

SEROPREVALENCE OF CHLAMYDOPHILA INFECTION IN SHEEP OF KRISHNA DISTRICT, ANDHRA PRADESH

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The present study was aimed to assess the seroprevalence of chlamydial infection in sheep. *Chlamydophila abortus* infection is characterized by reproductive losses in livestock posing heavy economic losses to farmers. Occurrence of the disease is high during the lambing season and *C. abortus* efficiently colonizes in the placental trophoblasts causing enzootic abortions. Moreover, it represents a zoonotic risk to humans who come in contact with aborted placental material. The present sero-prevalence study was conducted to identify the presence of chlamydial infection in sheep. A total of 28 serum samples were collected from the aborted cases in Krishna district of Andhra Pradesh. The seroprevalence of chlamydial infection was studied by enzyme linked immunosorbent assay (ELISA) for detection of antibodies in the serum samples of suspected chlamydial infection. Out of 28 serum samples tested, four samples were positive for the *Chlamydophila abortus* infection in sheep. The percentage of *Chlamydophila abortus* infection in sheep was 14. The present study indicated the prevalence of bacterial pathogen *Chlamydophila abortus* infection as a cause of abortions in sheep.

Key words: Andhra Pradesh, *Chlamydophila abortus*, Enzootic abortions, Ruminants, Seroprevalence

Chlamydial organisms, the fastidious obligatory intracellular bacterial pathogens, are responsible for a wide variety of infections in domestic ruminant livestock,

mostly the enzootic abortions (Markey, 2011). Chlamydiae are Gram negative diverse group of organisms belonging to the order *Chlamydiales* and family

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Chlamydiaceae. They were further grouped into two genera viz. *Chlamydia* and *Chlamydophila* (Everett *et al.*, 1999). *Chlamydophila abortus* (*C. abortus*) causes mastitis, epizootic bovine abortions in milking animals, endometritis, vaginitis, pneumonia, enteritis, conjunctivitis, encephalitis, (Twomey *et al.*, 2003) and localized infections like polyarthritis, orchitis, epididymitis and vesiculitis (Gomes *et al.*, 2001; Rekiki *et al.*, 2002).

Chlamydiae is also responsible for zoonosis in humans without the need of any intermediate host and causes abortions in pregnant women (Rodolakis and Mohamad, 2010). The aborted fetus, placenta and uterine discharges contains the elementary bodies which upon ingestion through contaminated feed and water is also responsible for the occurrence of disease (DeGraves *et al.*, 2004). Affected animals act as latent carriers with chances of abortions in the subsequent gestation period and remain as carriers for lifetime (Koehler *et al.*, 1997). *Chlamydophila abortus* is the causative pathogen for enzootic abortions in ewes and responsible for 20% to 50% of abortions and stillbirths, respectively in ovines all over the world (Aljumaah and Hussein, 2012). In small ruminants, the infection is mostly asymptomatic, except still births in the last trimester of gestation. The healthy ewes get the infection from the

breeding rams and plays an important role in the transmission of disease. In addition, wild animals serve as potential reservoirs and play an important role in environmental contamination and spread of *C. abortus* (Hotzel *et al.*, 2004). Seroprevalence of chlamydial infection is essential in monitoring the occurrence or presence of disease. Several diagnostic techniques have been in use worldwide since a decade to predict the chlamydial infections as it has been one of the economically important infections of livestock and of its zoonotic nature (Bandyopadhyay *et al.*, 2009). Isolation of the microorganism remains a difficult and time-consuming task. Thus serological methods like ELISA can effectively confirm the disease. Hence in the present study, the seroprevalence for the chlamydial infection is carried out by performing indirect ELISA

In the present study, a total of 28 serum samples from sheep were collected with a history of abortions and retained placenta cases in Krishna district of Andhra Pradesh. The blood samples were collected aseptically in vacutainers and allowed to clot for the separation of serum. The sera were aliquoted in 2mL vials and preserved at -40°C until further use. The samples were collected from different villages of Krishna district (Table. 1) during the months of August 2016 to January 2017.

