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RESEARCH ARTICLE

Sero-Prevalence and Associated Risk Factors of Q Fever in Cattle and Buffaloes Managed at Institutional Dairy Farms

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A B S T R A C T

The present study was conducted to determine the sero-prevalence of Q fever and associated risk factors in institutional dairy farms in Punjab, Pakistan. A total 11 dairy farms were investigated from different areas of Punjab province. Out of these farms, a total of 827 animals (cows n=419 and buffaloes n=408) were investigated. Presence of anti-*Coxiella burnetii* antibodies was determined by indirect ELISA. The overall prevalence in dairy animals (cattle and buffaloes) was 6.1% (95% CI: 4.5-7.9). In cattle prevalence was higher (7.6%; 95% CI: 5.3-10.6) than in buffaloes (4.4%; 95% CI: 2.6-6.9). The probability of occurrence of disease in cattle was higher (7.63%; OR=1.9, 95% CI: 0.99-3.25) than in buffalo (1.00%; OR=1.00) The difference in sero-prevalence between the two-dairy species was significant (p=0.052, $x^2 = 3.786$). Stratification of Q fever as per the breed of cattle indicated that the sero-prevalence was the highest (13%) in Cholistani followed by Sahiwal (7.5%) and cross bred (2.6%). Animals age between 2.1 to 5 year (OR=2.56, 95% CI 1.05, 6.20), contact with small ruminants (OR=1.43, 95% CI 0.63, 3.25), tick infestations (OR=4.91, 95% CI 2.40, 10.00), dry-dusty environment (OR=1.55, 95% CI 0.54, 4.43), retained fetal membranes (OR=1.68, 95% CI 0.94, 5.73) and abortion history (OR=2.17, 95% CI 0.88, 5.35) were risk factors for the seropositivity of Q fever. The results of the study indicated that Q fever is prevalent in cattle and buffalo managed at institutional dairy farms in Punjab.

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INTRODUCTION

Q fever (Query fever) or coxiellosis is ubiquitous zoonotic disease except in New Zealand (Hechemy, 2012). It is caused by a highly contagious Gram's negative obligate and intracellular bacterium *Coxiella burnetii*. The etiologic agent has erstwhile been classified as a rickettsial organism, however, currently, it is classified as an individual group (Seshadri *et al*., 2003). A bewildering variety of animal species are affected by *C. burnetii*. Nonetheless, dairy animals including cattle, buffaloes, goats as well as sheep are considered as the primary reservoirs of this pathogen (Radostits *et al*., 2007).

In most infected hosts, Q fever is usually an asymptomatic disease. Clinical manifestations of Q fever in animals are mostly referable to involvement of reproductive system and the disease may cause abortion, stillbirth, retained placenta, infertility and birth of weak newborn etc. Mastitis could be observed in dairy animals. Different animal species show different semiology (*i.e.* clinical picture). In cattle, sporadic abortions, low birth weights, and infertility are more common than in sheep and goats. Abortion in sheep and goats occurs in last trimester of pregnancy (Radostits *et al*., 2007). Humans exhibit a flu-like illness (characterized by fever, headache, chills and fatigue), followed by a typical pneumonia in acute cases, while chronic cases develop endocarditis and hepatitis (Agerholm, 2013).

Q fever is a zoonotic disease, which mainly affects the domestic animals. Affected animals get infection by biting of infected ticks and inhaling the Q fever causative organism from contaminated environment (de Valk, 2012). Q fever infection is often an occupational disease. Animal health personnel are exposed directly or indirectly

to infection during surgery and physical examination of animals (Meadows *et al*., 2017). Ever since its discovery, Q fever has been considered one of the important human and animal diseases. Humans having occupational contacts with animals have a higher risk of sero-positivity to *C. burnetii* (Hechemy, 2012; Meadows *et al*., 2017).

