SEROPREVALENCE OF LEPTOSPIROSIS IN GOATS IN TAMIL NADU

G. Balakrishnan
Leptospirosis Research Laboratory
Centre for Animal Health Studies
Tamil Nadu Veterinary and Animal Sciences University, Chennai - 600 051

ABSTRACT

A total of 318 goat serum samples were collected from different breeds of goats which suffered from abortion, repeat breeding, jaundice, haemorrhagic mastitis and also included apparently healthy goats belonging to different parts of Tamil Nadu during the period between 2005 and 2007. The samples were screened for the presence of antileptospiral antibodies by Microscopic Agglutination Test (MAT). Of which, 142 (44.65%) samples were found to have antileptospiral antibodies. The seropositivity of leptospirosis in goats with clinical symptoms was found to be 57.14 per cent with the involvement of 5 serovars namely hardjo, hebdomadis, australis icterohaemorrhagiae and pomona. The seropositivity in clinically suspected goats (57.14 per cent) was higher than that of apparently healthy goats (42.75 per cent). Among the goats the seropositivity was found to be more in Non-Descript breeds (72.92 per cent) and Kanni goats were least susceptible to leptospirosis (16.48 per cent). There was no variation in the seroprevalence rate of leptospirosis during the study period. The seroprevalence of leptospirosis was found to be more during monsoon and premonsoon periods.

Key words: Leptospirosis, Goats, Tamil Nadu

INTRODUCTION

Goats have been an integral component of rural farming to landless and marginal farmers. Goats are reared mainly by grazing, thus making them highly vulnerable to many infectious diseases including leptospirosis. Although goats are known to be less susceptible to leptospirosis than other domestic species like cattle (Leon, 1987), the disease in goats occurs in both acute and chronic forms with common clinical signs of pyrexia, anorexia, depression, jaundice and haemorrhagic syndromes (Faine, et al., 2000). The chronic form results in impaired fertility, neonatal deaths, abortions and decreased milk production leading to heavy economic losses (Cunha, et al., 1999 and Lilienbaum, et al., 2007). Very often chronic form of leptospirosis goes unnoticed, thus serosurveillance is essential. The involvement of leptospiral serovars with different clinical manifestations of leptospirosis is not common in literature. The present paper reports the seroprevalence of leptospirosis in different breeds of goat with different clinical manifestations encountered during 2005 - 2007 in Tamil Nadu state of India.

MATERIALS AND METHODS

Collection of samples

A total of 318 serum samples from goats of different breeds namely Barbari (70), Beetal (37),
Tellichery (12), Jamunapari (4), Boer (8), Kanni (91) and Non-Descript (96) goats which included clinically ill (abortion, repeat breeding, jaundice and haemorrhagic mastitis) and apparently healthy, were collected from different parts of Tamil Nadu during 2005-2007. All the serum samples were inactivated at 56°C for 30 minutes and stored at -20°C until tested by Microscopic Agglutination Test (MAT).

Microscopic agglutination test (MAT)

MAT was performed as described by Office International des Epizooties (OIE), 2004 as described below.

Preparation of antigens for MAT

The leptospiral reference strains representing 12 different serogroups (australis, ballum, canicola, grippotyphosa, hardjo, hebdomadis, icterohaemorrhagiae, javanica, pomona, pyrogenes, autumnalis and tarassovi) received from National Reference Laboratory, Indian Council of Medical Research, Andaman and Nicobar Islands, India and maintained in this laboratory were used for this study.

All these 12 strains were grown in Ellinghausen McCullough - Johnson - Harris (EMJH) medium containing 10% enrichment (Difco) and incubated at 29±1°C. Five to eight days liquid culture of live Leptospires containing density of $2 \times 10^8$ leptospires per ml was used as antigen. Precaution was taken to avoid clumps of leptospires for the preparation of antigen.

Single serum dilution method

This test was conducted as described by OIE (2004) in 96 well 'U' bottom titration plates (M/s. Laxbro, India). Serum dilutions were made in deep well (96 well) dilution plates (M/s. Laxbro, India). A 1:50 dilution of each serum (25 µl) was incubated with 25 µl (at the concentration of 2 x $10^8$ organisms per ml) of antigen at 37°C for 2 hours. A drop (5 µl) of mixture (final dilution of 1:100) was placed on a grease-free slide and the wet preparation without cover slip was screened using 20X objective of the dark field microscope (M/s. Nikon, 200E, Japan) for the presence of agglutination and/or reduction in number of organism in comparison with the respective antigen control. A 50 per cent reduction in the number of free leptospires in the test sample comparable with the respective antigen control was considered positive with or without agglutination.

