Serological investigation of chlamydial infection among ruminants in Krishna district of Andhra Pradesh, India


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Abstract

Chlamydiae are gram negative, obligatory intracellular pathogens, which are responsible abortions in animals, birds and humans. Infection occurs by ingestion of elementary bodies from aborted fetus, uterine discharge and placenta from infected animals or via contaminated feed and water. Lack of specific clinical signs and pathological lesions most often leads to difficulty in diagnosing chlamydial infections at the field level. In Andhra Pradesh, a study based on ELISA was conducted to assess the seroprevalence of chlamydiosis among ruminants, which revealed seropositivity of 68.18% in cattle, 33.76% in buffaloes with an overall prevalence of 41.41% in large ruminants. 35.38% of sheep and 25% of goat were found positive for chlamydial antibodies.

Key Words: Abortions; Andhra Pradesh; C. abortus; ruminants; seroprevalence.
Introduction

Chlamydiae are gram negative, obligatory intracellular pathogens, which are responsible for diverse disease conditions, majorly abortions in animals, birds and humans (Horn, 2011; Markey, 2011). Chlamydiae are genetically diverse group of organisms belonging to order Chlamydiales and family Chlamydiaceae. They were grouped into 2 genera namely Chlamydia and Chlamydophila, containing 9 species (Everett et al., 1999). Recently International committee on Systematics of prokaryotes adopted a single genus (Chlamydia) classification with 11 species, which is currently being followed (Greub, 2010; Horn 2011; Markey, 2011). Among ruminants Chlamydophila abortus (C. abortus) can cause orchitis, epididymitis and vesiculitis (Gomes et al., 2001), mastitis, endometritis, vaginitis, pneumonia, enteritis, conjunctivitis, encephalitis, polyarthritis (Twomey et al., 2003) and epizootic bovine abortion in milking animals (Rekiki et al., 2002). Chlamydiae can cause direct zoonosis in humans without involvement of any intermediate host and can cause abortions in pregnant women (Rodolakis and Mohamad, 2010). Infection may occur irrespective of season by ingestion of elementary bodies from aborted fetus, uterine discharge and placenta from infected animals or via contaminated feed and water (DeGraves et al., 2004). Infected animals are latent carriers with high chances of abortion in the subsequent gestation period and remain carriers for the rest of the reproductive life (Koehler et al., 1997).

Enzootic ovine abortion, caused by Chlamydophila abortus (formerly called as Chlamydia psittaci serotype 1) is believed to be responsible for 20 to 50% of abortion and still births in ovines all over the world (Aljumaah and Hussein, 2012). Chlamydial infection in small ruminants are mostly asymptomatic apart from late term abortion or still birth. Breeding rams may acquire the disease and spread to the healthy ewes in the flock. Abortions and infertility became serious economic problems in ruminant sector worldwide (Griffiths et al., 1995; Liao et al., 1997; Buxton and Henderson, 1999). There are many reports on chlamydial diseases in ruminants worldwide and use of improved diagnostic techniques since last decade lead to its recognition as one of the important problems in livestock sector (Bandyopadhyay et al., 2009). There are meagre reports on epidemiology and prevalence of chlamydial infections in Andhra Pradesh. Infertility and abortions are the major causes of economic loss to the livestock farmers loosing income in the form of milk and meat. In this regard, a study was conducted to assess the seroprevalence of chlamydiosis in Krishna district of Andhra Pradesh, India.

Materials and methods

Sample collection

184 samples (22 cattle, 77 buffaloes, 65 sheep and 20 goat) were collected randomly from various villages in the months of September to December, 2014 in Krishna district, Andhra Pradesh, India. Samples were collected aseptically from jugular vein using BD® vaccutainers. After collection serum was allowed to clot at room temperature and transferred to laboratory on ice at the earliest possible. Serum was separated and stored at -20°C until further use. The details of samples were as follows.

Table 1: Details of samples collected from Cattle and Buffaloes in Krishna district

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of the Village</th>
<th>Mandal</th>
<th>No. of samples collected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cattle</td>
</tr>
<tr>
<td>1</td>
<td>Atkur</td>
<td>Ungutur</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Chintalapadu</td>
<td>Tiruvuru</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Erukepadu</td>
<td>Tiruvuru</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Kotha Edara</td>
<td>Agiripalli</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>Seetharamapuram</td>
<td>Agiripalli</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>Veeravalli</td>
<td>Bapulapadu</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>Billanapally</td>
<td>Bapulapadu</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Rangannagudem</td>
<td>Bapulapadu</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>Reddygudem</td>
<td>Reddygudem</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>Velpucherla</td>
<td>Musunuru</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>Vellanki</td>
<td>Veerlupadu</td>
<td>15</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>22</strong></td>
</tr>
</tbody>
</table>

188
ELISA procedure
IDEXX® (Manufactured by IDEXX Europe B.V. (International Headquarters) Scorpius 60 Building F Hoofddorp, 2132 LR The Netherlands) Chlamydiosis total antibody kit works by detecting any antibody directed against C. abortus by binding antigen in the wells and thereby forming an antigen-antibody complex. Samples, positive and negative controls are prediluted at the rate of 1:400 and dispensed into appropriate wells of the microtitre plate and incubated for 60 minutes at 37°C. All wells were washed with approximately 300 µl of wash solution thrice. 100 µl of conjugate was added in to each well and incubated for 60 minutes at 37°C. All wells were washed again as before. 100 µl of TMB substrate N.12 was added in to each well and incubated at 37°C for 15 minutes. Color reaction was stopped by adding 100 µl of stop solution N.3 per well and optical density was read using BioTek® microplate reader at a wavelength of 450 nm. Results were calculated using xChekPlus® software.

