of an acute caprine mycoplasmal infection can be easily misinterpreted. Some animals also reduce the success of treatment being considered healthy based on clinical examination. Thus, the present work was aimed to study the prevalence, molecular detection and to evolve the suitable therapy against contagious agalactia in goats.

In this study, a total of 705 lactating goats belonging to organized and unorganized sectors of in and around areas of Jabalpur were screened between April 2014 to March 2015. From these, 282 goats with signs of clinical mastitis or suspected for mastitis were selected for further study. The confirmation of mastitis in suspected cases was done by California Mastitis test. Seroprevalence of Mycoplasma spp. causing contagious agalactia was done using slide agglutination test and the presence of Mycoplasma agalactiae was confirmed by polymerase chain reaction. The DNA required for PCR was extracted from the milk sample. On confirmatory diagnosis in goats suffering from CA, 24 clinical cases were randomly selected for therapeutic study and were grouped as T1, T2, T3 and T4 having 06 goats in each group. Moreover, 06 apparently healthy goats were selected as healthy control (Group C). The goats in groups T1, T2, T3 and T4 were treated with oxytetracycline @15 mg/kg b.wt, tylosin @ 20 mg/kg b.wt, combination of oxytetracyclines (@15 mg/kg b.wt), tocopherol (@ 2 mg/kg b.wt) and sodium selenite (@ 0.06 mg/kg b.wt), combination of tylosin (@ 20 mg/kg b.wt), tocopherol (@ 2 mg/kg b.wt) and sodium selenite (@ 0.06 mg/kg b.wt), respectively, I/M once a day for 05 days. In addition, symptomatic and
supportive therapy was done by administration of fluids, electrolytes, anti-inflammatory and antipyretic drugs as per need. The response of therapeutic study was evaluated on the basis of clinical condition which was assessed by score card, clinical parameters (temperature, pulse, respiration), CMT and PCR test on day 0 (pre-treatment), days 7 and 14 (post treatment).

Out of 705 goats screened, on the basis of clinical symptoms 282 goats were found suspected for contagious agalactia. From these, 137 goats showed mastitis, 52 showed respiratory signs, 33 showed ocular signs, 9 had a history of abortion and 51 goats showed mixed signs. The overall seroprevalence of mycoplasmosis in lactating goats using slide agglutination test was 9.50 per cent while, among mastitic cases it was 31.16 per cent. Seroprevalence of mycoplasmosis in organized goatry was significantly higher (19.65%) as compared to unorganized goatry (4.62%). A non-significant variation (p>0.05) among age wise seroprevalence of mycoplasmosis was noticed. However, it was highest (13.12 %) in females above 4 years of age followed by 3 to 4 years (9.34 %) and 2 to 3 years (7.42 %) of age. The breed wise seroprevalence of mycoplasmosis showed significant variation (p<0.05). Highest prevalence of 34.69 per cent in Barbari breed followed by prevalence of 21.43 per cent in Black Bengal, 17.48 per cent in Sirohi breed, 9.09 per cent in Jamunapari and lowest 2.77 per cent prevalence in non-descript breed of goats were observed. Out of 137 cases of clinical mastitis, 32.12 per cent goats was reported to be suffering from mycoplasmosis whereas, out of 78 goats suffering from sub clinical mastitis, 29.49 per cent goats were reported to be suffering from mycoplasmosis. However, the association of mycoplasmosis with status of mastitis in lactating goats was found to be non significant (p>0.05).

Confirmatory diagnosis of suspected cases of CA was done by PCR using specific primers of ma-mp 81 DNA gene of *Mycoplasma agalactiae*. The PCR product positive for *Mycoplasma agalactiae* yielded a band of 176bp when visualized in UV transiluminator gel documentation system after amplification and electrophoresis in 1.5% agarose.
The overall prevalence of CA in lactating goats was 4.39 per cent. The prevalence of CA was significantly higher in organized sector (10.92%) as compared to unorganized sector (1.26%) of goatry. The age wise prevalence of CA reported a non-significant variation (p>0.05) among various groups. Although a marginally higher prevalence was noticed in goats of above 4 years age group (6.87%) followed by 4.49 per cent in goats of 3 to 4 years of age group and lowest prevalence (2.73%) in goats of 2 to 3 years of age group. The prevalence of CA showed significant variation among various breeds. Barbari showed highest prevalence (12.24%), followed by 11.19 per cent in Sirohi, 7.14 per cent in Black Bengal, 3.41 per cent in Jamunapari and lowest prevalence in nondescript breeds (1.01%). The association of contagious agalactia with clinical and sub clinical mastitis in lactating goats was found to be non significant (p>0.05).

The response of therapeutic study revealed significant improvement in temperature, pulse and respiration in all treatment groups. On the basis of score card, goats of group T4 showed complete recovery on day 5 post treatment. Goats of group T2 and T3 showed complete recovery on day 14 while in group T1 five goats completely recovered on day 14 and one showed only mild illness. However, two goats in group T1 showed positive PCR results on day 7 whereas, all the goats under treatment were found negative for PCR on day 14. However, clinical recovery was noticed in all the goats under different therapeutic regimen but the combination of tylosin, tocopherol and sodium selenite was found to be most efficacious in the treatment of CA followed by tylosin alone, combination of oxytetracycline, tocopherol and sodium selenite and lastly oxytetracycline alone.
6.2 Conclusions

1. The overall seroprevalence of mycoplasmosis was 9.50 per cent in lactating goats and 31.16 per cent in mastitic goats in and nearby areas of Jabalpur. Higher seroprevalence was recorded in organized sector (19.65%) as compared to unorganized sector (4.62%).

2. *Mycoplasma agalactiae* was detected and confirmed as causative agent of contagious agalactia using PCR.

3. The overall prevalence of contagious agalactia in lactating goats was 4.39 per cent and among the goats suffering from mastitis was observed 14.42 per cent in and around Jabalpur. The association of contagious agalactia with clinical and subclinical mastitis was found to be non significant.

4. Among the different therapeutic regimens, combination of tylosin, tocopherol and sodium selenite was found to be most promising in the treatment of contagious agalactia.
6.3 Suggestions for further work

1. Detection of other Mycoplasma spp. responsible for contagious agalactia may be carried out.

2. Other fluids and secretions viz. synovial fluid, vaginal and nasal secretions may be tested for presence of *Mycoplasma agalactiae*.

3. Intensive research on molecular epidemiology is required to fully understand risk factors associated with the disease.

4. The clinical score card developed as new approach for evaluation of status of recovery under therapy should be accorded more importance.
7. REFERENCES