Quantitative assay

Quantitative assay was carried out in 'U' bottom microtitration plates against the reacting serovars of leptospires. All the 96 wells were charged with 25 µl PBS. In the first well of each row 25 µl of 1:25 diluted (Initially diluted in PBS in a separate deep well dilution plates) serum samples were added and mixed well. Then an equal volume (25 µl) was serially transferred upto 9 wells. From the 9th well 25 µl was discarded. A constant volume of 25 µl of the respective Leptospiral antigen ($2 \times 10^8$ per ml) was added in each row and incubated at 37°C for 2 hours. All final dilution mixtures (50, 100, 200, 400, 800, 1600, 3200, 6400 and 12800) were observed under dark field microscope and the results were recorded. A titre of 1 in 100 and above was considered as positive in this study.

RESULTS AND DISCUSSION

The over all leptospiral seropositivity was found to be 44.65 per cent (142/318) among goats (Table - 1). Among the serovars prevalent, australis (42.78 per cent) was predominant followed by canicola (8.96 per cent), poles (8.46 per cent), hebdomadis (5.97 per cent), javanica (5.47 per cent), pyrogenes (4.98 per cent), tarassovi (4.98 per cent), autumnalis (4.48 per cent), ballum (4.48 per cent), hardjo (3.48 per cent), grippotyphosa (3.0 per cent) and icterohaemorrhagiae (3.0 per cent).
Out of 318 serum samples, 42 samples were collected from goats with the history of abortion (20), repeat breeding (11), jaundice (6) and haemorrhagic mastitis (5) (Table - 2). Of the 42 serum samples, 24 (57.14 per cent) samples were found positive for leptospirosis. Out of 24 positive samples from clinical cases, 12 samples (50.0 per cent) were from abortion, 6 (25.0 per cent) from repeat breeding, 4 (16.67 per cent) from jaundice and 2 (8.33 per cent) from haemorrhagic mastitis (Table -1). Serovars involved in abortion included hardjo (4), pomona (3), australis (3) and hebdomadis (2). Serovars involved in repeat breeding included hebdomadis (3), hardjo (2) and australis (1). Similarly serovars involved with jaundice included icterohaemorrhagiae (3) and australis (1). Whereas serovars involved in haemorrhagic mastitis included hardjo (1) and hebdomadis (1).

Out of 318 serum samples, though 276 samples were from apparently healthy animals 118 animals showed seropositivity for leptospirosis. Eleven different serovars were found involved which included australis (81), autumnalis (9), ballum (9), canicola (18), grippotyphosa (6), hebdomadis (6), icterohaemorrhagiae (3), javanica (8), pomona (17), pyrogenes (10) and tarassovi (10).

Among the different breeds, seropositivity was found to be more in non-descript breeds (72.92 per cent) followed by Tellichery (66.67 per cent), Boer (50.00 per cent), Beetal (29.73 per cent), Barbari (48.57 per cent) and Kanni (16.48 per cent) respectively. All the 4 serum samples collected from Jamunapari goats were found to be negative.

In non descriptive breeds, 70 animals were seropositive, out of 96 animal screened. Eight animals were seropositive with clinical conditions like abortion (4), jaundice (2) and repeat breeding (2).

Only 12 samples from Tellichery and 8 samples from Boer goats were collected and results are presented in Table - 2. Out of 70 samples screened from Barbari goats, seropositivity to leptospirosis was associated with abortion (2), repeat breeding (2), jaundice (2) and haemorrhagic mastitis (1) from clinically suspected goats. Apparently healthy Barbari goats were also seropositive (27/60). Out of 37 Beetal goats screened, abortion (1) and repeat breeder (1) were observed in animals having antibodies to Leptospira. Apparently healthy goats (9) were also seropositive. Among 91 Kanni goats screened, only 15 goats suffered from clinical condition and leptospirosis was associated with abortion (3). Apparently healthy goats (12) were found positive to leptospirosis. All the 4 Jamunapari goats were found negative to leptospirosis. Among the 8 Boer goats, 2 suffered from abortion while 6 of them were apparently healthy goats, only 4 goats were found positive (abortion -1 and apparently healthy goats -3).

