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Occurrence of Bovine Coxiellosis in Small Livestock Farms of Uttar Pradesh, India

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ABSTRACT

The present study aimed at assessing the occurrence of coxiellosis in cattle and buffaloes on 117 small-sized livestock farms owned by small or marginal farmers of 36 villages in Uttar Pradesh of India. The samples of blood and serum (250 each) collected from 86 cattle and 164 buffaloes were screened for *Coxiella burnetii* infection by PCR assay targeting *IS1111* transposase gene of the pathogen (trans-PCR), Latex Agglutination Test employing synthetic linear antigenic peptide (LAP) of Com-1 protein of *C. burnetii* (Com-1 LAP-LAT) and commercial ELISA kit (IDEXX), taken as a reference. In our study, none of the bovine blood or serum samples showed the presence of *C. burnetii* DNA in trans-PCR, however, antibodies against the pathogen could be detected in sera of 5.6% (14/250) animals by Com-1 LAP-LAT and 5.13% (13/250) animals by ELISA kit. A way forward is suggested for developing improvised LAT as a simple, pragmatic, affordable diagnostic test having field applicability by employing multiple antigenic peptides of potential immunogenic proteins of *C. burnetii*. The risk of bovine coxiellosis under small livestock settings seems to be less than that reported on organised farms in India; however, studies involving many farms with varying holdings of large ruminants, which constitute the main proportion of milch animals, are needed for assessing the risks in bovines and their handlers in the right perspective.

HIGHLIGHTS

- A seropositivity of 5.13% for coxiellosis was observed among bovines in small livestock farms of Uttar Pradesh.
- Developing an improvised LAT based on multiple antigenic peptides is suggested as a way forward.

Keywords: *C. burnetii*, Coxiellosis, Bovines, Latex, ELISA

Q fever (coxiellosis in animals) caused by *Coxiella burnetii* is a highly infectious bacterial zoonotic disease of great public health importance, which came into public and research prominence through a massive outbreak that occurred in the Netherland during the year 2007 to 2010 resulting in 4000 notified human cases and culling of 58,150 goats (Schneeberger *et al*., 2014). However, in the absence of an active disease surveillance, insufficient reliable epidemiological data, limited diagnostic facilities as well as low level of awareness about this disease among the general public, veterinarians and clinicians, it largely remains a masked and neglected zoonosis in India (Malik *et al.,* 2013; Sahu *et al.,* 2018; Sahu *et al.,* 2021).

The causal agent- *C. burnetii* is an obligate intracellular,

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gram-negative bacteria (Eldin *et al*., 2017) having a worldwide presence except New Zealand and Antarctica (Eldin *et al.,* 2017). The disease ranks among the 13 globally prioritized zoonoses, categorized by International Livestock Research Institute, Kenya (Grace *et al.,* 2012). Potentially, a single bacterium is capable of infecting humans and animals and therefore, requires biosafety level-3 laboratory facilities to handle it (Fournier *et al*., 1998). Recent scientific evidence shows that the infection can actively persist for more than 5 years in animal reservoirs like *Coxiella* infected sheep flocks and the contaminated dust in their environment if efficient control and biosafety methods are not properly implemented (Álvarez-Alonso *et al.,* 2020).

The clinical nature of coxiellosis in ruminants is frequently sub-clinical and can be manifested in the form of late abortions, stillbirths and reproductive disorders (Arricau- Bouvery and Rodolakis, 2005; Guatteoet *al.,* 2011; Mohabati Mobarez *et al.*, 2021). The key reservoirs of *C. burnetii* are the domestic ruminants, including goats (Rodolakis *et al.,* 2007; Van den Brom *et al.,* 2015), Sheep (Álvarez-Alonso *et al.,* 2020) and cattle (Mccaughey *et al.,* 2010) as they excrete high numbers of pathogen, primarily in their reproductive discharges (Eldin *et al*., 2017; Álvarez-Alonso *et al.,* 2020). It is worth mentioning that shedding of this bacteria in cattle milk also lasts for a long time(Plummer *et al.,* 2018; Szymanska-Czerwinska *et al*., 2019), which potentially poses higher risk to other healthy animals and their handlers (Dhaka *et al.,* 2019).

