Correspondence

Seroprevalence of coxiellosis (Q fever) in sheep & goat in Puducherry & neighbouring Tamil Nadu

Sir,

Coxiellosis caused by an intracellular bacterium Coxiella burnetii is an important zoonosis prevalent throughout the world, with the notable exception of Antarctica and New Zealand^{1,2}. The outbreaks of coxiellosis in goats in The Netherlands, which spread to humans has renewed the interest in this zoonosis³. In India, reports of coxiellosis in domestic livestock from several States appeared during the fifties with a spurt in the seventies and eighties⁴⁻¹². Barring an isolated report of threatened abortion in livestock by Vaidya *et al*¹³ in northern India, there was gap of three decades when coxiellosis in these ruminants was not documented in the Indian literature. This investigation was, therefore, undertaken to study the present seroprevalence status of C.burnetii in ovines and caprines of Puducherry and neighbouring Tamil Nadu State in India.

This work was carried out at the department of Microbiology, Mahatma Gandhi Medical College & Research Institute, Puducherry, during July 2012 to June 2013. The protocol was approved by the Institutional Research and Ethical Committee. Blood samples from 216 sheep and 195 goats were collected from private and municipal slaughter houses in and around Puducherry. None of the animals tested in this study had received Q fever vaccination. The serum was separated on the same day, aliquoted and kept frozen at -20°C till the time of testing. Q fever (C.burnetii) antibody test kit, IDEXX Switzerland AG, was used in the study. The ELISA plates were coated with inactivated phase I and phase II C. burnetii antigens. The test was performed with strict adherence to the instructions of the kit's manufacturers. Sheep and goat serum samples were initially diluted to1:400 with the sample diluent provided in the kit. Positive

and negative controls were included in each run in duplicate. At the end of the test, the absorbance values (optical density-OD) were measured using 450 nm filter in Bio-Rad ELISA Reader (Japan). Results were expressed in percentage. OD reading of the test sample $(S/P) = 100 \times (S-N) / (P-N)$, where S, N, and P are the OD of test sample, negative control, and positive control, respectively. Results were interpreted as per the kit's guidelines as $S/P \le 30$ per cent were negative, 30-40 per cent were suspect and ≥ 40 per cent were considered as positive. Samples in the suspect zone were repeated twice to decide whether those were positive or negative.

Eleven of the 195 goats (5.64 %) and four of the 216 sheep (1.85%) had antibodies to phase I and II *C.burnetii*. Statistical analysis using χ^2 test showed is a significant difference between these two groups of small ruminants (*P*<0.05). The observed seropositivity for coxiellosis in the present study was significantly lower than that reported earlier ranging from 1.9 to 60 per cent (mean 13.1%) in case of goats and 3.7 to 49.75 per cent (mean 17.4%) in sheep from different parts of India⁴⁻¹².

Until the eighties, three serological tests were commonly used for the diagnosis of *C.burnetii* in animals: Luoto's capillary agglutination test¹⁴ (CAT), microagglutination test¹⁵ (MAT) and complement fixation test¹⁶. Because of false positive results observed in CAT and the need for large amounts of antigens for MAT, these two tests are no longer used. Complement fixation test [though OIE (Office International des Epizooties) recommended test for animals], is a specific test, but has poor sensitivity. While immunofluorescence test (IFA) is the gold standard serological test for Q fever in humans only, ELISA test for C.burnetii is considered highly specific and equally sensitive^{1,17,18}. The major drawback of the ELISA kit used in this study is that it can be only used for testing the ruminants. Other animals like dogs, cats, birds, etc., cannot be examined using this kit. In seroprevalence studies, mostly phase I antigen and less commonly both phase I (CAT) and phase II antigens (MAT) were used¹². Two Indian studies have reported on the role of C.burnetii causing abortion in humans and animals^{13,19}. According to this report, many seropositive ruminants do not shed C.burnetii in their secretions and excretions, and in contrast, seronegative animals harbour these parasites and shed them in their vaginal secretions/milk. This presents the possibility that the frequency of seroprevalence of Q fever in ruminants could be greater than what has been reported. Early reports of higher prevalence of C. burnetii in India could be due to the use of capillary agglutination test where positivity was based on undiluted neat serum. The kit used in the present study has been reported to give satisfactory results^{13,19,20}.

Sheep, goat, cattle and buffaloes as meat animals in Puducherry are procured from the neighboring States of Tamil Nadu and Andhra Pradesh. Thus the seroprevalence of C.burnetii as observed in this study may be considered as reflecting the status for other States of south India. McQuiston and Childs² reported C.burnetii seroprevalence of 41.6 per cent for goat and 16.5 per cent for sheep in USA. Researchers from Turkery²¹ reported seropositivity of 38.6 and 25.4 per cent for goat and sheep, respectively. Knobel et al 22 observed a seropositivity of 30 per cent for goat and 18.2 per cent for sheep in Kenya. In Iran, positivity rates of 27.2 per cent for goats and 19.5 per cent for sheep have been reported²⁰. A low prevalence of 6.3 per cent for goats and 11.8 per cent for sheep was shown from Italy²³. The lowest seropositivity has been reported from Switzerland by Hunninghaus and coworkers²⁴ with 3.4 per cent for goats and 1.8 per cent for sheep, which is comparable to our findings.

In view of our finding of a low seroprevalence of *C.burnetii* in caprines and ovines, a regular surveillance of this zoonosis is required.

Acknowledgment

The authors acknowledge the Indian Council of Medical Research (ICMR), New Delhi, for funding this *ad-hoc* research project. Authors are grateful to the Chairman, Vice-Chancellor and Dean of MGMC & RI for providing the facilities.

