OPEN ACCESS Freely available online

Journal of Bacteriology & Parasitology

Research Article

A Cross-Sectional Survey of Brucellosis in Small Ruminants of District Jhang, Punjab, Pakistan

Rizwan Saeed[1], Hafiz Zaid Mahmood[1*], Zulqarnain Baqar[2], Sanaullah[1]

[1]Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan; [2]Department of Pathobiology, the University of Veterinary and Animal Sciences, Lahore, Pakistan

ABSTRACT

Background: Brucellosis is a bacterial zoonotic disease having a wide host range and global zoonotic importance. It has great public health importance in most of the countries, where livestock is a major source of food and income. High-risk individuals include animal handlers that are at great risk of getting an infection because bacterial transmission occurs from all body fluids from an infected animal.

Objective: A randomized cross sectional survey was conducted to check the period prevalence of brucellosis in small ruminants in different areas of district Jhang.

Methods: Serum samples were collected along with a questionnaire for this purpose. Different risk factors like age, sex, specie, feeding protocol, abortion history, type of herd, herd size, location were observed using a questionnaire. A total of 280 serum samples (136 caprine and 144 ovine) were collected and subjected to Rose Bengal precipitation test for screening of brucellosis.

Results: Overall sero-positivity was 5.5% after confirmation with Indirect ELISA. 21 samples out of 280 were seropositive after RBPT screening and 14 out of 21 were confirmed seropositive for brucellosis by indirect ELISA. According to p value after statistical analysis, all the risk factors except feeding protocols, abortion and age in the case of sheep had no significant results. According to the odds ratio, all the selected risk factors have an association with disease prevalence. In females (6.25%) there is more sero-positivity than male (1.39%). Sheep (8.09%) had more seropositivity than goats (2.08%). Out of three age groups (<2 years, 3-4 years and >5 years) >5 years (6.78%) animals had more seropositivity than 50 animals (10.94%) had more sero- positivity than ≤ 10 (3.17%), 10-30 (1.61%) and 30-50 (10.34%). Mix animal species within-herd had more chance of sero-positivity than the pure herd. Grazing practice for feeding of animals (7.02%) had more sero-positivity than stall feeding (1.83%).

Conclusion: Brucellosis was endemic in the study design area which is a risk not only for the animal's population but also for humans.

Keywords: Brucellosis; Zoonotic; Rose bengal precipitation test; Chi-square

INTRODUCTION

Livestock plays an integral role in the socio-economic development in the agricultural economy of Pakistan. In 2018-19, the total livestock share in the agriculture sector was 58.9% with an 11.1% share in overall Gross Domestic Product (GDP). The total livestock population goat population was 430,871 and sheep was 241,742. The total milk production from small ruminants was 955 thousand tons and available for human consumption on a small scale. The total mutton production in 2017-18 was 717,000 tons in Pakistan. Brucellosis is one of the

Correspondence to: Hafiz Zaid Mahmood, Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan, Tel: 923366528706; E-mail: zaibizee@gmail.com

Received: July 08 2020; **Accepted:** July 22, 2020; **Published:** July 29, 2020

Citation: Saeed R, Mahmood HZ, Baqar Z, Sanaullah (2020) A Cross-Sectional Survey of Brucellosis in Small Ruminants of District Jhang, Punjab, Pakistan. J Bacteriol Parasitol. 11: 376. DOI: 10.35248/ 2155-9597.20.11.376.

important zoonotic diseases in Pakistan. It is the second most important zoonotic disease after rabies [1].

Bacteria from genus *Brucella* are the cause of brucellosis having a wide host range. *Brucella abortus* causes infection in cattle and other bovidae, *B. melitensis* causes infection in goat and sheep, *B. canis* causes infection in dogs and *B. suis* causes infection in pigs [2,3]. *Brucella* was first isolated by Sir David Bruce [4]. It causes abortion and infertility in different mammalian species. It's a public health issue [5].

Different names are given to this disease e.g. Bang's disease, Malta fever, undulating fever, epizootic abortion and contagious abortion. *Brucella species* are non-motile, Gram-negative, cocco-bacilli and facultative intracellular microorganisms [6,7]. It can grow in the presence of oxygen and some are also carbon dioxide loving. Produces gas bubbles when reacted with hydrogen peroxide (catalase test), urease positive and target reproductive organs of many species [2,8].