Q fever is endemic in many countries of the world. A survey conducted in 51 countries including Pakistan confirmed the prevalence of Q fever (Kaplan and Bertagna, 1955). The prevalence of Q fever in animals has also been reported from countries neighboring Pakistan i.e. Iran (Nokhodian *et al*., 2017), India (Yadev and Sethi, 1979) and China (Li *et al.,* 2018). Reports on the prevalence of Q fever in Pakistani dairy animals are extremely sparse and limited only to 4 studies thus far over the decade of 63 years (Ahmad, 1987; Shabbir *et al*., 2016; Zahid *et al*., 2016). Although Q fever is a ubiquitous zoonotic disease, so far it has been neglected in Pakistan. The present study was planned to determine the sero-prevalence of Q fever and associated risk factors in cows and buffaloes managed at institutional dairy farms in Punjab, Pakistan.

MATERIALS AND METHODS

Study locales and herd structure profiles: A total of 11 institutional dairy farms under the administered control of Livestock and Dairy Development (L&DD) Department, Government of the Punjab and University of Agriculture, Faisalabad were selected for sero-prevalence of Q fever in cows and buffaloes from different areas of the Punjab province, Pakistan. These farms were selected on the basis of their geographical distribution and number of animals managed. Farms that managed less than 70 dairy animals were not included in the research on these livestock farms. Dairy animals were identified by ear tag.

Sample size estimation and collection of samples: Sample size was estimated by considering an unknown status of Q fever in dairy animals in Punjab province. Number of animals to be sampled was calculated by keeping the expected prevalence as 50% with 95% confidence interval (CI) and 5% desired absolute precision (Thrusfield, 2007). Sampling period spanned over 6 and embraced 3 seasons (spring, rainy and winter). Data regarding herds were recorded on a pre-designed questionnaire.

Four ml blood was drawn from the jugular vein of each animal with a 16 G (3.81 cm long) needle into commercially available blood collecting vials with vacuum and clot activating gel. Sera were harvested by spinning the blood collecting vials in centrifuge machine (Hettich™ Zentrifugen, Germany) at 6000 rpm for 15 minutes, and stored in freezer at -80°C till analysis.

Enzyme Linked Immunosorbent Assay (ELISA) for determination of prevalence of *C. burnetii* antibodies. The sera were tested for the presence of anti-*C. burnetii* antibodies using a commercially available enzyme linked immunosorbent assay kit (LSI Vet.®, France) following the instructions given by the manufacturer. Briefly, the reagents of ELISA kit were placed at room temperature prior to test. First two wells (A1 and B1) were filled with positive control serum while next two wells (i.e. C1 and D1) were inoculated with negative control serum. Optical density (OD) of ELISA plates were read at the wavelength of 450 nm in ELISA reader (BioTeck®, BioTeck Instruments, Inc., USA). Sample positive percentage (SP%) for each serum sample was determined using the following formula:

$$\frac{OD_{sample} - OD_{negative}}{OD_{positive} - OD_{negative}} \times 100$$

The interpretation of SP values described by manufacturer is as follows: ≤40%=negative; ≥40% SP ≤50%=doubtful; >50%=positive.

Associated risk factors: Associated risk factors *viz.* age, species (cattle and buffalo), sex, breed, feeding pattern, type of herd, contact with small ruminants, tick infestation, breeding methods, farm environment, reproductive disorders and abortion history were recorded through pre-designed proforma.

Statistical analysis: Prevalence (%), 95% confidence interval (CI) and Chi-square testing were performed to find out significant difference among sex, age and associated factors. Univariate analysis was performed to calculate Odds ratio (OR) for different determinants of disease. The associations between the outcome response variables (sero-prevalence) and explanatory variables were determined using binary logistic regression for prediction of sero-prevalnce of Q fever were calculated using IBM SPSS Statistics 17.0 for Windows®, IBM Corporation, New York, USA.

