Leptospriosis in sheep and its diagnosis

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Abstract

The present study was carried out to screen or detect the leptospires in the migratory flocks of sheep in Karnataka. A total of 60 blood, tissue and urine samples were collected from the migratory sheep flock in the area of Shimogga and Belgaum districts of Karnataka with clinical manifestation. The samples were subjected to screening for leptospirosis by Dark Field Microscopy (DFM), Polymerase chain reaction (PCR), isolation and identification studies. It was found that out of total 60 samples 2 samples were positive by PCR. However none of the samples were found positive by other tests.

Keywords: DFM, Isolation and identification studies, Leptospirosis, PCR, Sheep.

Introduction

Leptospirosis is an acute febrile septicaemic disease of zoonotic importance caused by spirochetes of species *Leptospira interrogans* having a broad spectrum of host range including wildlife. Leptospirosis is a complex disease caused by more than 226 known serovars each of which can independently infect any susceptible host species. Advances in the zoonosis research have greatly contributed to mans continues effort to wipe endemic, epidemic and panzootic communicable disease throughout the world, which took considerable loss of human and animal life in the past.

Among sheep most of the outbreak goes unnoticed due to lack of proper clinical signs and they usually react asymptomatically to the infection (Leovinz *et al.,* 1987). Different leptospiral serovars have been reported from different countries (Hathaway *et al.,* 1982, Faine *et al.,* 1999, Ciceroni *et al.,* 2000). In India leptospirosis has been recorded in cattle, Buffalo, Horse, Sheep, Goat and dog (Arora, 1977; Uppal and Singh 198). The pathogenic leptospirosis can colonize in the kidneys and shed in the urine for prolonged periods. The clinical manifestation of leptospires ranges from mildness to severe life threatening disease with jaundice, renal failure or abortion during pregnancy. Sheep may acquire the disease from contaminated urine of rodents, cattle or other farm animals. Serological studies suggest that the most prevalent sero groups associated with sheep worldwide are *autumnalis, grippotyphosa* and *pomona* (Faine *et al.,* 1999).

Materials and Methods

The present study was carried out to screen or detect the leptospires in the migratory flocks of sheep in Karnataka. A total of 60 samples were collected from the migratory sheep flocks in the area of shimogga and Belgaum districts of Karnataka with a flock history of abortion, hemoglobinuria, and intermittent fever. The samples from the aborted animals viz. kidney, liver, placenta, were collected and blood, were collected from other ailing animals with haemoglobinuria. The blood, tissue and urine samples were subjected to DFM, PCR and culture studies.

All the samples were initially subjected to dark field microscopic examination using Dark field microscope. Dark field microscopy was done as per Chandrasekhar and Pankajalakshmi, 1997 with minor modification. It was conducted on a minute drop, approximately 10 µl of the processed sample and covering it with cover slip to meticulously look for the typical spiral leptospira organisms with spinning hooked ends and showing high corkscrew motility.

Then the samples were subjected to the polymerase chain reaction as per the method of Grave kemp *et al.* 1993 using G1 (5' CTG AAT CGC TGT ATA AAA GT 3') and G2 (5' GGA AAA CAA ATG CTC GGA AG 3') primers. The reaction was set up in 50 µl reaction volumes, containing 5 µl of 10X buffer, 1 µl of primers each, 0.5 µl of dNTPs, 0.5 µl of Taq DNA polymerase, 5 µl of template and final volume was made up using double distilled water. The PCR was performed in a MJ research thermal cycler, for 32 cycles, each

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consisting of denaturation at 94°C for 90 sec, annealing at 55°C for 60 sec and polymerization at 72°C for 2 min. PCR products were finally electrophoresed on 1.5% agarose gels after staining with ethidium bromide and then visualized with ultraviolet light using Gel documentation system (Biorad). If the template is amplified then it will yield a product of 285 bp. In order to avoid false positive results positive and negative controls were used as false positive results might occur due to DNA contamination.

Apart from this the samples were filtered and inoculated into EMJH (Difco) semisolid and liquid medium for isolation as per the procedure of Venkatesh *et al.* 1997. A loopful of processed sample was inoculated aseptically into screw cap tubes containing EMJH semisolid medium. The tubes were incubated at the room temperature for 4-6 weeks and were examined at weekly interval for the presence of Dingers ring to check the growth of any leptospires. The cultures were also observed under DFM for viable organisms and to ensure purity and cultural stability of the isolation.

Results and Discussion

Out of the total 60 samples subjected to DFM, PCR and isolation studies none of the samples were found positive by DFM and cultural studies. The detail of the results is given in table 1.

Ellis (1986) reported that DFM was not useful for diagnosis as there were many serum proteins and cell debris, artifacts that may resemble intact or partially intact leptospires. Bolin *et al.* (1989) reported that DFM was insensitive and required a skilled observer to differentiate leptospires from artifacts. Smith *et al.* (1994) also quoted that DFM cannot reveal the serovars involved.

The culturing of leptospires from clinical materials is of paramount importance both for definitive diagnosis of individual cases and epidemiological purposes but the isolation of leptospires is a difficult procedure due to their fastidious nature, fragility for artificial media and overgrowth of contaminants. The probable reason for not obtaining any isolates in the present study could be attributed to heavy contamination of the samples as revealed by Kaveri and Upadhya (1980) who also attempted for isolation of leptospires from 100 samples of dogs.

However, two blood samples were found positive by PCR with an amplicon size of 285bp (Fig.1). This observation was in agreement with the findings of Sreenivas (2003) and Brown *et al.,* 1995. The probable reason for leptospires detection by PCR in comparison with the cultural studies could be that PCR detects DNA from both viable and non viable organisms whereas bacteriological culturing has an absolute requirement of viable organisms in the samples. Zuerner *et al.* (1995) reported that viable cells were likely to constitute a fraction of the total number of cells in a give samples and this fraction was influenced by the method of collection, transport and storage conditions prior to inoculation into the media. Bolin *et al.,* 1989 also reported that bacteriological culturing required fresh samples and was the least sensitive technique for detection of leptospires when compared to DNA based techniques.

Table-1. Details of results of the samples screened

Type of samples	DFM	Results	PCR	Results	Cultural studies	Results
Blood	12	Negative	122	Positive	12	Negative
Tissue	8	Negative	8	Negative	8	Negative
serum	27	Negative	27	Negative	27	Negative
Urine	13	Negative	13	Negative	13	Negative
Total samples	60	Negative	60	2 positive	60	Negative

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