Direct contact of humans with body fluid of small ruminants can transmit infection [8]. The udder is the main site in females from where the bacterium can secrete through milk to other animals and humans. After entry of the bacterium through oral route the bacterium localized in the regional lymph nodes and multiply in spleen and mesenteric and supramamary lymph nodes. The second bacterium stage spread bacterium to different organs like udder and pregnant uterus [9]. The entry of the bacterium can take place from wounds on the skin, the mucous membrane of the respiratory tract and GIT (Gastrointestinal tract). The main portal of entry for the bacterium is the oral mucosa in case of ruminants [10].

In 1952, *Brucella ovis* was first time recognized and isolated as a pathogen for ovine population in New Zealand [11]. *Brucella melitensis* mainly causes brucellosis in small ruminants but *Brucella ovis* can cause orchitis and epididymitis in ram and sometimes in infected ewes. Clinical manifestations due to brucellosis in the female of ovine are loss of pregnancy (abortion), stillbirth, fetal membrane retention and offspring having weak body condition. In males, *Brucella ovis* mainly cause acute testicular and epididymis inflammation, which may lead to fertility loss [12].

Goats are the main cause of worldwide zoonotic. A serological study conducted in photohar plateau region and Peshawar region by Ali et al. and Rashid et al. respectively showed that there is more chance of sero-positivity in the goat population for brucellosis [3,13]. Oral mucosa is the main route of transmission in goats. Brucella mainly infects placenta and fetus in goats. Abortion in last trimester is main manifestation and aborted fetus has no gross lesions. In male goats; main clinical manifestations are restricted to genital tract [14].

This is prerequisite that only those tests are recommended which can detect acute (recent) infection. According to OIE, the RBPT test is reliable for the detection of brucellosis and the positive samples must be confirmed by CFT (Complement Fixation Test) or ELISA (Enzyme-Linked Immunosorbent Assay) [15,16]. Bacterial culturing is the most reliable for accurate diagnosis but it is very time consuming and requires BSL 3 for isolation [17]. In the case of the RBPT test for detection, there are chances of cross-reactivity between different bacteria like *Vibrio cholera*, *Bordetella bronchiseptica*, *Yersinia enterocolitica*, and the *Salmonella species* [18]. ELISA test is more sensitive than the RBPT test and more accurately diagnosis positive diseased samples without any chance of cross-reactivity. All these limitations can be overcome by using the PCR technique, which is very sensitive, rapid, reliable and specific [19]. Keeping in view the importance of brucellosis diagnosis and lab facilities, we used RBPT for detection and Indirect ELISA for confirmation.

MATERIALS AND METHODS

Study area

The study was carried out in district Jhang Punjab Pakistan. Jhang has four different Tehsils (Athara Hazari, Jhang Sadar, Shorkot and Ahmad Pur Sial). District Jhang is situated on the east bank of the Chenab River and at the center of Punjab. Thal desert also located in the Jhang district, which is located 10 km from Athara Hazari town. Usama et al. in 2019 conducted an epidemiological survey of different districts of Punjab including Faislabad, Okara, Lahore and Kasur [9]. Association with different risk factors was also developed. Jhang district also has a huge livestock population and contribution in livestock, so to check the present status of the disease sero-positivity in a small ruminant population and to develop an association with different risk factors, that area was selected as cross sectional study planarea.

Study animals

The study animals were indigenous sheep and goat population. Animals of all ages, breed sex were sampled. History of vaccination against brucellosis was also recorded while sampling and not a single animal was found vaccinated with Brucella vaccine. Then individual animal age, sex, breed, specie and flock size were recorded. A total of 280 small ruminants were sampled, out of which, 136 sheep and 144 goats, randomly from four Tehsils of district Jhang.