The leptospiral seropositivity was found to be 42.47 per cent in males and 33.61 per cent in females.

The prevalence of leptospirosis among goats of Tamil Nadu was 75.81% during June - September followed by 53.85% during October - December, 42.25% during January - March and 27.72% during April - May.

The year wise leptospiral seropositivities were found to be 45.45%, 38.89% and 44.59% during the year 2005, 2006 and 2007 respectively.

Leptospirosis is a zoonotic disease. A variety of wild and domestic animals can act as reservoir hosts for one or more serotypes and can shed the organisms in their urine for months or years after being infected. Animals may be infected when come into contact with leptospires through activities such as swimming, drinking or walking through contaminated water, soil or mud. Study of seroprevalence is important to implement control measures.
The leptospiral seropositivity was found to be from 38.89 per cent to 45.45 per cent among goats during 3 years of study and similar percentage of seropositivity (47.40 per cent) has been recorded earlier (Ratnam et al., 1994). Among the serovars, australis (42.78 per cent) was predominantly distributed among goats in Tamil Nadu followed by canicola (8.96 per cent), pomona (8.46 per cent), hebdomadis (5.97 per cent), javanica (5.47 per cent), pyrogenes (4.98 per cent), tarassovi (4.98 per cent), autumnalis (3.48 per cent), ballum (4.48 per cent), hardjo (3.48 per cent) and grippotyphosa (3.0 per cent). The prevalence of australis, autumnalis, pyrogenes, pomona, ballum and hardjo was earlier reported in Tamil Nadu (Ratnam et al., 1994).

The seropositivity of leptospirosis in clinically affected goats was found to be 57.14 per cent with the involvement of 5 serovars namely hardjo, hebdomadis, australis, icterohaemorrhagiae and pomona.

Out of clinically affected animals (42) only 57.14 per cent (24) were seropositive to leptospirosis. Among Seropositive and clinically affected animals, 50 per cent (12) had abortion, 25 per cent (6) repeat breeding, 16 per cent (4) jaundice and 8 per cent (2) haemorrhagic mastitis. From the results of seropositivity it could be observed that more abortion was due to hardjo (33 per cent) followed by australis (25 per cent), pomona (25 percent) and hebdomadis (16 per cent). Repeat breeding was due to hebdomadis (50 per cent) followed by hardjo (33 per cent) and australis (25 per cent). Jaundice could be attributed to Icterohaemorrhagiae (75 per cent) followed by australis (1 per cent). Hemorrhagic mastitis was due to hardjo and hebdomadis each (50 per cent).

It could be observed that australis serovar was involved in abortion, repeat breeding and jaundice. Hardjo and hebdomadis were involved in abortion, repeat breeding and haemorrhagic mastitis. Pomona was involved only in cases of abortion. Hence it could be concluded that organisms are not condition specific. Further, conclusion could not be arrived based on a limited number of samples screened.

Breed wise study revealed, 72.92 per cent of non-descript goats were seropositive. Clinically affected goats suffered from abortion, repeat breeding and jaundice. Least affected breed was Kanni (16.48 per cent). Although goats are known to be less susceptible to leptospirosis (Leon, 1987) which was also in agreement with the present study and was also true for Kanni goats, the local breed of Tamil Nadu state. Only a few aborted animals were seropositive to leptospirosis. Abortion was seen in all the six breeds except Janunapari. Repeat breeder was seen only in Barbari, Beetal, Tellicherry and Non-Descript. Jaundice was seen in Barbari and Non-Descript whereas Haemorrhagic mastitis was seen in Barbari and Tellicherry. This study highlights the susceptibility of different breeds to leptospirosis.

Male goats were infected with the disease at a higher level than females. Infection of male goats was 42.47 per cent (31 / 73) and in female goats 33.61 per cent (40 / 119). Similar earlier reports are available (Agunloye, 2002). The higher seropositivity in male goats was possibly attributed to high metabolic and sexual activity of male goats and lack of nutritious feed given to male compared to female.

The prevalence of leptospirosis among goats of Tamil Nadu was 75.81% during June - September followed by 53.85 % during October - December, 42.25 % during January - March and 27.72 % during April - May. Leptospirosis outbreaks occurred all the year round but mainly in monsoon and pre monsoon.
seasons, which favour the survival and development of leptospirosis. The results of this study provided some information on the prevalence of leptospirosis among goats in Tamil Nadu state which will be helpful to develop a suitable vaccine to control the disease.