RESULTS

ELISA based sero-prevalence of Q fever: A total of 827 cows (n=419) and buffaloes (n=408) were investigated for the sero-prevalence of Q fever. The overall sero-prevalence of Q fever in cows and buffaloes was 6.1% (95% CI: 4.5-7.9). Ten of 11 farms (90.90%) were found as positive for Q fever antibodies in ELISA. The percent Q fever prevalence on the farms ranged from 0% to 13%. The sero-prevalence of Q fever in GLF-J was the highest (13%) followed by LPRI (12.9%), BRI (7.1%), LES-KB (5.3%), GLF-F (4.4%), LES-UAF (3.9%), LES-H (2.9), LES-JA (2.4%), GLF-K (1.3%) and LES-RG (1.1%). None of the animal from LES-CK was seropositive for Q fever (Fig. 1). The optical density (OD) values of ELISA positive animals were ranged from (40.207 to 207.230).

Stratification of Q fever sero-prevalence as per the breed of cattle indicated that the sero-prevalence was the highest (13%) in Cholistani followed by Sahiwal (7.5%) and cross bred (2.6%). All buffaloes (n=408) belonged to Nili-Ravi breed. Sero-prevalence of Q fever in buffalo was 4.4% (18 out of 408 animals, 95% CI: 2.6-6.9). Sero-prevalence of Q fever was the highest (7.8%) in age group 2 (2.1-5year old) followed by group 2 (<5 year old; 3.2%) and 1 (≥2 years old; 5.1%). Only female animals tested positive for Q fever with sero-prevalence of 6.5% (95% CI: 4.8-8.4). None of male animal (0%; 95% CI: 0-6.7) was positive for the antibodies against *C. burnetii*. The sex specific variable was non-significant P=0.067.

Table 1: Univariable analysis of the Q fever sero-prevalence at 11 institutional farms in Punjab province, Pakistan