The global prevalence of coxiellosis has been approximated to be 20% at the individual level in cattle (Guatteo *et al*., 2011). In case of India, the apparent prevalence of bovine coxiellosis in the diverse geographical areas of four states (Uttar Pradesh, Rajasthan, Haryana and Kerala), as revealed by molecular and/or serological tests, has been reported to be 24.5% and 8.9%, respectively (Dhaka *et al.*, 2020); while the overall prevalence has been recorded as 7.0% in Punjab (Keshavamurthy *et al*., 2019), 27.1% in Bihar and 5.8% in Assam (Shome *et al*., 2019). Animal farming having the predominance of small-scale dairy farming, which comprises of barely 5% of farmers having more than 5 dairy animals (Kumar, 2016),remains a major source of livelihood in rural parts of India (Douphrate *et al.,* 2013).

The diagnosis of coxiellosis under proper laboratory settings is commonly performed by employing either molecular tests (commonly as Trans-PCR, Com1-PCR, Real-time PCR, qPCR or LAMP); or serological tests (commonly as IFAT, ELISA or LAT) (Sahu *et al.,* 2018); or preferably, as a combination of these two approaches (Schneeberger *et al*., 2010). The IFAT is used as a reference test for serodiagnosis of Q fever in man (AFSSA, 2004); however, sero-screening of ruminants for coxiellosis is routinely and preferentially done by ELISA (Lurier *et al*., 2021). Recently, development a latex agglutination test (LAT) employing synthetic linear antigenic peptide (LAP) of Com-1 protein of *C. burnetii* has been reported for rapid, economical, reliable and on-site seroscreening for coxiellosis in cattle (Kumar *et al.,* 2019; Yadav *et al.,* 2020) and buffaloes (Yadav *et al.,* 2020).

The state of Uttar Pradesh (U.P.) has produced the highest amount of milk in India at about 30.5 million metric tons in the financial year 2019, which increased to 318.630 lakh metric tonnes in 2019-20 (DAHD report, 2020). In recent times, seroprevalence of bovine coxiellosis in U.P. have been carried out among slaughtered animals (Yadav *et al*.,2020) and on the organised dairy farms (Kumar *et al*., 2018; Dhaka *et al.,* 2020) as well as gaushalas or cattle shelters (Yadav *et al*., 2020); but not in the bovines reared in the backyard settings. Therefore, the present investigation was envisaged to assess the occurrence of bovine coxiellosis in small livestock farms owned by small or marginal farmers in U.P., using a combination of molecular and serological diagnostics.

MATERIALS AND METHODS

In the present investigation, a total of 500 clinical samples (blood-250, serum-250) were collected from 86 cattle and 164 buffaloes on some small livestock farms (117) owned by small or marginal farmers of 36 villages falling under 9 districts of U.P. State of India, namely Baghpat, Raibareilly Gautam Buddha Nagar, Ghaziabad, Mathura, Mau, Muzaffarnagar, Pratapgarh and Saharanpur; and subsequently screened for assessing *C. burnetii* infection by employing molecular and serological tests.

The blood sample (10 ml) from each animal was drawn aseptically in 2 aliquots (5 ml each) under the expert supervision of veterinarians. One aliquot was transferred to

a 5 ml vacutainer tube coated with K2EDTA (Vacutainer®), for whole blood, while another aliquot transferred to a 5 ml clot activator tube (BD Vacutainer® SST II Advance, Becton Dickinson, USA)) for serum separation. The samples were brought to the laboratory at the earliest in a thermocol box containing ice packs for maintaining cold chain. The blood samples in clot activator tubes were kept at 4˚C and centrifuged for 10 min at 2500 × *g* for the separation of serum. The serum and whole blood samples were kept at -20˚C, until analyzed by serological tests and PCR.