Sample size determination

To calculate the sample size thrusfield method for simple random sampling was used. Recent serological survey results were considered as an expected prevalence in the respective formula:

According to the serological survey conducted by Ali et al. in 2015 and Rashid et al., overall seroprevalence was 11.6% and 4.33% respectively in the small ruminant population. Accordingly minimum sample size would be either 157 or 63 with 95% confidence interval and 5% marginal error, but to get more accuracy and to interpret risk factor association, we collected 280 samples randomly from both species [20,21].

d2: absolute precision (5%) CI: confidence interval (95%)

Study design and sampling strategies

This study was preceded under the Institutional Biosafety/ Bioethical Committee (IBC of the University of Agriculture, Faisalabad) keeping all the national and institutional legislation

regarding animal protection and welfare of laboratory animals. Moreover, the Directorate of Graduate Studies (University of Agriculture, Faisalabad, Pakistan) approved my thesis with reference#680 dated 06-August-2019. Sampling was done after taking consent from the owners of the animals in the form of a questionnaire. A written document having name, address and phone numbers of all owners was recorded and saved.

A serological and questionnaire survey was performed in this cross-sectional study. A total of 280 blood samples were collected in gel clotting vacutainers with the help of a separate disposable needle. Samples were stored at 4°C in the icebox and transported to the University of Agriculture Faisalabad microbiology lab for further proceedings. Serum samples were stored in a deep freezer at -20°C.

Serum samples were screened by RBPT (RBPT antigen by lillidale) and confirmation was done by indirect ELISA (ID screen® Brucellosis serum samples by ID Vet) as per manufacturer's recommendations and cut off values (24).

A questionnaire was established in which different information about the experimental unit was recorded. The following information was recorded before sampling management conditions, age, sex, breed, flock size, vaccination history, Herd Size, Type of feeding, abortion history, presences of insects and dogs and owner's biodata [22]. Chi-square analysis was performed using the statistics software version 8.1 [23]. To check the association between different risk factors and the brucellosis prevalence in small ruminant's population Chi-square test was performed.

Preparation of the sera

Blood samples from sheep and goats were collected from the jugular vein and stored in vacutainer having clotting gel. Blood samples were transported to the lab in an icebox to maintain cool chain. Serum was extracted after centrifugation and stored at 4°C for further serological testing at the University of Agriculture Faisalabad.

RESULTS

After RBPT testing over seropositive was 21 which were detected based on precipitation. These positive samples were subjected to indirect ELISA for confirmation, then because of cross-reactivity in RBPT and cull off a value given by ELIZA kit manufacturers confirmed positive samples were 14 with 5% seropositivity. According to the ELISA results it was observed that there are 6.01% more chances of brucellosis in sheep than in goat. There are 4.86% time higher chances of brucellosis in female animals (6.25%) than in male animals (1.39). In the case of species sheep population had 8.09% and in goat was 2.08%. It was recorded that there is 6.78% seropositivity within the age limit of >5 years, after >5 years most seropositivity was recorded in <2 years (4.54%), then 3-4 years (4.51%). It was recorded that there is 11.57% seropositivity within the age limit of >5 years for sheep, after >5 years most of the seropositivity was recorded in 3-4 years (8.57%), then <2 (4.51%). It was recorded that there is 5.08% seropositivity within the age limit of <2 years, remaining groups had no positive sample in case of goat population. In the case of aborted total 69 samples were collected out of which 9 were detected positive. In the case of animals with no abortion history, total samples were 211 and positive was 5. In animals having abortion history (13.04%), there is 4.34% more chance of seropositivity for brucellosis than an animal with no abortion history (8.70%). It was recorded that animals feed by grazing protocol has comparatively more chances of getting brucellosis than stall feeding. According to the Confirmatory results by indirect ELISA, grazing had 5.19% more chance of brucellosis than Stall feeding. Four groups (≤ 10, 10-30, 30-50 and>50) were developed to interpret herd size as a risk factor for brucellosis in small ruminants. According to the confirmatory results after indirect ELISA, it was recorded that herd size>50 animals (10.94%) had more chance of brucellosis than 30-50 (10.34%), ≤ 10 (3.17%) and 10-30 (1.61%). It was recorded that keeping Mix herd had 2.16% more chance of getting brucellosis than the pure herd (Table 1).

Table 1: Statistical table for all risk factors.

