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

H. Didugu[1*], Ch.E. Narasimha Reddy[1], R.N Ramanipushpa[2], S.S.B. Ramaraju[3], M.V. Reddy[4], M. Satyanarayana[5], K.N. Kishore[6], A. Saimahesh Reddy[7]

[1]Veterinary Assistant Surgeon, Animal Disease Diagnostic Laboratory, Vijayawada, [2]Associate Professor, Department of Veterinary Microbiology, NTR College of Veterinary Science, Gannavaram, [3]Veterinary Assistant Surgeon, State Institute of Animal Health, Tanuku, [4]Veterinary Assistant Surgeon, Veterinary Dispensary, Pedakakani, [5]Technical Manager, Sneha feeds, Tanuku, [6]Veterinary Pathologist, Laila Neutraceuticals, Vijayawada, [7]PhD Scholar, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, India

* Corresponding author's e- mail: hareesh.vet@gmail.com

Journal of Livestock Science (ISSN online 2277-6214) 7: 187-191
Received on 13/6/2016; Accepted on 12/7/2016

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, epididimytis 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 *Chalmydia 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^{0}C until further use. The details of samples were as follows.

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

S. No	Name of the Village	Mandal	No. of samples collected	
			Cattle	Buffalo
1	Atkur	Ungutur	1	5
2	Chintalapadu	Tiruvuru	0	10
3	Erukepadu	Tiruvuru	1	2
4	Kotha Edara	Agiripalli	3	7
5	Seetharamapuram	Agiripalli	1	11
6	Veeravalli	Bapulapadu	1	10
7	Billanapally	Bapulapadu	0	3
8	Rangannagudem	Bapulapadu	0	10
9	Reddygudem	Reddygudem	0	8
10	Velpucherla	Musunuru	0	11
11	Vellanki	Veerlupadu	15	0
		Total	**22**	**77**

Table 2: Details of samples collected from Sheep and Goat in Krishna district

S. No	Name of the Village	Mandal	No. of samples collected	
			Sheep	Goat
1	Ketanakonda	Ibrahimpatnam	9	1
2	Kuntamukkala	G. konduru	8	2
3	Gannavaram	Gannavaram	9	1
4	Pulluru	Mylavaram	8	2
5	Pothireddypalli	Agiripalli	14	11
6	Anumanchipalli	Jaggaihpet	8	2
7	Sher M Peta	Jaggaihpet	9	1
		Total	**65**	**20**

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 in to appropriate wells of the microtitre plate and incubated for 60 minutes at 37^0C. 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 37^0C. All wells were washed again as before. 100 µl of TMB substrate N.12 was added in to each well and incubated at 37^0C 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 testes 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 most objective and less cumbersome method. Hence, use of ELISA for diagnosing chlamydial infections n 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.

Acknowledgements

Authors are thankful to Director of Animal Husbandry, Government of Andhra Pradesh, India for providing facilities for conducting this research work.

References

1) Aljumaah, R. S. and Hussein, M. F. 2012. Serological prevalence of ovine and caprine chlamydophilosis in Riyadh region, Saudi Arabia. *African Journal of Microbiology Research*, 6(11): 2654-2658.
2) Bandyopadhyay, S., Sasmal, D., Biswas, T. K., Samanta, I. and Ghosh, M. K. 2009. Serological evidence of antibodies against Chlamydophila abortus in free-ranging yak (Poephagus grunniens) in Arunachal Pradesh, India. *Revue scientifique et technique (International Office of Epizootics)*, 28(3): 1051-1055.
3) Buxton, D. and Henderson, D. 1999. Infectious abortion in sheep. *In Practice*, 21(7): 360-368.
4) Chahota, R., Gupta, S., Bhardwaj, B., Malik, P., Verma, S. and Sharma, M. 2015. Seroprevalence studies on animal chlamydiosis amongst ruminants in five states of India. *Veterinary World*, 8(1): 72-75.
5) Čisláková, L., Halánová, M., Kováčová, D. and Štefančíková, A. 2007. Occurrence of antibodies against Chlamydophila abortus in sheep and goats in the Slovak Republic. *Annals of Agriculture and Environmental Medicine*, 14: 243-245.
6) Czopowicz, M., Kaba, J., Szaluś-Jordanow, O., Nowicki, M., Witkowski, L., Nowicka, D. and Frymus, T. 2010. Prevalence of antibodies against Chlamydophila abortus and Coxiella burnetii in goat herds in Poland. *Polish Journal of Veterinary Sciences*, 13: 175-179.
7) DeGraves, F. J., Kim, T., Jee, J., Schlapp, T., Hehnen, H. R. and Kaltenboeck, B. 2004. Reinfection with Chlamydophila abortus by uterine and indirect cohort routes reduces fertility in cattle preexposed to Chlamydophila. *Infection and Immunity*, 72(5): 2538-2545.
8) El-Razik, K. A., AL-Humiany, A. A., Ahmed, W. M., Barakat, A. M. A. and ELfadaly, H. A. 2011. Investigations on non Brucella Abortifacients in Small Ruminants in Saudi Arabia with Emphasis on Zoonotic Causes. *Global Veterinari*, 6(1): 25-32.