Variable	Category	Q Fever					P Value
		Pos. / Tested	Prev. %	95% CI	OR	95% CI	
Farm	GLFJ, Bahawalpur	6/46	13.0	4.9,26.3	13.35	1.56, 114.56	chi=32.476, P<0.001
	LPRI, BN, Okara	25/194	12.9	8.5,18.4	13.17	1.76, 98.77	
	BRI, Pattoki	5/71	7.0	2.3,15.7	6.74	0.77, 59.08	
	LES, Khushab	4/77	5.2	1.4,12.8	4.88	0.53,44.59	
	GLF, Fazil Pur	1/21	4.8	0.1,23.8	4.45	0.27, 74.21	
	LES, UAF	4/103	3.9	1.1,9.6	3.59	0.39, 32.78	
	LES, Haroonabad	1/35	2.9	0.1,14.9	2.62	0.16, 43.04	
	LES, Jahangirabad	2/84	2.4	0.3,8.3	2.17	0.19, 24.39	
	GLF, Kallurkot	1/75	1.3	0.0,7.2	1.20	0.07, 19.56	
	LES,R.Ghulaman	1/90	1.1	0.0,6.0	1.00	-	
	LES, CK, Hasilpur	0/31	0.0	0.0,11.2	-	-	
Age Groups	Above 5 years	11/217	5.1	2.6,8.9	1.61	0.58, 4.44	chi=5.310, P=0.070
	2.1 to 5 years	33/423	7.8	5.4,10.8	2.56	1.05, 6.20	
	≤ 2 years	6/187	3.2	1.2,6.9	1.00	-	
Sex	Female	50/774	6.5	4.8,8.4	-	-	chi=3.644, P=0.056
	Male	0/53	0.0	0.0,6.7	-	-	
Breed	Cross bred	1/38	2.6	0.1,13.8	1.00	-	chi=7.847, P=0.049
	Buffalo	18/408	4.4	2.6,6.9	1.71	0.22, 13.16	
	Sahiwal	25/335	7.5	4.9,10.8	2.98	0.39, 22.67	
	Cholistani	6/46	13.0	4.9,26.3	5.55	0.64, 48.30	
Species	Buffalo	18/408	4.4	2.6,6.9	1.00	-	chi=3.786, P=0.052
	Cattle	32/419	7.6	5.3,10.6	1.79	0.99, 3.25	
Feed pattern	Pen	4/103	3.9	1.1,9.6	1.00	-	chi=0.969, P=0.325
	Mixed	46/724	6.4	4.7,8.4	1.68	0.59, 4.77	
Herd Type	Mixed	3/159	1.9	0.4-5.4	1.00	-	chi=5.995, P=0.014
	Dairy	47/668	7.0	5.2-9.2	3.94	1.21, 12.81	
Species Contact	Alone	7/154	4.5	1.8-9.1	1.00	-	chi=0.750, P=0.386
	with small ruminants	43/673	6.4	4.7-8.5	1.43	0.63, 3.25	
Tick	Yes	12/59	20.3	11-32.8	4.91	2.40, 10.00	chi=22.849, P<0.001
	No	38/768	4.9	3.5-6.7	1.00		
Breeding	Artificial insemination (AI)	46/724	6.4	4.7,8.4	1.68	0.59, 4.77	chi=0.969, P=0.325
	Mixed	4/103	3.9	1.1,9.6	1.00	-	
Environment	Dry, Hot	6/46	13.0	4.9,26.3	3.71	0.99, 13.86	chi=4.839, P=0.089
	Dry, Dusty	40/678	5.9	4.2,7.9	1.55	0.54, 4.43	
	Dry	4/103	3.9	1.1,9.6	1.00	-	
Reproductive Disorders	No	40/758	5.3	3.8,7.1	1.00	-	chi=19.410, P=0.002
	RFM	3/35	8.6	1.8,23.1	1.68	0.94, 5.73	
	Ovarian Problem	1/11	9.1	0.2-41.3	1.79	0.22, 14.37	
	Infertility	1/6	16.7	0.4-64.1	3.59	0.41, 31.46	
	Uterine Prob.	2/8	25.0	3.2-65.1	5.98	1.17, 30.59	
	Premature / Repeat Breeder	3/9	33.3	7.5-70.1	8.98	2.17, 37.21	
Abortion	Yes	6/52	11.5	4.4-23.4	2.17	0.88, 5.35	chi=2.947, P=0.086
	No	44/775	5.7	4.2-7.5	1.00	-	

BRI= Buffalo Research Institute, Pattoki, GLF-F= Livestock Experiment Station, Fazilpur, GLF-K = Government Livestock Farm, Kallurkot, GLF-J = Government Livestock Farm, Jogaitpeer, LES-CK = Livestock Experiment Station, Chak Katora, LES-H = Livestock Experiment Station, Haroonabad, LES-JA = Livestock Experiment Station, Jahangirabad, LES-KB = Livestock Experiment Station, Khushab, LES-UAF = Livestock Experiment Station, University of Agriculture, Faisalabad, LES-RG = Livestock Experiment Station, Rakh Ghulaman, LPRI = Livestock Production and Research Institute, Bahadar Nagar, Okara.

Table 2: Binary logistic regression model for the prediction of Q fever in cattle and buffalo sampled at various farms

Exposure Variable	Comparison	Odds Ratio	95% CI	p value
Age				0.010
2.1 to 5 years old	<= 2 years old	3.87	1.49, 10.10	0.006
Above 5 years old	<= 2 years old	1.93	0.65, 5.70	0.234
Cattle	Buffalo	3.31	1.72, 6.36	<0.001
Dairy Herd	Mixed Herd	8.64	2.45, 30.47	0.001
Tick Infestation	No tick infestation	6.43	2.96, 13.97	<0.001
Reproductive Disorders				0.004
RFM	No disorder	11.20	2.40, 52.24	0.002
Ovarian Problem	No disorder	12.22	2.14, 69.69	0.005
Infertility	No disorder	3.76	0.37, 38.57	0.265
Uterine Problem	No disorder	0.86	0.10, 7.13	0.885
Premature / Repeat Breeder	No disorder	1.01	0.28, 3.69	0.989