The bovine samples (blood and serum) were processed for genomic DNA extraction and purification by using DNeasy blood and tissue kit (Qiagen, Germany) and as per the appropriate protocols given by the manufacturer. The purity of DNA was checked using a Biospectrometer (Eppendorf GmbH, Germany). The DNA samples with an absorption ratio (A260/A280) of more than or equal to 1.80 were tested by trans-PCR, targeting the transposons-like regions in the chromosomal DNA of *C. burnetii*. The DNA of standard *C. burnetii* Nine Mile strain kindly supplied by Dr Eric Ghigo, URMITE-IRD, Faculté de Médecine, France was taken as a positive control in PCR. The trans-PCR assay employed the primer set comprising trans-1 (5'-TAT GTA TCC ACC GTA GCC AGT C-3') and trans-2 (5'-CCC AAC AAC ACC TCC TTA TTC-3') targeting the transposase gene of *C. burnetii*, with an expected PCR product of 687bp size (Berri *et al.,* 2000). Subsequent to the amplification of the target DNA by PCR, the resultant PCR products were analyzed by agarose gel electrophoresis and visualized by the gel documentation system (UVP Gel Seq Software). All the serum samples were tested for detecting anti-*Coxiella* antibodies by the LAT employing synthetic linear antigenic peptide (LAP) of Com-1 protein of *C. burnetii.,* i.e., Com1LAP-LAT (Kumar *et al*., 2019; Yadav *et al*., 2020) as well as a commercial indirect ELISA kit (IDEXX, Switzerland) taken as a reference method (Yadav *et al*., 2020). The Com1LAP-LAT has been reported to have a relative diagnostic sensitivity of 82.7% and diagnostic specificity of 79.9%, as compared to the commercial indirect ELISA kit manufactured by IDEXX, Switzerland, which is reported to have 100% specificity and 100% sensitivity (Emery *et al*., 2012; Yadav *et al*., 2020). The Com1LAP-LAT was standardized as per the protocol outlined by Kumar *et al.* (2019); Yadav *et al.* (2020) and used for the seroscreening of bovine samples. The commercial indirect ELISA kit (IDEXX, Switzerland) was procured and used as per the procedure given by the manufacturer.

RESULTS AND DISCUSSION

Of late, coxiellosis in humans and animals has been emerging or re-emerging in various parts of the globe (Angelakis and Raoult, 2011; Sahu *et al.,* 2020), including India (Sahu *et al.,* 2021).The positivity of large ruminants for coxiellosis in larger settings, such as organised dairy farms (Kumar *et al*., 2018; Dhaka *et al.,* 2020) and gaushalas or cattle shelters (Yadav *et al*., 2020) has been reported to range from 0.97% to 29.91% (Sahu *et al.,* 2021), however, studies conducted in small animal holdings are limited to certain geographical area (Shome *et al.,* 2019). In the present study, large ruminants (250) reared on small or marginal livestock farms (117) in Uttar Pradesh, the state having sizeable population of bovines as milch animals that contribute to make it the largest producer of milk in India, were tested for coxiellosis by molecular and serological tests performed on their blood (n= 250) and serum (n= 250) samples (Table 1).

The routinely employed sero-diagnostic assays for coxiellosis, such as IFA and ELISA not only have complex test protocols, need sophisticated laboratory facilities and technical expertise (Yadav *et al*., 2020) but also exhibit inconsistent sensitivity and specificity (Kumar *et al*., 2019). These limitations can be effectively addressed to a greater extent by resorting to novel diagnostics, such as synthetic peptide-based latex LAT diagnostic assays developed for the serodiagnosis of bovines coxiellosis, on account of being quick, affordable, reliable and user-friendly with on-site applicability (Kumar *et al.,* 2019). The cost of analyzing a single serum sample by Com-1 LAP LAT has been claimed to be nearly ten times cheaper compared to the commercial indirect-ELISA kit (Yadav *et al*., 2020). Therefore, a recently reported format of LAT (Com-1 LAP-LAT) and a commercial ELISA kit (IDEXX), which was taken as a reference for seroscreening of bovines for coxiellosis, were employed in the present research study, along with the Trans-PCR, as a highly sensitive and specific molecular test for the detection of pathogen in the blood and serum of selected bovine population.

In our study, none of the bovine blood or serum samples screened for the presence of *C. burnetii* DNA was found

Table 1: Screening of selected small livestock farms of U.P. for bovine coxiellosis by molecular and serological tests

Animal Species	Total Animals	Total number of samples screened		Number of samples detected as positive in molecular and serological tests (% positivity)		
		Blood	Serum	PCR (on blood & serum)	LAT (on serum)	I-ELISA (on serum)
Cattle	86	86	86	0	7 (8.13%)	8 (9.30%)
Buffaloes	164	164	164	0	7 (4.27%)	5 (3.05%)

positive in Trans-PCR, however, antibodies against the pathogen could be detected in sera of 5.6% (14/250) animals by Com-1 LAP-LAT and 5.13% (13/250) animals by ELISA kit. (Table 1).