Table 1. Details of the serum samples collected randomly from various villages of Krishna District

S.No	Village	Mandal	Number of samples collected
1	Korukollu	Kalidindi	5
2	Bobbiligudem	Kalidindi	9
3	Gollagudem	Kalidindi	4
4	Jammigolvepalli	Pamarru	7
5	Nimmakuru	Pamarru	3
Total			28

ELISA procedure: The detection of antibody levels to *Chlamydomphila abortus* infection in the test serum samples was estimated by indirect ELISA test kit (IDEXX Chlamydiosis total Ab kit, Ireland). In brief, microwells coated with *Chlamydomphila abortus* specific antigen were added with 1: 10 diluted test sera with dilution buffer. Positive and negative controls were also placed that were provided in the kit. The ELISA plate was incubated at 20°C for 45 min. The wells were emptied and the microwells were washed thrice with 200µL wash solution. Then 100 µL of diluted conjugate (1:10) was added and incubated at 20°C for 30 min. The wells were emptied and washed thrice with 200µL of wash solution. Substrate solution of OPD (O-Phenylenediamine dihydrochloride) @ 100µL was added to each well and incubated for 15 min at 20°C in dark. Finally, the reaction was stopped by adding

100µL of stopping solution and the results were read with optical density at a wave length 450nm in an ELISA reader (Thermo, 51119000, Germany).

Interpretation for each sample was done by calculating the S/P percentage (S/P%)

$$S/P\% = \frac{\text{OD of the sample}}{\text{OD of the positive control}} \times 100$$

Samples presenting a (S/P%):

- Less than or equal to 50% are considered negative
- Less than 60% and greater than 50% are considered doubtful
- Greater than or equal to 60% are considered positive

The ELISA results revealed seropositivity of 14% (4/28) in sheep for chlamydial antibodies. Only four sera samples of sheep (one from Korukollu and three from Bobbiligudem village) produced a S/P percentage of greater than 60%. Among all

the villages, highest incidence of the chlamydial infection was seen in Bobbiligudem with three positive sera. Statistical analysis was carried out to find out any significant difference in the prevalence of disease by using Chi-square test. Bobbiligudem had the highest prevalence of chlamydial infection with three positive cases out of nine serum samples collected.

Serological tests play a significant role in detecting the presence of antibodies against the chlamydial infection despite various other tests like isolation, immunohistochemistry and highly sensitive polymerase chain reaction (Didugu *et al.*, 2016). Indirect ELISA is easy to perform and does not require highly qualified persons. The other methods are cumbersome and require skilled persons. It is very difficult to diagnose the infection in the clinical cases as the infection is more asymptomatic and only abortions are the characteristic pathognomic clinical sign that could be detected. But abortions in livestock are caused by a large number of reasons and it is difficult to differentiate as chlamydial infection. Hence, indirect ELISA is the best method with high specificity without any cross reactions and is less cumbersome (Gokce *et al.*, 2007).

Earlier, several authors reported seroprevalence of chlamydiosis among

large ruminant species, viz. cattle using ELISA that revealed the seroprevalence rates as zero percent (Ozturk *et al.*, 2012), 4.75% (Wilson *et al.*, 2012), 8.33% (Igayara-Souza *et al.*, 2004), 26.92% (Gokce *et al.*, 2007), 35% (Bandyopadhyay *et al.*, 2009) and 68.18% (Didugu *et al.*, 2016). In the present study, the chlamydial infection in small ruminant species, e.g. sheep was 14% and the reports of seroprevalence of other authors were 7.52% (Aljumaah and Hussein, 2012), 4.55% (El-Razik *et al.*, 2011), 5.4% (Otlu *et al.*, 2007) and 11.7% (Cislakova *et al.*, 2007). Statistical analysis was carried out using Chi-square test to study seroprevalence of *Chlamydophila abortus* in relation to village affected. Highest incidence of chlamydial infection was observed in Bobbilligudem and the differences in occurrence of the disease was significant as the p value is less than 0.05. In the present study, the seroprevalence in sheep was high and it might be due to the low sample number and high susceptibility of infection. The presence of infection in sheep indicates that detailed study should be performed in future with large number of samples to explore the real status of chlamydial infection of Krishna district, Andhra Pradesh.

Studies on seroprevalence of chlamydial antibodies in livestock using reliable serodiagnostic methods like indirect ELISA

aids in identifying infected and carrier animals, and also in assessing the status of infection in a particular geographical studied area, so that appropriate preventive measures can be undertaken for minimizing the disease and to reduce zoonosis.

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