Univariable analysis was performed to evaluate the relationship between individual and herd level variables and sero-prevalence. A total of 12 variables, *viz*., age, species (cattle and buffalo), sex, breed, feeding pattern, type of herd, contact with small ruminants, tick infestation, breeding methods, farm environment, reproductive disorders and abortion history were analyzed. Sero-prevalence in cattle and buffalo was influenced by age, species/breed, geographical location, reproductive disorders and environment (Table 1).

Briefly, animals between 2.1 and 5 years of age (OR=2.56, 95% CI 1.05, 6.20) and above 5 years of age (OR=1.61, 95% CI 0.58, 4.44) were more likely to test positive as compared to young (OR=1.00; less than equal to 2 years) animals. The Cholistani (OR=5.55, 95% CI 0.64, 48.30), Sahiwal (OR=2.98, 95% CI 0.39, 22.67)

breeds of cattle were more likely to be seropositive as compared to cross bred cattle. Moreover, the chances of being seropositive were higher in animals belonging to dairy herds (OR=3.94, 95% CI 1.21, 12.81) as compared to mixed herds. The cattle and buffaloes having contact with small ruminants (OR=1.43, 95% CI 0.63, 3.25), tick infested animals (OR=4.91, 95% CI 2.40, 10.00) and the animals bred using artificial insemination (AI) were more likely to test positive (OR=1.68, 95% CI 0.59, 4.77). Farms with dry and hot (OR=3.71, 95% CI 0.99, 13.86) and dry and dusty environment (OR=1.55, 95% CI 0.54, 4.43) were more likely to test positive as compared to dry and clean (OR=1.00).

Animals suffering from reproductive problems including retained fetal membranes (OR=1.68, 95% CI 0.94, 5.73), ovarian disorders (OR=1.79, 95% CI 0.22, 14.37), infertility (OR=3.59, 95% CI 0.41, 31.46), uterine disorders (OR=5.98, 95% CI 1.17, 30.59), premature birth / repeat breeders (OR=8.98, 95% CI 2.17, 37.21) and history of abortion (OR=2.17, 95% CI 0.88, 5.35) were more likely to test positive.

For the multivariate analysis and all variables with $p \leq 0.20$ found in bivariable analysis were used in a binary logistic regression model (Table 2). Animals between 2.1 to 5 years of age (OR=3.87, 95% CI 1.49, 10.10), single dairy herds (OR=8.64, 95% CI 2.45, 30.47) and tick infestation (OR=6.43, 95% CI 2.96, 13.97) retention of fetal membrane (OR=11.20, 95% CI 2.40, 52.24), ovarian problems (OR=12.22, 95% CI 2.14, 69.69) and infertility (OR=3.76, 95% CI 0.37, 38.57) were the risk factors for the sero-positivity of Q fever at institutional dairy farms.