It was noted that out of 250 bovine sera, 14 samples tested positive in Com-1 LAP-LAT, whereas 13 samples showed positivity for anti-*C. burnetii* IgG in indirect commercial ELISA kit, indicating chronic nature of coxiellosis in these animals. The higher seropositivity for coxiellosis in Com-1 LAP-LAT as compared to ELISA kit observed in our study can be ascribed to the enhanced diagnostic ability of LAT, as it could detect both IgG and IgM antibodies (Ybañez *et al*., 2020), while the commercial indirect-ELISA kit employed in the study could detect only IgG antibodies. Conversely, 3 bovine serum samples showing negativity in Com-1 LAP-LAT tested positive in ELISA, while 4 ELISA-negative samples showed positivity in LAT (Table 2; Table 3). In this context, it is significant to note that synthetic linear antigenic peptides (LAPs) suffer from some inherent drawbacks, such as poor coating efficiency and reactivity with test antibodies (Joshi *et al*., 2013), leading to lesser sensitivity and specificity of LAT in comparison to i-ELISA kit (Yadav *et al.,* 2020).

In view of the more positive cases detected in Com-1 LAP-LAT as compared to the commercial indirect ELISA kit (IDEXX, Switzerland) in our study, the diagnostic efficacy of LAT and ELISA kit needs to be validated on a large number of bovine serum samples, especially from the known positive cases from whom the *C. burnetii* has been successfully isolated, before recommending it as a routine seroscreening and/or confirmatory test for bovine coxiellosis. Moreover, the diagnostic efficacy of the LAT needs be improved either by employing alternative immunogenic peptides of *C. burnetii* constituent protein, such as chaperonin GroEL, Chaperone DnaK and 34 kDa outer member protein (ybgF) (Skultety *et al.,* 2011; Xiong *et al.*, 2012), or another orientation of the linear antigenic peptide (LAP) of Com1 protein of *C. burnetii*, wherein LAP molecules are conjugated in different forms for creating a branched structure, called as multiple antigenic peptides or MAP (Joshi *et al*., 2013). The use of MAP has been suggested to be the most promising approach for improving the diagnostic sensitivity and specificity of LAT, as the multimeric peptide mimics the native antigenic structure and provides enhanced surface-binding and epitope projection, moreover, it shows superior reactivity to monoclonal and polyclonal antibodies at lesser concentrations (Tam and Zavala, 1989).

The negativity of ELISA-positive serum in trans-PCR observed in our study might be due to the seroconversion following the immune response to *C. burnetii* infection in such animals, leading to the purging of the pathogen from the bloodstream of the infected animals (Schneeberger *et al*., 2010). In Uttar Pradesh, based on the seroprevalence reports in general, the occurrence of coxiellosis in cattle**,** has been recorded from a low of 2.85% (in bullock) and 5.58% (in cow) by capillary agglutination test (Ghosh *et al.*, 1976) to a high of 29.91 % by ELISA (Dhaka *et al*., 2019) and 29.90% by Com1-LAT (Yadav *et al*., 2020); whereas in case of buffaloes, it ranged from a low of 4.00% by complement fixation test (Agarwal and Negi, 1983) to a high of 18.18% by ELISA (Vaidya *et al.,* 2010). The positivity of cattle for coxiellosis on organised dairy farms in U.P. has been reported to be 12.03% in PCR and 14.81%, 16.2% and 23.1% in ELISA (Kumar *et al*., 2019; Dhaka *et al.,* 2020). In our study that was oriented to small livestock holdings, the seropositivity for coxiellosis recorded was rather low in case of cattle (9.30%) and buffaloes (3.05%), however, the difference between the seropositivity of these species was significant in chi-square test with a P value of 0.034 at 95% confidence interval (CI), which is in agreement with an earlier report, wherein similar trend was noticed at a significant level (Dhaka *et al.,* 2020). Moreover, the observed lower seropositivity of cattle and buffaloes for coxiellosis in the small or marginal livestock farms as compared to that reported by other workers on organised farms can be explained in the light of similar

Table 2: Comparative seropositivity for bovine coxiellosis in diagnostic tests

Com-1 LAP-LAT and commercial i-ELISA kit	Positive (+) sera		Negative (-) sera	
	Cattle	Buffalo	Cattle	Buffalo
Positive sera **(+)**	6(a)	4(a)	2(b)	2(b)
Negative sera **(-)**	2(c)	1(c)	79(d)	154(d)

Denotations:

Com-1- LAP: Com1 synthetic peptide in linear antigenic peptide (LAP) orientation

LAT: Latex Agglutination Test;

I-ELISA: Indirect immunosorbent assay

(a): Samples detected as true positives; (b): Samples detected as false positives; (c): Samples detected as false negatives; (d): Samples detected as true negatives.