Risk factors	Variables	Total samples	Negative	Positive	Range	Prevalence	p value	OR	95% confidence interval	RR	95% confidence interval
Species	Sheep	136	125	11	7	8.09%	0.032*	4.13	(.067-.886)	3.88	(1.11-13.62)
	Goat	144	141	3	7	2.08%	Ref	NA	NA	NA	NA
Sex	Male	72	71	1	7	1.39%	Ref	NA	NA	NA	NA
	Female	208	195	13	7	6.25%	0.001*	4.73	(.61-36.84)	4.5	(.60-33.80)
Age groups for small ruminant	1-2 Years	88	84	4	4.67	4.54%	Ref	NA	NA	NA	NA
	3-4 Years	133	127	6	4.67	4.51%	0.752	1.52	(.276-3.68)	1.008	(.29-3.42)
	>5 Years	59	55	4	4.67	6.78%	0.49	1	(.157-2.73)	1.49	(.39-5.73)

Age groups for sheep	1-2 Years	32	31	1	3.67	3.13%	Ref	NA	NA	NA	NA
	3-4 Years	70	64	6	3.67	8.57%	0.178	2.9	(.040-2.98)	2.74	(.34-21.85)
	>5 Years	34	30	4	3.67	11.57%	0.16	4.13	(.025-2.29)	3.76	(.44-31.92)
Age groups for goat	1-2 Years	59	56	3	1	5.08%	0.049*	0	(0-0)	0	(0-0)
	3-4 Years	64	64	0	1	0.00%	0.157	0	(0-0)	0	(0-0)
	>5 Years	21	21	0	1	0.00%	Ref	NA	NA	NA	NA
Feeding protocols	Grazing	171	159	12	7	7.02%	0.007*	4.03	(.054-1.13)	3.82	(.87-16.76)
	Stall	109	107	2	7	1.83%	Ref	NA	NA	NA	NA
Herd sizes	≤ 10	63	61	2	3.5	3.17%	Ref	NA	NA	NA	NA
	30-Oct	124	122	2	3.5	1.61%	0.256	2	(.275-14.54)	2	(.07-3.52)
	30-50	29	26	3	3.5	10.34%	0.398	3.51	(.045-1.80)	3.26	(.58-18.46)
	>50	64	57	7	3.5	10.94%	0.182	3.74	(.053-1.34)	3.75	(.74-15.95)
Type of herd***	Pure	14	13	1	7	7.14%	Ref	NA	NA	NA	NA
	Mix	266	253	13	7	4.88%	0.001*	1.51	(.067-.886)	1.49	(.10-4.87)
Abortion samples	Sheep	40	33	7	4.5	27.50%	0.095	2.86	(.067-1.821)	2.54	(.57-11.34)
	Goat	29	27	2	4.5	10.34%	Ref	NA	NA	NA	NA
Abortion history	Aborted	69	60	9	7	13.04%	0.285	6.18	(2.25-22.5)	5.5	(1.91-15.87)
	Non aborted	211	206	5	7	8.70%	Ref	NA	NA	NA	NA

OR: Odds ratio (If=1, no association; if>1, have an association with risk factor; if<1, have an association with protective factor) checked by Binary logistic regression;

RR: Relative ratio (<1 have no association with disease prevalence; >1 have an association with disease prevalence) Checked by Binary logistic regression;

Ref: Reference category;

*: Shows significant results;

NA: Not Applicable

DISCUSSION

Brucellosis is one of these diseases which cause health and reproductive problems in animals. This disease is the second most important zoonotic disease after rabies. It causes a great declining effect on the economics of the country in developing countries. Developed countries applied mass culling strategy for eradication of brucellosis in their livestock animals after giving incentives to the livestock farmers. In this present study questionnaire survey and serological analysis were conducted to check overall sero-positivity of Brucella antibodies in the serum samples of indigenous sheep and the goat population of district Jhang. Small ruminants are in great numbers in this district. Out of the total livestock population, the goat population was 430,871 and sheep was 241,742 [24].