Results and discussion
ELISA revealed seropositivity of 68.18% (15/22) in cattle, 33.76 (26/77) in buffaloes with an overall prevalence of 41.41% (41/99) in large ruminants. 35.38% (23/65) in sheep and 25% (5/20) in goat were found positive for chlamydial antibodies with an overall prevalence of 37.5% (69/184) among all ruminants. Lack of specific clinical signs and pathological lesions most often leads to difficulty in diagnosing chlamydial infections at the field level. Common clinical sign like abortion was caused by various pathogenic organisms including C. abortus. Numerous diagnostic techniques for detecting chlamydial infections are available including histochemical, immunological staining of placental tissue smears, isolation of organism in cell culture and PCR assay (Nietfeld, 2001; Rekiki et al., 2002). But all these techniques are in need of well preserved, high quality material or specialized persons for isolation and identification (Gokce et al., 2007). Hence serological techniques like immunofluorescence tests (IFATs), ELISA and complement fixation test (CFT) are widely used, owing to their easiness. Even then antigen cross-reactivity between C. abortus and C. pecorum and also with gram negative bacteria like Acinetobacter may lead to false positive results in CFT and IFAT (Niemczuk, 2005). These false positive results can be avoided by using ELISA, a sensitive and specific method without any cross reactivity with C. pecorum. Vlahovic et al. (2001) also reported ELISA as a superior method compared to IIF and CFT with higher sensitivity and reported ELISA as a more objective and less cumbersome method. Hence, use of ELISA for diagnosing chlamydial infections in animals and humans was recommended by various authors (Gokce et al., 2007).

Many authors reported seroprevalence of Chlamydiosis among cattle using ELISA and reported infection rates ranging from zero percent (Ozturk et al., 2012), 4.75% (Wilson et al., 2012), 8.33% (Igayara-Spuza et al., 2004), 26.92% (Gokce et al., 2007) and 35% (Bandyopadhyay et al., 2009). 68.18% seropositivity observed in this study was higher than other seroprevalence reports using ELISA as a diagnostic method and on contrary, low infection rate of 2.8% was reported by Chahota et al. (2015) in Andhra Pradesh using AGPT. Low sample number and greater susceptibility of cross bred cattle to infection might have contributed high prevalence observed in this study. Buffalo contributes most of the bovine population in the studied area. Most of the reports concerning chlamydial infection in buffaloes used AGPT as a serological method and reports using ELISA were almost nil. Prevalence of 33.76% observed in buffaloes using ELISA in this study was higher than 0.93% (Chahota et al., 2015) reported in the same state and also higher than the other reports (Paul et al., 2002).

Zoonotic potential of C. abortus is significant especially in pregnant women, who are in close contact with infected and carrier animals (Nietfeld, 2001). So it is essential to undertake serological studies to detect infected and
carrier animals to avoid environmental contamination, zoonotic transmission and spread of disease to remaining healthy animals. Placenta and uterine discharges of infected animals are reservoirs of chlamydial organisms, which subsequently enter milk, feces, ocular and nasal discharges. Infected bulls spread the disease to healthy cows via semen, causing embryonic death and infertility (Nietfeld, 2001; DeGraves et al., 2004). In addition wild animals serve as potential reservoirs and play a vital role in environmental contamination and spread of C. abortus (Hotzel et al., 2004).

Healthy animal acquire disease by ingestion or inhalation of infected material, especially fetal membranes and discharges. In heifers, organism remain in latent form until the onset of pregnancy, probably in lymphoid tissue (Nietfeld, 2001). Being intracellular, organism can’t be detected by direct methods (PCR, Ziehl-Neelsen staining) or by serological methods until infectious organisms are excreted, rising antibody titers at the time of abortion. In the present study, 35.38% of sheep were found to be effected with chlamydial infection. On contrary lower seroprevalence of 7.52% (Aljumaah and Hussein, 2012), 4.55% (El-Razik et al., 2011), 5.4% (Otlu et al., 2007), 11.7% (Čisláková et al., 2007) was reported by various authors. Aljumaah and Hussein, (2012) reported a seroprevalence of 34.5% in goats, which was proximate to the findings of this study (25%). In disagreement, lower seroprevalence of 4.2%, 5.66% and 7.7% was reported by Czopowicz et al. (2010); El-Razik et al. (2011) and Čisláková et al. (2007), respectively. Even though ewes don’t get aborted subsequently by developing immunity, they excrete C. abortus during estrous and subsequent lambing, contaminating environment and spreading infection (Nietfeld, 2001). Housing of all animals irrespective of age, sex, species, pregnant and non-pregnant lead to increased spread of the disease in India. Furthermore exhaustive epidemiological studies may be needed (Gokce et al., 2007) to characterize indigenous chlamydial strains.

Seroprevalence of chlamydial antibodies among ruminants using a reliable serodiagnostic method helps in identifying infected and carrier animals and also in assessing the status of infection in the studied area, so that appropriate measures can be undertaken for controlling the disease and to reduce zoonosis.

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References