Table 3: Animal-wise positivity for bovine coxiellosis in serological tests

Sl. No.	Animal ID	Animal species	I-ELISA	Com-1 LAP-LAT
1	3789	Cattle	+	+
2	Budson 6	Cattle	+	+
3	3623	Cattle	+	+
4	2855	Cattle	+	+
5	2060	Cattle	+	+
6	1082	Cattle	+	+
7	1286	Buffalo	+	+
8	1024	Buffalo	+	+
9	DAH 14	Buffalo	+	+
10	7671	Buffalo	+	+
11	4115	Cattle	+	-
12	No 42	Cattle	+	-
13	No 48	Buffalo	+	-
14	6378	Cattle	-	+
15	9468	Cattle	-	+
16	2292	Buffalo	-	+
17	5368	Buffalo	-	+

remarks made by others in terms of the difference in the farm management conditions, such as stall-feeding system and inadequate floor spacing in cattle sheds commonly observed in large dairy farms as well as higher odds of coxiellosis occurrence compared to semi extensive and backyard rearing of cattle (Dhaka *et al*., 2020). Large herd size present in a confined area has been reported to more frequently favour clinical conditions such as mastitis and reproductive disorders that are easily maintained in the farm settings (Bastan *et al.,* 2015; Patel *et al.,* 2014). Therefore, such conditions on the farm might also account for relatively higher positivity rate than the backyard settings (Dhaka *et al*., 2020). Earlier studies have also indicated that large herd size is positively associated *C. burnetii* infection in the herd (Van Engelen *et al.,* 2014). Moreover, the animals screened in our study had a lower average age (3.56 years), which might have resulted in the lower positivity rate, since older animals have been reported to have higher odds of *Coxiella* infection, especially after the first calving (McCaughey *et al*., 2010) and the probability of getting exposed to this pathogen also increases with the age of the animal (Barlozzari *et al*., 2020).

CONCLUSION

The risk of bovine coxiellosis under small livestock farm settings in U.P. appears to be of lesser magnitude than that reported earlier on the organised farms in U.P. as well as other states of India. However, studies involving more farms with different livestock holdings of large ruminants need to be carried out for the realistic appraisal of the hidden risk posed by *C. burnetii* infection in bovines and their handlers. Moreover, in view of encouraging results observed with Com-1 LAP-LAT used in our study, the development of an improvised LAT employing MAP of Com-1 or other potential immunogenic proteins of *C. burnetii* is suggested for a simple, pragmatic and affordable diagnostic solution in the field settings, as an adjunct or alternative to costly and lab-based tests, like ELISA, currently used for seroscreening of animals for coxiellosis.

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REFERENCES

AFSSA. 2004. Fièvre Q: Rapport sur l'évaluation des risques pour la santépublique et des outils de gestion des risquesenélevage de ruminants, pp. 1-88.

Agarwal, J.P. and Negi, S.K. 1983. Complement-fixing Q-fever antibodies in domestic animals and man. *Indian J. Comp. Microbiol. Immunol. Infect Dis*., **4**: 149–151.

Angelakis, E. and Raoult, D. 2011. Q fever. *Vet. Microbiol.,* **140**: 297–309.

Álvarez-Alonso, R., Zendoia, I.I., Barandika, J.F., Jado, I., Hurtado, A., López, C.M. and García-Pérez, A.L. 2020. Monitoring *Coxiella burnetii* infection in naturally infected dairy sheep flocks throughout four lambing seasons and investigation of viable Bacteria. *Frontiers in Vet. Sci.*, **7**: 352.

Arricau-Bouvery, N. and Rodolakis, A. 2005. Is Q fever an emerging or re-emerging zoonosis? *Vet. Res*., **36**: 327–349.

Barlozzari, G., Sala, M., Iacoponi, F., Volpi, C., Polinori, N., Rombolà, P., Vairo, F., Macrì, G. and Scarpulla, M., 2020. Cross-sectional serosurvey of *Coxiella burnetii* in healthy cattle and sheep from extensive grazing system in central Italy., *Epidemiol. Infect*, **148**.