9) Everett, K. D., Bush, R. M. and Andersen, A. A. 1999. Emended description of the order Chlamydiales, proposal of Parachlamydiaceae fam. nov. and Simkaniaceae fam. nov., each containing one monotypic genus, revised taxonomy of the family Chlamydiaceae, including a new genus and five new species and standards for the identification of organisms. *International Journal of Systemic Bacteriology*, 49(2): 415-440.
10) Gokce, H., Kacar, C., Genc, O. and Sozmen, M. 2007. Seroprevalence of Chlamydophila abortus in aborting ewes and dairy cattle. *Bulletin of the Veterinary Institute in Pulawy*, 51: 9-13.
11) Gomes, M. J. P. O., Wald, V. B. O., Machado, R. D. O. and Silveira, M. C. 2001. Isolamento de Chlamydia psittaci em touros com vesiculite seminal, no Rio Grande do Sul. *A Hora Veterinária*, 119: 43-46.
12) Greub, G. 2010. International Committee on Systematics of ProkaryotesSubcommittee on the taxonomy of the Chlamydiae Minutes of the closed meeting, 21 June 2010, Hof bei Salzburg, Austria. *International Journal of Systematic and Evolutionary Microbiology*, 60(11): 2694-2694.
13) Griffiths, P. C., Plater, J. M., Martin, T. C., Hughes, S. L., Hughes, K. J., Hewinson, R. G. and Dawson, M. 1995. Epizootic bovine abortion in a dairy herd: characterization of a Chlamydia psittaci isolate and antibody response. *The British Veterinary Journal*, 151(6): 683-693.
14) Horn, M. 2011. Phylum XXIV. Chlamydiae Garrity and Holt 2001. *Bergey's manual of systematic bacteriology. New York. Bergey's Manual Trust*, 843-844.
15) Hotzel, H., Berndt, A., Melzer, F. and Sachse, K. 2004. Occurrence of Chlamydiaceae spp. in a wild boar (Sus scrofa L.) population in Thuringia (Germany). *Veterinary Microbiology*, 103(1): 121-126.
16) Igayara-Souza, C. A., Genovez, M. E., Ferreira, F., Paulin, L. M., Scarcelli, E., Cardoso, M. V. and Turilli, C. 2004. Ocorrência de anticorpos anti-Chlamydophila em bovinos e sua relação com distúrbios reprodutivos. *Revista brasileira de reprodução animal*, 28(1): 28-33.
17) Koehler, L., Nettelnbreker, E., Hudson, A. P., Ott, N., Gerard, H. C., Branigan, P. J., Schumacher, H. R., Drommer, W. and Zeidler, H. 1997. Ultrastructural and molecular analyses of the persistence of Chlamydia trachomatis (serovar K) in human monocytes. *Microbial Pathogenesis*, 22(3): 133-142.
18) Liao, Y. K., Chain, C. Y., Lu, Y. S., Li, N. J., Tsai, H. J. and Liou, P. P. 1997. Epizootic of Chlamydia psittaci infection in goats in Taiwan. *Journal of Basic Microbiology*,. 37(5): 327-333.
19) Markey, B. 2011. Chlamydial infection in cattle: The burden of proof?. *Veterinary Journal*, 189(3): 246-247.
20) Nanda, N. K., Rao, A. T., Nayak, B. C., Rao, A. G. and Mishra, P. R. 1992. Diagnosis of bovine chlamydial abortion in cattle. *Indian Veterinary Journal, 69*(6): 483-486.
21) Niemczuk, K. 2005. Prevalence of antibodies against Chlamydophila psittaci and Chlamydophila abortus in cattle in Poland. A preliminary report. *Bulletin of the Veterinary Institute in Pulawy*, 49(3): 293.
22) Nietfeld, J. C. 2001. Chlamydial infections in small ruminants. *Veterinary Clinics of North America: Food Animal Practice*, 17(2): 301-14.
23) Otlu, S., ŞAHİN, M., Unver, A. and Celebi, O. 2007. Detection of Brucella melitensis and Chlamydophila abortus antibodies. *Bulletin of the Veterinary Institute in Pulawy*, 51: 493-495.
24) Öztürk, D., Kale, M., Pehlivanoğlu, F., Hasırcıoğlu, S. and Türütoğlu, H. 2012. Evaluation for some bacterial and viral abortions of dairy cattle farms in Burdur District of Turkey. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*. 18 (2): 255-258
25) Paul, R., Katoch, R. C., Chahota, R. and Mahajan, A. 2002. Seroprevalence of chlamydiosis among cows and buffaloes in Himachal Pradesh. *Indian Journal of Animal Science*, 72(6): 434-435.
26) Rekiki, A., Sidi-Boumedine, K., Souriau, A., Jemli, J., Hammami, S. and Rodolakis, A. 2002. Isolation and characterisation of local strains of Chlamydophila abortus (Chlamydia psittaci serotype 1) from Tunisia. *Veterinary Research*, 33(2): 215-222.
27) Rodolakis, A. and Mohamad, K. Y. 2010. Zoonotic potential of Chlamydophila. *Veterinary Microbiology*, 140(3): 382-391.
28) Twomey, D. F., Griffiths, P. C., Hignett, B. C. and Martin, T. P. 2003. Suspected chlamydial polyarthritis in a calf in the UK. *Veterinary Record*, 152(11): 340-340.
29) Vlahović, K., Dovč, A., Župančić, Ž., Pavlak, M. and Jerčić, J. 2001. Comparison of serological procedures for diagnosis of infection with Chlamydophila sp. in bovines. *Veterinary Arhives*, 71(6): 367-379.
30) Wilson, K., Sammin, D., Harmeyer, S., Nath, M., Livingstone, M. and Longbottom, D. 2012. Seroprevalence of chlamydial infection in cattle in Ireland. *The Veterinary Journal*, 193(2): 583-585.