The study was carried out in four different Tehsils (Athara Hazari, Jhang Sadar, Shorkot and Ahmad Pur Sial) of district Jhang Punjab Pakistan. Its geographical coordinates are 31°16′ 10″ N 72°18′ 58″ E. District Jhang is situated on the east bank of the Chenab River and at the center of Punjab. Thal desert also located in the Jhang district, which is located 10 km from Athara Hazari town. It is the 18th largest city in Pakistan. It is known for the shrine of Sultan Bahoo and Heer and Ranjha's Tomb. During monsoon season most of the areas of the Jhang district are under great risk of flood so people move to safe areas along with their animals and most of the people

depend on small ruminants for their likelihood. Up till now no serological survey was conducted on the small ruminant population of district Jhang. Usama et al. in 2019 conducted an epidemiological survey of brucellosis in livestock population of different districts of Punjab including Faislabad, Okara, Lahore and Kasur [25-27].

A total of 280 samples were collected and the overall prevalence was 7.5% (21 seropositive samples) after RBPT screening and after confirmation of positive samples, sero-positivity was 5%. Iqbal et al., surveyed the small ruminant population of Layyah district Punjab, Pakistan [11]. According to their finding overall prevalence was 7% after modified RBPT screening. This difference might be due to climate change, sampling strategy and study design. Rajala et al. in 2016 had interpreted seroprevalence of 6.7% in small ruminants of four districts of Tajikistan [27]. Ali et al., recorded 8.6% prevalence in different areas of Islamabad, Potohar plateau, Rawat [3]. The results of these three kinds of literature showed that high temperature might decrease brucellosis prevalence. Hussain et al. in 2014 conducted in Kohat district, to check the seroprevalence of brucellosis in sheep and the human population [25]. Results indicated that the overall prevalence in the sheep population of the district was 10% and in the human population was 6%. Their results showed the public health aspect of brucellosis. Another study was conducted by Shahzad et al., on the camel population of different districts [12]. The present area of research (Jhang district) was also surveyed for brucellosis and the overall prevalence was 3.54% in the camel population of district Jhang. In Mirpur Azad Kashmir over prevalence was found out to be 8.6% by RBPT and 6.5% by ELISA, Its temperature was comparatively lower than Jhang [27]. Dadar, et al., in 2020 explained that extreme hot temperature and extreme frosty days had a negative effect on brucellosis prevalence. In Temperature above 30°C and scanty frosty days incidence of brucellosis is very low [16].

On comparing different species (sheep and goat) for brucellosis prevalence, present results showed that there are 6.01% (sheep (8.09%) and goats (2.087%) prevalence) more chances of having seropositivity for Brucella antibodies in serum sample of sheep than in goat. That might be due to random sampling design used for calculated prevalence between sheep and goat or might be sheep hygienic conditions are the predisposing factor for increase prevalence in sheep. Although p value showed no significant results but chances in sheep population are more than goat according to the present study. The main reason is that in this research work serum samples were collected randomly without discrimination of herd size. Rajala et al., also reported that sheep (11%) has more chances of brucellosis than goat (5%). Results of Ali et al. in 2015 were different from my findings because of different goat breeds in their area ofresearch. Tegegn et al., reported that those are two times more chance of Brucella infection in goats than in sheep [24]. That is opposite to the present study results. That might be due to a random sampling strategy in my study plan. In this research work, random sampling was done without discrimination of abortion history, so more aborted sheep samples were collected. This randomization in sampling affected the results and deviate from previous studies.

In the present study herd size (≤ 10, 10-30, 30-50 and >50) was evaluated as a risk factor for brucellosis prevalence in the small ruminant population. p value (.0192) after a statistical analysis showed significant results. According to the statistical data, there is more chance of brucellosis in larger herds than smaller herds. These findings were correlated with the findings of Abdallah et al., and Iqbal et al., who reported a statistical association of herd size with seropositive serum samples for brucellosis is significant [3,7]. Ali et al. recorded a difference in the prevalence percentage of Brucella antibodies in small ruminants of different areas of Potohar plateau [6]. They reported that areas having large herd size had more prevalence than small herd areas. The main reason is a well-known fact that in large herd size, there is more chance of getting infection from the infected animal. If an infected male animal is present in the herd, the disease will transmit like a storm in the herd. Most of the farmers are unaware of clinical signs because of a lack of knowledge about the brucellosis in animals, which is the main reason for the persistence of Brucella affected animals in the herd.