Baştan, A., Salar, S., Cengiz, M., Darbaz, I., Demirel, M.A. and Özen, D. 2015. The prediction of the prevalence and risk factors for subclinical heifer mastitis in Turkish dairy farms. *Turkish J. Vet. Anim. Sci*., **39**(6): 682-687.

Berri, M., Laroucau, K. and Rodolakis, A. 2000.The detection of *Coxiella burnetii* from ovine genital swabs, milk and fecal samples by the use of a single touchdown polymerase chain reaction. *Vet. Microbiol*., **72**(3-4): 285-293.

DAHD report. 2020. State-wise estimates of milk production during 2014-15 to 2019-2020 (Figures in '000' Tonnes). *In:* Basic Animal Husbandry Statistics-2020. Department of Animal Husbandry and Dairying, Ministry of Fisheries Animal Husbandry and Dairying, Krishi Bhavan, New Delhi, pp. 30.

Dhaka, P., Malik, S.S., Yadav, J.P., Kumar, M., Baranwal, A., Barbuddhe, S.B. and Rawool, D.B. 2019. Seroprevalence and molecular detection of coxiellosis among cattle and their human contacts in an organized dairy farm. *J. Infect. Public Heal*., **12**(2): 190-194.

Dhaka, P., Malik, S.V.S., Yadav, J.P., Kumar, M., Barbuddhe, S.B. and Rawool, D.B. 2020. Apparent prevalence and risk factors of coxiellosis (Q fever) among dairy herds in India. *PloS one*., **15**(9): p.e0239260.

Douphrate, D.I., Hagevoort, G.R., Nonnenmann, M.W., Lunner Kolstrup, C., Reynolds, S.J., Jakob, M. and Kinsel, M. 2013. The dairy industry: a brief description of production practices, trends and farm characteristics around the world. *J. Agromedicine*., **18** (3): 187-197.

Eldin, C., Mélenotte, C., Mediannikov, O., Ghigo, E., Million, M., Edouard, S., Mege, J.L., Maurin, M. and Raoult, D. 2017. From Q fever to *Coxiella burnetii* infection: a paradigm change. *Clin. Microbiol. Rev*., **30**(1): 115-190.

Emery, M.P., Ostlund, E.N. and Schmitt, B.J. 2012. Comparison of Q fever serology methods in cattle, goats and sheep. *J. Vet. Diagn. Invest.,* **24**(2): 379-382.

Fournier, P.E., Marrie, T.J. and Raoult, D. 1998. Diagnosis of Q fever. *J. Clin. Microbiol*., **36**: 1823-1834.

Ghosh, S.S., Mittal, K.R. and Sen, G.P. 1976. Incidence of Q-fever in man and animals. *Indian J. Anim. Heal*., **15**: 79–80.

Grace, D., Mutua, F., Ochungo, P., Kruska, R., Jones, K., Brierley, L., Lapar, L., Said, M., Herrero, M., Phuc, P.M., Thao, N.B.,

Akuku, I. and Ogutu, F. 2012. Mapping of poverty and likely zoonoses hotspots. Zoonoses Project 4. *In: Report to the UK Department for International Development. Nairobi, ILRI*., pp. 119.

Guatteo, R., Seegers, H., Taurel, A.F., Joly, A. and Beaudeau, F. 2011. Prevalence of *Coxiella burnetii* infection in domestic ruminants: A critical review. *Vet. Microbiol*., **149**(1-2): 1-16.

Joshi, V.G., Dighe, V.D., Thakuria, D., Malik, Y.S. and Kumar, S. 2013. Multiple antigenic peptide (MAP): a synthetic peptide dendrimer for diagnostic, antiviral and vaccine strategies for emerging and re-emerging viral diseases. *Indian J. Virol.,* **24**(3): 312-320.

Keshavamurthy, R., Singh, B.B., Kalambhe, D.G., Aulakh, R.S. and Dhand, N.K. 2019. Prevalence of *Coxiella burnetii* in cattle and buffalo populations in Punjab, India. *Prev. Vet. Med*., **166**: 16-20.

Kumar, A.2016. A Field Approach to Mastitis Control in India. *In: Proceedings of 6th IDF International Mastitis Conference*. Sept. 2016, Nantes, France, pp. 7-9.