DISCUSSION

Q fever is endemic in many countries of the world. A perusal of literature indicated that so far, only 4 reports have documented the prevalence of Q fever in Pakistan (Kaplan and Bertagna, 1955; Ahmad, 1987; Zahid *et al*., 2016; Shabbir *et al*., 2016). These studies have limitations in study design. To address this void, the present study was designed to investigate the sero-prevalence and was carried out. A total of 827 animals (n=419 cows and n=408 buffaloes) were investigated. Sero-prevalence of Q fever was determined in two species *viz.* cow and buffalo. In cattle prevalence of Q fever was highest (7.63%) than in buffaloes (4.4%). The overall sero-prevalence of Q fever was 6.1%. With the exception of HS, the overall susceptibility of buffalo to infectious diseases is lower than those in cattle (Dua, 2003). The reports on prevalence of Q fever in water buffalo (*Bubalus bubalis*) are by far fewer than those on cow. The prevalence of Q fever was ranged from 4.8% to 17.5% in water buffalo (Kaplan and Bertagna, 1955; Perugini *et al*., 2009; Gunaydin and Pekkaya, 2016; Klemmer *et al.*, 2018). Results of present study are in agreement with those of an Indian study (cattle=24.29%, buffalo=16.02%; Yadav and Sethi, 1979) and Egyptian study (cattle=19.3%, Buffalo= 11.2%; Klemmar *et al.*, 2018) that sero-prevalence of Q fever in cattle was higher than buffaloes. A lower susceptibility of dairy buffalo than that of cow is not limited to Q fever only. Compared with cow, buffalo is less susceptible to severe other infectious diseases like mastitis (Muhammad *et al*., 2010).

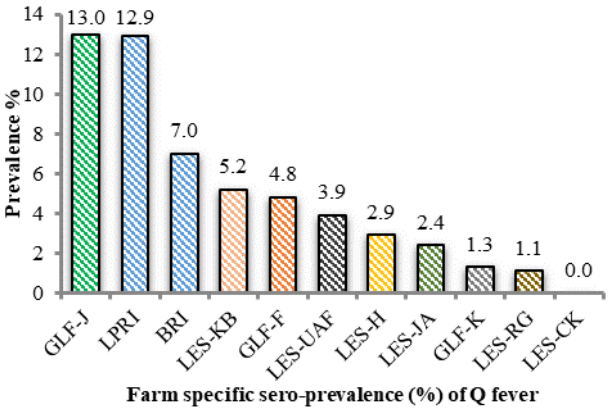

Fig. 1: Graphical presentation of farm specific ELISA-based sero-prevalence of Q fever on 11 institutional dairy farms in Punjab province, Pakistan. BRI= Buffalo Research Institute, Pattoki, GLF-F= Livestock Experiment Station, Fazilpur, GLF-K=Government Livestock Farm, Kallurkot, GLF-J=Government Livestock Farm, Jogaitpeer, LES-CK= Livestock Experiment Station, Chak Katora, LES-H=Livestock Experiment Station, Haroonabad, LES-JA=Livestock Experiment Station, Jahangirabad, LES-KB=Livestock Experiment Station, Khushab, LES-UAF=Livestock Experiment Station, University of Agriculture, Faisalabad, LES-RG=Livestock Experiment Station, Rakh Ghulaman, LPRI=Livestock Production and Research Institute, Bahadar Nagar, Okara.

Ten of 11 farms (90.90%) tested positive for Q fever antibodies in ELISA. The percent Q fever prevalence on the 11 dairy farms ranged from 0 to 13%. Sero-prevalence was higher in those areas where herd mix with small ruminants. The difference among 11 dairy farms can be attributed to dry and dusty weather, low vegetation, unusual dusty winds, harsh environment, poor management, un-planned construction of dairy sheds and un-hygienic practices. These associated factors were noted on pre-designed questionnaire during the sampling. These findings are in agreement with those of previous studies that transmission of *C. burnetii* is associated with dry environment (van der Hoek *et al*., 2011). The difference among the herds might be associated with difference in management and hygienic conditions.

Breed specific sero-prevalence of Q fever was determined. The sero-prevalence of Q fever among the Cholistani cows was the highest (13%) and lowest in cross bred cow (2.6%). Cholistani breed was raised in Livestock Experimental Station, Jogaitper, Bahawalpur. It was situated near desert and desert having dusty environment due to low rainfall. This kind of environment is associated with aerosol transmission. Transmission of the organism through contaminated dust in animals is very common (Tissot-Dupont *et al*., 2004).