Selvaraj Stephen^{1,*}, Balakrishnan Sangeetha¹ & Prabakhar X. Antony²

¹Department of Microbiology Mahatma Gandhi Medical College & Research Institute, Pondy-Cuddalore Main Road Pillaiyarkuppam, Puducherry 607 402 & ²Vetarinary Microbiology Rajiv Gandhi College of Veterinary & Animal Sciences (Government of Puducherry) Puducherry 605 009, India **For correspondence:* stephens4950@gmail.com

References

- 1. Fournier PE, Marrie TJ, Raoult D. Diagnosis of Q fever. *J Clin Microbiol* 1998; *36* : 1823-34.
- 2. McQuiston JH, Childs JE. Q fever in humans and animals in the United States. *Vector Borne Zoonotic Dis* 2002; 2 : 179-91.
- Roest HI, Tilburg JJ, van der Hoek W, Vellema P, van Zijderveld FG, Klaassen CH, *et al.* The Q fever epidemic in The Netherlands: history, onset, response and reflection. *Epidemiol Infect* 2011; *139*: 1-12.
- 4. Padbidri VS, Gupta NP. Riskettsiosis in India: a review. *J Indian Med Assoc* 1978; 71 : 104-7.
- 5. Stephen S, Achyutha Rao KN. Q fever in India: a review. *J Indian Med Assoc* 1980; 74 : 200-3.
- Randhawa AS, Dhillon SS, Jolley WB. Serologic prevalence of Q fever in the state of Punjab, India. *Am J Epidemiol* 1973; 97: 131-4.
- Joshi MV, Menon RD, Padbidri VS, Manjrekar SL. A note on the serological evidence of 'Q' fever in sheep from Karnataka state. *Indian J Anim Sci* 1975; 45: 54-5.
- Yadav MP, Sethi MS. Sero-epidemiological studies on coxiellosis in animals and man in the state of Uttar Pradesh and Delhi (India). *Int J Zoonoses* 1979; 6: 67-74.
- Padbidri VS, Rodrigues FM, Vidyasagar J, Joshi MV. Prevalence of antibodies to *Coxiella burnetii* among the domestic animal population in Orissa. *Indian J Med Vet* 1981; 5:99.
- Pathak PN, Tanwani SK. Serological investigations into Q fever. *Indian Vet J* 1969; 46: 551-3.
- Choudhury S, Balaya S, Mohapatra LN. Serological evidence of *Coxiella burnetii* infection in domestic animals in Delhi and surrounding areas. *Indian J Med Res* 1971; 59: 1194-202.
- 12. Shukla CL, Negi SK. Sero-prevalence of Q fever in Nainital tarai. *Indian J Med Res* 1978; 67: 535-7.
- Vaidya VM, Malik SV, Bhilegaonkar KN, Rathore RS, Kaur S, Barbuddhe SB. Prevalence of Q fever in domestic animals with reproductive disorders. *Comp Immunol Microbiol Infect Dis* 2010; 33: 307-21.
- Luoto L. A capillary agglutination test for bovine Q fever. J Immunol 1953; 71: 226-31.

- Fiset P, Ormsbee RA, Silberman R, Peacock M, Spielman SH. A microagglutination technique for detection and measurement of rickettsial antibodies. *Acta Virol* 1969; 13: 60-6.
- 16. Kittelberger R, Mars J, Wibberley G, Sting R, Henning K, Horner GW, et al. Comparision of the Q-fever complement fixation test and two commercial enzyme-linked immunosorbent assays for the detection of serum antibodies against *Coxiella burnetii* (Q-fever) in ruminants: recommendations for use of serological tests on imported animals in New Zeland. N Z Vet J 2009; 57 : 262-8.
- 17. Cowley R, Fernandez F, Freemantle W, Rutter D. Enzyme immunoassay for Q fever: comparison with complement fixation and immunofluorescence tests and dot immunoblotting. *J Clin Microbiol* 1992; *30* : 2451-5.
- Kovácová E, Kazár J. Rickettsial diseases and their serological diagnosis. Clin Lab 2000; 46: 239-45.
- 19. Vaidya VM, Malik SV, Kaur S, Kumar S, Barbuddhe SB. Comparison of PCR, immunofluorescence assay, and pathogen isolation for diagnosis of Q fever in humans with spontaneous abortions. *J Clin Microbiol* 2008; *46* : 2038-44.

- 20. Asadi J, Kafi M, Khalili M. Seroprevalence of Q fever in sheep and goat flocks with a history of abortion in Iran between 2011 and 2012. *Vet Ital* 2013; *49* : 163-8.
- Arserim NB, Yeşilmen S, Yasar O, Özekinci T, Keskin O, Pulat H, et al. Seroprevalance of Coxiellosis in cows, sheep, goats and humans in Diyarbakir region of Turkey. *Afr J Microbiol Res* 2011; 5: 2041-3.
- 22. Knobel DL, Maina AN, Cutler SJ, Ogola E, Feikin DR, Junghae M, et al. Coxiella burnetii in humans, domestic ruminants, and ticks in rural western Kenya. Am J Trop Med Hyg 2013; 88 : 513-8.
- 23. Capuano F, Parisi A, Cafiero MA, Pitaro L, Fenizia D. *Coxiella burnetii*: what is the reality? *Parassitologia* 2004; *46* : 131-4.
- Hunninghaus J, Schüpbach-Regula G, Wittenbrink MM, Hamburger A, Scherrer S, Stärk K, *et al.* Q fever in Switzerland: seroprevalence in small ruminants and risk assessment for humans. In: *Q fever in Switzerland, 2012 - 2012.* Zurichm, Switzerland: Zurich Open Repository and Archive: University of Zurich; 2012.