Introduction

Leptospirosis is a zoonotic disease of worldwide distribution and causes heavy economic losses due to abortion, stillbirth, infertility and reduced productivity in livestock including goat (Vamshi Krishna et al., 2012). Diagnosis based on culture technique of *Leptospira* is difficult and fastidious (Limmathurotsakul et al., 2012), therefore, serological tests like microscopic agglutination test (MAT), enzyme-linked immunosorbent assay (ELISA) are more reliable and easy. MAT is highly specific but due to its inherent difficulties the test is restricted to a few reference laboratories (Bourhy et al., 2013).

Recombinant leptospiral lipoprotein rLipL41 antigen based ELISA has been developed for diagnosis of leptospirosis in man and animals. Among various recombinant leptospiral lipoprotein rLipL41 is one of the promising one, used as antigen in ELISA for serodiagnosis of caprine leptospirosis in the present study. The rLipL41 was evaluated as diagnostic antigen for bovine leptospirosis using ELISA against MAT, and found to be 100% sensitive and 85.3% specific (Mariya et al., 2006) and 100% sensitive and 78.8% specific (Srivastava et al., 2006). However, Narendran (2007) used rLipL32 in ELISA and found higher specificity of 94.01% against MAT.

Materials and Methods

Goat serum samples

Altogether 402 goat serum samples (145 diseased and 257 apparently healthy) of five different breeds (Barbari, Beetal, Black Bengal, Jamunapari, Non-descript) were collected from different parts of the country [Bareilly, Mathura, Rampur (Uttar Pradesh), Pantnagar (Uttarakhand), Seikhpura (Punjab)] during October 2008 to March 2009, kept in small aliquots after adding 0.01% sodium azide and stored at -20°C till use. These sera were screened for the presence of leptospiral antibodies.

Microscopic agglutination test

The MAT was performed as per Faine (1982). A total of 10 *Leptospira* serogroups viz., Australis, Autumnalis, Ballum, Canicola, Grippotyphosa, Icterohaemorrhagiae, Javanica, Pomona, Pyrogenes, and Tarassovi maintained at *Leptospira* Laboratory, Division of Bacteriology and Mycology, Indian Veterinary Research Institute, Izatnagar, India were used as antigen. Serum samples showing MAT titre equals to or higher than 1:100 were considered positive.

Indirect enzyme-linked immunosorbent assay (I-ELISA)

The ELISA was done (Flannery et al. 2001) by using...
rLipL41 antigen provided by Leptospira Laboratory, Division of Bacteriology and Mycology, Indian Veterinary Research Institute, Izatnagar, India. The protein content of this antigen was 0.5 mg/ml (Lowry et al., 1951). The optimum concentration of the antigen (100 ng/well), serum dilution (1:100) and rabbit anti-goat IgG HRPO conjugate (1:6000) were attained by checkerboard titration (Kumar et al., 1985). The plates were read at 490 nm in ELISA reader (ECIL, India) along with controls (positive, negative, conjugate and substrate controls). The cut off optical density (OD) value for positive serum sample was taken as equal to or more than two times standard deviation (SD) of OD value of known negative sample.

Evaluation of MAT and ELISA

Relative sensitivity and specificity (McDiarmid and Hellstrom, 1987) of the rLipL41 ELISA for serodiagnosis of caprine leptospirosis were evaluated in comparison to MAT. The concordance percentage and Kappa statistics were also calculated (Thrusfield, 2005).

Results and Discussion

Among outer membrane proteins of Leptospira, LipL41 is an important protein expressed only in pathogenic Leptospira spp. (Matsunga et al., 2003) and has been identified as a sero-diagnostic marker for screening leptospiral infection (Haake et al., 1999). Therefore, recombinant LipL41 protein as an antigen was used in Indirect ELISA for the serodiagnosis of the caprine leptospirosis in this study.