Kumar, M. 2018. Screening of bovines and their human contacts for coxiellosis in farms and gaushalas by molecular and serological tests. Thesis, PhD. Deemed University, Indian Veterinary Research Institute, Izatnagar, India.

Kumar, M., Malik, S.S., Vergis, J., Ramanjeneya, S., Sahu, R., Pathak, R. and Rawool, D.B. 2019. Development of the Com1 synthetic peptide-based Latex Agglutination Test (LAT) and its comparative evaluation with commercial indirect-ELISA for sero-screening of coxiellosis in cattle. *J. Microbiol. Methods*., **162**: 83-85.

Lurier, T., Rousset, E., Gasqui, P., Sala, C., Claustre, C., Abrial, D., Dufour, P., de Crémoux, R., Gache, K., Delignette-Muller, M.L. and Ayral, F. 2021. Evaluation using latent class models of the diagnostic performances of three ELISA tests commercialized for the serological diagnosis of *Coxiella burnetii* infection in domestic ruminants. *Vet. Res*., **52**(1):1-17.

Malik, S.V.S., Das, D.P., Suryawanshi, R.D. and Vergis, J. 2013. Global perspective of zoonotic diseases and their management. *In:* Training Manual on "Recent Advances in Animal Disease Diagnosis and their Treatment", Feb, 2013, Indian Veterinary Research Institute, Izatnagar, India, pp. 90-104.

Mccaughey, C., Murray, L.J., McKenna, J.P., Menzies, F.D., McCullough, S.J., O'neill, H.J., Wyatt, D.E., Cardwell, C.R. and Coyle, P.V. 2010. *Coxiella burnetii* (Q fever) seroprevalence in cattle. *Epidemiol. Infect.*, **138**(1): 21-27.

Mohabati Mobarez, A., Khalili, M., Mostafavi, E. and Esmaeili, S, 2021. Molecular detection of *Coxiella burnetii* infection in aborted samples of domestic ruminants in Iran. *PLoS ONE.,* **16**(4): e0250116

Patel, M.D., Patel, P.R., Prajapati, M.G., Kanani, A.N., Tyagi, K.K. and Fulsoundar, A.B. 2014. Prevalence and risk factor's analysis of bovine brucellosis in peri-urban areas under intensive system of production in Gujarat, India. *Vet. World.*, **7**(7).

Plummer, P.J., McClure, J.T., Menzies, P., Morley, P.S., Van den Brom, R., Van Metre, D.C., Heilmann, R.M., Steiner, J.M., Hezzell, M.J., Block, C.L. and Laughlin, D.S. 2018. Management of *Coxiella burnetii* infection in livestock populations and the associated zoonotic risk: A consensus statement. *Consensus Statement*., **32(**5).

Rodolakis, A., Berri, M., Hechard, C., Caudron, C., Souriau, A., Bodier, C.C., Blanchard, B., Camuset, P., Devillechaise, P., Natorp, J.C., Vadet, J.P. and Arricau-Bouvery, N. 2007. Comparison of *Coxiella burnetii* shedding in milk of dairy bovine, caprine and ovine herds. *J. Dairy Sci.,* **90**: 5352-5360.

Skultety, L., Hajduch, M., Flores-Ramirez, G., Miernyk, J.A., Ciampor, F., Toman, R. and Sekeyova, Z. 2011. Proteomic comparison of virulent phase I and avirulent phase II of *Coxiella burnetii*, the causative agent of Q fever. *J. Proteomics.,* **74**(10): 1974-1984.

Sahu, R., Kale, S.B., Vergis, J., Dhaka, P., Kumar, M., Choudhary, M. and Kurkure, N.V. 2018. Apparent prevalence and risk factors associated with occurrence of *Coxiella burnetii* infection in goats and humans in Chhattisgarh and Odisha, India. *Comp. Immunol. Microbiol. Infect. Dis*., **60**: 46-51.

Sahu, R., Rawool, D.B., Vinod, V.K., Malik, S.V.S. and Barbuddhe, S.B. 2020. Current approaches for the detection of *Coxiella burnetii* infection in humans and animals. *J. Microbiol. Methods.*, **179**: 106087.