As we compare different age groups (<2 years, 3-4 years and >5 years) p value (.7794) showed no significant association with prevailing Brucella antibodies in the small ruminant population but within age groups increasing trend was observed from younger to older age groups (<2 years >3-4 years >>5 years). Iqbal et al., also found related results on comparing age groups. Their age groups were 1-2.5 years, 2.5–4 years and >4 years. P value was also non-significant in their study [22]. Tegegn et al. reported that elders were more prone to infection than younger animals [24]. Increasing maturity level is the main factor which is responsible for high brucellosis susceptibility because erytritolconcentration after maturity starts increasing and that's responsible for the attraction of bacteria. Abdallah et al., estimated that small animal having undergone three birth stages have more prevalence cases than younger animals. Rajala et al.,also reported that increase in age there is greater chances of seropositivity of serums sample for brucellosis [27].

The present study results after a statistical analysis showed that in female animals (6.25%) chance of Brucella infection is more as compared to the male (1.39%). Ali et al. 2015 recorded 10.4% prevalence in females and 3.3 prevalence in the male in Different areas of Potohar plateau in Pakistan [3]. Diab et al., recorded 11.36% in females and 6.88 in males. The odds ratio showed a significant association with the disease occurrence in that small ruminant [7]. The main reason is sacrificing male animals after 2 years of age and female animals spend more life within the farm environment. Because of the random sampling number of female animals sampled are 3 fold greater than male animals. Ram population is lower than ewes because of the faster culling rate. Erythritol concentration in the fetal tissues during pregnancy is the factor for the tropism of the bacterium toward the gravid horn. Erythritol is present in the fetal tissues which attract bacterium toward the placenta. During pregnancy cause of abortion might be the tropism of bacterium toward the placental tissues, where bacterium multiplies and became a source of contamination in the environment and the bacterial load in the dam`s circulation also increase. The female

reproductive tract is the main contributing factor toward increase prevalence in the female.

CONCLUSION

In this present study cross-sectional survey was conducted to test the overall sero-positivity of brucellosis within small ruminants of district Jhang. This cross-sectional survey had shown the present status of such a devastating zoonotic disease in the small ruminant population of the study design area. To eradicate the disease, the culling of positive animals and awareness to the farmers about signs and symptoms must bepracticed.