REFERENCES


Lilenbaum, W.; Souza, G.N.; Distow, P.; Moreira, M.C.; Fraguas, S.; Cardoso, V.S.;


Table 1

Seroprevalence of leptospirosis in clinically ailing and apparently healthy goats

<table>
<thead>
<tr>
<th>Clinical Conditions</th>
<th>Total Screened</th>
<th>Total Positive</th>
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<th>2</th>
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<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>Total</th>
</tr>
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<tr>
<td>Abortion</td>
<td>20</td>
<td>12</td>
<td>3</td>
<td>25%</td>
<td></td>
<td></td>
<td>4</td>
<td>33%</td>
<td></td>
<td>1%</td>
<td></td>
<td>3</td>
<td>2%</td>
<td>2%</td>
<td>12</td>
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<tr>
<td>Recent illness</td>
<td>11</td>
<td>6</td>
<td>1</td>
<td>10%</td>
<td></td>
<td></td>
<td>2</td>
<td>33%</td>
<td></td>
<td>1%</td>
<td></td>
<td>1</td>
<td>0%</td>
<td>0%</td>
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<tr>
<td>Jaundice</td>
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<td>4</td>
<td>1</td>
<td>25%</td>
<td></td>
<td></td>
<td>1</td>
<td>25%</td>
<td></td>
<td>1%</td>
<td></td>
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<td>0%</td>
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<td>Hemorrhagic urinaria</td>
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<td></td>
<td></td>
<td>1</td>
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<td>50%</td>
<td></td>
<td>0</td>
<td>0%</td>
<td>0%</td>
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</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>24</td>
<td>5</td>
<td>20%</td>
<td></td>
<td></td>
<td>7</td>
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<td></td>
<td>2%</td>
<td></td>
<td>3</td>
<td>25%</td>
<td>2%</td>
<td>24</td>
</tr>
<tr>
<td>Apparently healthy</td>
<td>206</td>
<td>118</td>
<td>91</td>
<td>44.29%</td>
<td>81</td>
<td>3.90%</td>
<td>54</td>
<td>26.29%</td>
<td>23</td>
<td>11.17%</td>
<td>18</td>
<td>8.72%</td>
<td>11</td>
<td>5.34%</td>
<td>177</td>
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<td>Healthy</td>
<td>318</td>
<td>142</td>
<td>96</td>
<td>4.48%</td>
<td>96</td>
<td>3.08%</td>
<td>86</td>
<td>26.89%</td>
<td>54</td>
<td>17.02%</td>
<td>48</td>
<td>15.22%</td>
<td>48</td>
<td>15.22%</td>
<td>311</td>
</tr>
</tbody>
</table>

Table - 2
Seropositivity of leptospirosis in different breeds of goats associated with different clinical signs

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Goat breeds</th>
<th>Clinically ailing goats</th>
<th>Apparently healthy goats</th>
<th>Total</th>
<th>Percentage</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Abortion</td>
<td>Repeat breeding</td>
<td>Jaundice</td>
<td>Haemorrhagic mastitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. of Positive/Total screened</td>
<td>No. of Positive/Total screened</td>
<td>No. of Positive/Total screened</td>
<td>No. of Positive/Total screened</td>
</tr>
<tr>
<td>1</td>
<td>Barbari</td>
<td>2/3</td>
<td>2/4</td>
<td>2/2</td>
<td>1/1</td>
</tr>
<tr>
<td>2</td>
<td>D-vel</td>
<td>1/2</td>
<td>1/2</td>
<td>0/1</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Tilichery</td>
<td>1/2</td>
<td>1/1</td>
<td>0/1</td>
<td>1/1</td>
</tr>
<tr>
<td>4</td>
<td>Kuni</td>
<td>3/5</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>5</td>
<td>Jumunpuri</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>Bser</td>
<td>1/2</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>Non-Descript</td>
<td>4/6</td>
<td>2/3</td>
<td>2/2</td>
<td>–</td>
</tr>
<tr>
<td>Total in No.</td>
<td>12/20</td>
<td>6/11</td>
<td>4/6</td>
<td>2/5</td>
<td>118/278</td>
</tr>
<tr>
<td>Total in Percentage</td>
<td>60.00</td>
<td>54.55</td>
<td>66.67</td>
<td>40.00</td>
<td>42.75</td>
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