In a present study, age related seropositivity was not-significant (P=0.070). However, sero-prevalence of Q fever was increased with age i.e. highest in 5-year (7.8%). These findings are in line with the findings of Astobiza *et al.* (2012). This might be due to the more exposure of healthy animals to infected animals and animals remain sero-positive for many years after exposure (McQuiston and Childs, 2002).

Q fever is reportedly an important cause of abortion in cattle (Lee *et al*., 2018), buffalo (Vaidya *et al*., 2010) and goats (Lee *et al*., 2018). Q fever as a cause of abortion has not yet caught the imagination of Pakistani investigators. Paradoxically, however, in present study, 11.5% of Q fever sero-positive animals had a history of

abortion during 1 year, whereas the overall sero-prevalence was 6.1%. the higher percentile of sero-positive animals with a history of abortion (11.5%) than the overall prevalence (6.1%) is probably a reflection of an association between Q fever and odds of abortion in the study population. However, linking Q fever with abortion requires execution of case-control and longitudinal epidemiologic studies (Thrusfiled, 2007). Further Q fever studies on Pakistani dairy animals may address these important pitfalls of the present maiden study on coxiellosis in institutional dairy herds. The results of present study are also in line with Rahman *et al*. (2016) that abortion in cattle was evident. However, abortion in cattle due to Q fever was reportedly less (To, 1998).

Among all samples tested sera from animals, females were more sero-positive (6.5%) than male (0%). These findings are in line with the previous results documenting the higher prevalence in females than males. The higher sero-activity in females might be caused by an increased susceptibility of pregnant cow/buffalo and constant shedding of organism into the environments following normal parturition or abortion through placenta, amniotic fluid, vaginal discharge, fetal membranes and milk (Sakhaee and Khalili, 2010).

Statistically, a significant association (P=0.001) was recorded between the presence of *C. burnetii* specific antibodies and tick infestations. These findings are in line with the results of previous researchers (Angelakis and Roult, 2010) who documented the significant role of ticks in transmission and maintenance of the disease among animals and humans. In sum, Q fever is present on institutional dairy farms in Punjab province, Pakistan. As far as could be ascertained from the available literature, the present study is maiden attempt to document the sero-prevalence of Q fever in institutional dairy animals (cow and buffalo) in Punjab province Pakistan. Ticks, presence of sheep and goat along with dairy animals, unhygienic conditions, and environment are the main associated factors. Reproductive problems are also associated with the Q fever.

REFERENCES

Agerholm JS, 2013. *Coxiella burnetii* associated reproductive disorders in domestic animals-a critical review. Acta Vet Scandinavic 55:1-11.

Ahmad IP, 1987. A serological investigation of Q fever in Pakistan. J Pak Med Assoc 37:126-9.

Angelakis E and Raoult D, 2010. Q fever. In: Veterinary Microbiology, 140:297-309.

Astobiza I, Ruiz-Fons F, Pinero A *et al*., 2012. Estimation of *Coxiella burnetii* prevalence in dairy cattle in intensive system by serological and molecular analyses of bulk-tank milk samples. J Dairy Sci 95: 1632-8.

de Valk H, 2012. Q fever: new insights, still many queries. Euro Survill 17:1-3.

Dua K, 2003. Comparative Disease susceptibility of Cattle and Buffalo in Punjab (India). Proc 10th International symposium on veterinary epidemiology and economics, 2003.

Gunaydin E and Pekkaya S, 2016. Serological and molecular investigation of Q fever on water buffalo in Afyan. Van Vet J 27:17-9.

Hechemy KE, 2012. History and prospects of *Coxiella burnetii* research. In: *Coxiella burnetii*: Recent advances and new perspectives in research of the Q fever bacterium. Advances in Experimental Medicine and Biology 984. Toman R, Heinzen RA, Samuel JE, Mege J-L (eds.). Springer Science.

Kaplan MM and Bertagna P, 1955. The geographic distribution of Q fever. Bulletin World Health Organ 13:829-60.