REFERENCES

1. *Brucella melitensis* in Eurasia and the Middle East. FAO Animal production and Health proceedings Rome. 2010.
2. Ali S, Akhter S, Neubauer H, Melzer F, Khan I, Ali Q, et al. Serological, cultural, and molecular evidence of Brucella infection in small ruminants in Pakistan. J Infect DevCtries. 2015;9(5): 470-475.
3. Ali S, Nawaz Z, Akhtar A, Aslam R, Zahoor MA, Ashraf M. Epidemiological investigation of human brucellosis in pakistan. Jundishapur J Microbiol. 2018;11(7): e61764.
4. Abubakar M, Mansoor M, Arshed MJ. Bovine brucellosis: old and new concepts with pakistan perspective. Pak Vet J. 2012;32(2): 147-155.
5. Elfaki MG, Al-Hokail A, Nakeeb S, Al-Rabiah F. Evaluation of culture, tube agglutination and PCR methods for the diagnosis of brucellosis in humans. Med SciMonit. 2005;11(11):69-74.
6. Godfroid J, Al Dahouk S, Pappas G, Roth F, Matope G, Muma J, et al. A "One Health" surveillance and control of brucellosis in developing countries: Moving away from improvisation. Radiology. 2020;294(2):1-7.
7. Yu P, Zhu J, Zhang Z, Han Y. A familial cluster of infection associated with the 2019 novel coronavirus indicating potential person-to-person transmission during the incubation period. J Infect Dis. 2020;221(11):1757-1761.
8. Hussain B. Study on Seroprevalence of Brucellosis in Caprine in Kohat (Khyber Pakhtoon Khwa). lasbelaUni J Tech. 2014;3(2): 14-20.
9. Usama S, Ali S, TM Khan, Adawy HE, Iftikhar A. Seroepidemiology and the molecular detection of animal brucellosis in punjab, pakistan. Microorgainsms. 2019;7(10):449.
10. Abdallah AA, Elfadil AAM, Elsanosi EM, Shuaib YA. Seroprevalence and risk factors of brucellosis in sheep in north kordofan state. J Agric Vet Sci. 2015;8(1):31-39.
11. Moreno E, Cloeckaert A, Moriyón I. Brucella evolution and taxonomy. Vet Microbiol. 2002;90(1):209-227.
12. Bruce D. Note on the discovery of a microoragnism in Malta fever. Practitionar. 1887;39(3):161-170.
13. Diab MS, Elnaker YF, Ibrahim NA, Sedeek EK, Zidan SAA. Seroprevalence and associated risk factorss of brucellosis in sheep and human in four regions in matrouhgonvernorate, egypt. World Vet J. 2018;8(4):65-72.
14. Ducrotoy MJ, Conde-Álvarez R, Blasco JM, Moriyón I. A review of the basis of the immunological diagnosis of ruminant brucellosis. Vet Immunol Immunopathol. 2016;171(1):81-102.
15. Bovine brucellosis. Manual of diagnostic tests and vaccines for terrestial animals. OIE. 2008.
16. Bernheim A, Mei X, Huang M, Yang Y, Fayad ZA, Zhang N, et al. Chest ct findings in coronavirus disease-19 (covid-19): relationship to duration of infection. Radiology. 2020;295(3):5-10.
17. Chan JFW, Yuan S, Kok KH, To KKW, Chu H, Yang J, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet. 2020;395(10223):514-523.
18. Centers for Disease Control and Prevention. Public Health Guidance for Community-Related Exposure: Coronavirus Disease 2019 (COVID-19). 2020.
19. Hu Z, Song C, Xu C, Jin G, Chen Y, Xu X, et al. Clinical characteristics of 24 asymptomatic infections with covid-19 screened among close contacts in nanjing, china. Sci China Life Sci. 2020;63(5):706-711.
20. Inui S, Fujikawa A, Jitsu M, Kunishima N, Watanabe S, Suzuki Y, et al. Chest CT Findings in Cases from the Cruise Ship "Diamond Princess" withCoronavirus Disease 2019 (COVID-19). Radiology Cardiothoracic Imaging. 2020;2(2):2-10.
21. American College of Radiology. ACR Recommendations for the use of Chest Radiography and Computed Tomography (CT) for Suspected COVID-19 Infection. 2020.
22. Simpson S, Kay FU, Abbara S, Bhalla S, Chung JH, Chung M, et al. Radiological Society of North America Expert Consensus Statement on Reporting Chest CT Findings Related to COVID-19. Endorsed by the Society of Thoracic Radiology, the American College of Radiology, and RSNA. Radiology Cardiothoracic Imaging. 2020;2(2):6-15.
23. Iqbal ZH, Jamil ZI, Qureshi M, Saqib LA, Lodhi, Waqas MS. Seroprevalence of ovine brucellosis by modified rose bengal test and elisa in southern punjab, pakistan. Pak Vet J. 2013;33(4): 455-457.
24. Tuli ATG, Kassa T, Kebede N. Seroprevalence and risk factors of Brucellosis in small ruminants slaughtered at DebreZiet and Modjo export abattoirs, Ethiopia. J Infect Dev Ctries. 2015;9(4): 373-380.
25. Tegegn AHA, Feleke W, Adugna, Melaku SK. Small ruminant brucellosis and public health awareness in two districts of Afar Region, Ethiopia. J Vet Sci Technol. 2016;7(4):2-5.
26. Mubeen H, Khan I, Saleem MH, Akhtar R, Ali S, Saqib M, et al. Brucellosis in Mirpur, Azad Kashmir Pakistan: a livestock threat forneighboring zones. Med Weter. 2018;74(7):449-451.
27. Rajala EJ, Grahn C, Ljung I, Sattorov N, Boqvist S, Magnusson U. Prevalence and risk factors for Brucella seropositivity among sheep and goats in a peri-urban region of Tajikistan. Trop Anim Health Prod. 2016; 48(2):553-558.