Sahu, R., Rawool, D.B., Dhaka, P., Yadav, J.P., Mishra, S.P., Kumar, M., Vergis, J., Malik, S.V. S. and Barbuddhe, S.B. 2021. Current perspectives on the occurrence of Q fever: highlighting the need for systematic surveillance for a neglected zoonotic disease in Indian subcontinent. *Environ. Microbiol. Rep.,* **13**(2): 138-158.

Schneeberger, P.M., Hermans, M.H., van Hannen, E.J., Schellekens, J.J., Leenders, A.C. and Wever, P.C. 2010. Real-time PCR with serum samples is indispensable for early diagnosis of acute Q fever. *Clin*. *Vaccine Immunol*., **17**: 286–90.

Schneeberger, P.M., Wintenberger, C., van der Hoek, W. and Stahl, J.P. 2014. Q fever in the Netherlands—2007–2010: What we learned from the largest outbreak ever. *Med. Mal. Infect*., **44**: 339–353.

Shome, R., Deka, R.P., Milesh, L., Sahay, S., Grace, D. and Lindahl, J.F. 2019. *Coxiella* seroprevalence and risk factors in large ruminants in Bihar and Assam, India. *Acta Tropica*., **194**: 41-46.

Szymańska-Czerwińska, M., Jodełko, A., Zaręba-Marchewka, K. and Niemczuk, K. 2019. Shedding and genetic diversity of *Coxiella burnetii* in Polish dairy cattle. *PLoS One*., **14**: e0210244

Tam, J.P. and Zavala, F. 1989. Multiple antigen peptide. A novel approach to increase detection sensitivity of synthetic peptides in solid-phase immunoassays. *J. Immunol. Methods*, **124**: 53– 61

Vaidya, V.M., Malik, S.V.S., Bhilegaonkar, K.N., Rathore, R.S., Kaur, S. and Barbuddhe, S.B. 2010. Prevalence of Q fever in domestic animals with reproductive disorders. *Comp. Immunol. Microbiol. Infect. Dis*., **33**: 307-321.

Van den Brom, R., Van Engelen, E., Roest, H.I.J., Van der Hoek, W. and Vellema, P. 2015. *Coxiella burnetii* infections in sheep or goats: an opinionated review. *Vet. Microb*., **181**(1-2): 119-129.

Van Engelen, E., Schotten, N., Schimmer, B., Hautvast, J.L. A., Van Schaik, G. and Van Duijnhoven, Y.T.H.P. 2014. Prevalence and risk factors for *Coxiella burnetii* (Q fever) in Dutch dairy cattle herds based on bulk tank milk testing. *Prev. Vet. Med*., **117**(1): 103-109.

Xiong, X., Wang, X., Wen, B., Graves, S. and Stenos, J. 2012. Potential serodiagnostic markers for Q fever identified in *Coxiella burnetii* by immunoproteomic and protein microarray approaches. *BMC Microbiol*., **12**(1): 35.

Yadav, J.P., Malik, S.V.S., Dhaka, P., Kumar, M., Sirsant, B., Gourkhede, D., Barbuddhe, S.B. and Rawool, D.B. 2020. Comparison of two new in-house Latex Agglutination Tests (LATs), based on the DnaK and Com1 synthetic peptides of *Coxiella burnetii*, with a commercial indirect-ELISA, for sero-screening of coxiellosis in bovines. *J. Microbiol. Methods*., **170**: 105859.

Yadav, J.P., Malik, S.V.S., Dhaka, P., Kumar, M., Bhoomika, S., Gourkhede, D., Kumar, B., Ram Verma, M., Barbuddhe, S.B. and Rawool, D.B. 2019. Seasonal variation in occurrence of Coxiella *burnetii* infection in buffaloes slaughtered in India. *Biol. Rhythm Res*., **52**(4): 615-621.

Yadav, J.P., Malik, S.V.S., Dhaka, P., Kumar, A., Kumar, M., Bhoomika, S., Gourkhede, D., Singh, R.V., Barbuddhe, S.B. and Rawool, D.B. 2020. *Coxiella burnetii* in cattle and their human contacts in a gaushala (cattle shelter) from India and its partial *com* 1 gene sequence-based phylogenetic analysis. *Anim.Biotechnol*.,:1-10.

Ybañez, R.H.D., Ybañez, A.P. and Nishikawa, Y. 2020. Review on the Current Trends of Toxoplasmosis Serodiagnosis in Humans. *Front. Cell. Infect. Microbiol*., **10**: 204.