Klemmer J, Njeru J, Eman A, *et al*., 2018. Q fever in Egypt: Epidemiological survey of *Coxiella burnetii* specific antibodies in cattle, buffaloes, sheep, goats and camel. PLoS One, https://doi.org/10.1371/journl. pone.0192188.

Lee KH, Lee HK, Baek KH, *et al.,* 2018. Abortion caused by *Coxiella burnetii* in a cow and oat in Korea. J Vet Sci Tech 9:1-3.

Li K, Luo H and Shahzad M, 2018. Epidemiology of Q-fever in goats in Hubei province of China. Trop Anim Heallth Prod 50:1395-8.

McQuiston JH and Childs JE, 2002. Q fever in humans and animals in the United States. Vect Borne Zoon Dis 2:179-91.

Meadows SL, Jones-Bitton A, McEwsn SA, *et al.,* 2017. Prevalence and risk factors for *Coxiella burnetii* seropositivity in small ruminant veterinarians and veterinary students in Ontario, Canada. Can Vet J 58:397-9.

Muhammad G, Athar M, Rehman F, *et al*., 1995. Clinical and therapeutic *Escherichia coli* mastitis in water buffaloes. Ind J Dairy Sci 48:581-6.

Nokhodian Z, Feizi A, Ataei B, *et al.,* 2017. Epidemiology of Q fever in Iran: A systematic review and meta-analysis for estimating serological and molecular prevalence. J Res Med Sci 22:121.

Perugini AG, Capuano F, Esposito A, *et al.,* 2009. Detection of *Coxiella burnetii* in buffaloes aborted fetuses by IS111 DNA amplification: a preliminary report. Res Vet Sci 87:189-91.

Radostits OM, Gay CC, Hinchcliff KW *et al*., 2007. Veterinary Medicine: A Textbook of the diseases of Cattle, Sheep, Pigs, Goats and Horses. 10th Ed. Saunders-Elsevier, London, UK.

Rahman MA, Alam MM, Islam MA, *et al*., 2016. Serological and molecular evidence of Q fever in domestic ruminants in Bangaladesh. Vet Med Int pp: 1-8.

Sakhaee E and Khalili M, 2010. The first serologic study of Q fever in sheep in Iran. Trop Anim Health Prod 42:1561-4.

Seshadri R, Paulsen IT, Eisen JA, *et al.,* 2003. Complete genome sequence of the Q-fever pathogen *Coxiella burnetii*. Proc Natl Acad Sci USA 100:5455-60.

Shabbir MZ, Akram S, Hassan Z, *et al*., 2016. Evidence of *Coxiella burnetii* in Punjab province, Pakistan. Acta Tropica 163:61-9.

Thrusfield M, 2007. Veterinary Epidemiology. 3rd Ed., Blackwell Science Foundation, Oxford, UK.

Tissot-Dupont H, Amadei MA, Nezri M, *et al.,* 2004. Wind in November, Q fever in December. Emerg Infect Dis 10:1264-9.

To H, 1998. Prevalence of Coxiella *burnetii* infection in dairy cattle with reproductive disorders. J Vet Med Sci 60:859-86.

Vaidya VM, Malik SV, Bhilegaonkar KN, *et al.,* 2010. Prevalence of Q fever in domestic animals with reproductive disorders. Comp Immunol Microbiol Infect Dis 33:307-21.

van der Hoek, Hunink J, Vellema P, *et al*., 2011. Q fever in The Netherlands: The role of local environmental conditions. Int J Environ Health Res 21:441-51.

Yadav MP and Sethi MS, 1979. Sero-epidemiological studies on coxiellosis in animals and man in the state of Uttar Pradesh and Delhi (India). Int J Zoonoses 6:67-74.

Zahid MU, Hussain MH, Saqib M, *et al*., 2016. Sero-prevalence of Q fever (*Coxiellosis*) in small ruminants of two districts in Punjab, Pakistan. Vect Borne and Zoo Dis 7:449-54.