Out of 402 goat serum samples screened, 42 showed evidence of leptospiral antibodies at a titre of 1:100 or more by MAT and 63 by rLipL41 ELISA. Thus, overall seroprevalence rate of leptospirosis among caprine was found to 10.44% and 15.67% by MAT and rLipL41 ELISA, respectively. Several investigators have reported the seroprevalence of leptospirosis in goat on the basis of MAT in India (Natarajaseenivasan and Ratnam, 1997; Agrawal et al., 2005; Vamshi Krishna et al., 2012) and abroad (Moch et al., 1975; Lindsay et al., 1995). In India, the reported seroprevalence of leptospirosis in goat varied from as low as 4.6% (Agrawal et al., 2005) to as high as 75.00% (Natarajaseenivasan and Ratnam, 1997), while in other countries the reported seroprevalence varied from 2.6% (Lindsay et al., 1995) to 47.30 % (Moch et al., 1975). In present study, seroprevalence was found higher in diseased goats (12.41% & 18.62%) than apparently healthy goats (9.34% and 14.00%) by MAT and rLipL41 ELISA, respectively. In MAT, goat serum samples reacted maximum with serovar Icterohaemorrhagiae (23.07%) followed by Canicola (19.23%), Grippotyphosa (19.23%), Autumnalis (11.53%), Javanica (7.69%), Pomona (7.69%). Earlier studies indicated, Icterohaemorrhagiae as a predominant serovar among sheep and goats in Uttar Pradesh (Mukherjee et al., 1962), Icterohaemorrhagiae and Grippotyphosa in goats in Madurai district of Tamilnadu (Sivaseelam et al., 2003), Autumnalis in Pernambuco state of Brazil (Cunha et al., 1999) and Pomona in Spain (Lindsay et al., 1995). The predominance of different Leptospira serovars varies in different geographical areas, and accordingly circulating serovars should be selected for use in vaccines for that region.

Source-wise, the highest seroprevalence of leptospirosis 23.52% and 26.47% by MAT and rLipL41 ELISA, respectively, was found at Sheikhpura village, Punjab (Fig. 1). Most of the Beetal goats of this village had the history of abortion, which may be caused by Leptospira spp. as its association with abortion was
reported (Al-Badrawi et al., 2010). Breed-wise seroprevalence was found to be maximum in Beetal, followed by Black Bengal, Non-descript, Jamunapari and Barbari by both MAT and rLipL41 ELISA (Fig. 2). Age-wise the maximum seroprevalence was among 3-4 years age group (Fig. 3). In a recent study conducted in Brazil (dos Santos et al., 2012), adult goats had three times more risk of acquiring leptospiral infection than younger animals. These risks could be explained by the level of opportunities for contact with older animals as sources of infection. The seroprevalence of caprine leptospirosis was found higher in case of female than male by both MAT and ELISA (Fig. 4). Agunloye et al. (1996) also reported similar findings. In aborted goats, Leptospira can persist for long duration in the uterus and venereal transmission of leptospirosis in these species has also been suggested (Lilenbaum et al., 2008).

In ELISA more goat sera were found positive, with higher serum dilution (1:100) and very low antigen concentration (100 ng/well) indicating its high analytical sensitivity than MAT. Flannery et al. (2001) opined that recombinant protein-based serological test may achieve high sensitivity and specificity because of the high concentration of immuno-reactive antigens used in this assay and the lack of non-specific moieties present in whole-cell preparations. The relative sensitivity of ELISA in the present study was found to be 100% suggesting that it was as effective as MAT in detecting true positive animals. But the specificity of ELISA was 94.17% signifying the inability of the ELISA to detect all MAT negative goats as healthy. The lower relative specificity of rLipL41 ELISA found in this study could be explained on the basis that all those sera turned out to be MAT negative may not actually be negative as only 10 Leptospira serovars were used as antigens. Recombinant LipL41 antigen, which is used in ELISA is genus specific and therefore is more sensitive and specific than MAT (Mariya et al., 2006). The specificity observed in the present study is higher than that reported by Mariya et al. (2006) because they had used only eight Leptospira serovars in MAT. A high concordance percentage (94.77%) was observed between MAT and ELISA, which is also corroborated by a substantial agreement between these two tests by kappa value (0.77). The MAT is inadequate for rapid case identification at early stage of infection besides being complex and unsafe to perform because live cultures of all serovars are required for use as antigens. Whereas, ELISA is safe to conduct and diagnosis can be made during the early phase of the disease (Vijayachari et al., 2001). The rLipL41 protein used as antigenic preparation is highly conserved among more than 200 pathogenic serovars of Leptospira spp. (Haake et al., 2000) and exhibit similar performance regardless of the locally predominant serovar. The bulk amount of the purified rLipL41 antigen could be obtained without handling live Leptospira organisms. Thus, rLipL41 as antigen in ELISA has the potential to become a useful tool for serodiagnosis of leptospirosis.

Goats in India are mostly kept by landless labourers and small farmers in close proximity with their families, therefore goat infected with Leptospira may pose a human health hazard. In present study, a high seroprevalence of the leptospirosis was observed even in apparently healthy goats which indicate the role of goat as a carrier. Therefore, it is essential to adopt control measures and the best method is to use a suitable vaccine in the affected flock incorporating the circulating serovars of Leptospira prevalent in the geographical location.
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References


