Research Article

Sero-epidemiological study of brucellosis in small ruminants and associated human beings in district Quetta, Balochistan

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Abstract

Brucellosis is an occupational hazard in animal handlers and medical practitioners. This study was aimed to estimate the sero-prevalence of brucellosis in goats, sheep and butchers (slaughterhouse worker) in Quetta and its suburb. A total of 530 peripheral blood samples were collected randomly from goats (n=250), sheep (n=250) and butchers (n=30). The serum samples were subjected for Rose Bengal Precipitation Test and Slide Agglutination Test, whereas hematological test was performed for (n=30) samples collected from slaughterhouse workers. Butchers were interviewed with the help of a predesigned questionnaire. Results showed that the overall prevalence of brucellosis in the area was about 3.4 % out of 530 serum samples. The antibody titer through SAT for *Barbari* breed was ranging 1:525 to 1:2625. For *Khurasani* and *Mengali* breeds, it was 1:125. In Complete Blood Count leukopenia was detected (WBC<4300/mm³) 21/30 (70 %), anemia 21/30 (70 %) thrombocytopenia (Platelets <150.000/mm³) within samples of butchers. Hemoglobin deficiency (HGB< 12mg/dl) 24/30 (80%) were found. It is concluded that brucellosis is more prevalent in female animals as compared to males. *Barbari*, *Khurasani* and *Lehri* breeds in goats and *Mengali*, *Shinwari* and *Bibri* in sheep were more affected by brucellosis. Although insignificant association between prevalence of brucellosis in sheep and goat (P <0.05) was observed. Butchers and slaughter house workers are found to be at high risk due to their low immunity level.

Keywords: Sero-prevalence; RBPT; SAT; Slaughterhouse; *Brucella*

Introduction

Brucellosis is one of the important zoonotic diseases among livestock. It is a contagious disease that infects animals and can be transmitted to humans [1]. *Brucella* has been categorized into a genus, which includes ten species namely *B. suis*, *B. melitensis*, *B. ceti*,

B. canis, *B. microti*, *B. abortus*, *B. pinnipedialis*, *B. ovis*, *B. neotomae* and *B. inopinata* [2]. Brucellosis occurs worldwide, particularly in developing and underdeveloped countries, but it is well controlled in developed countries. Sheep (*Ovis aries*) and goats (*Capra hircus*) are

more frequently found infected with *brucella* spp. [3]. The clinical signs of brucellosis are abortion, stillbirth, orchitis and retained placenta in animals and causes undulant fever in humans [4]. Humans are severely infected by brucellosis especially with *B. melitensis* and the *B. suis*. All in all, certain species of brucella can also infect cattle, pigs and most of all sheep and goats respectively. Wild life animals and domestic animals both are equally infected by brucella [5]. The infection is also acquired by humans either in contact with infected animals or consumption of contaminated milk or dairy products. Brucellosis has major economic impact by time constraints of patients from normal daily activities and causes major loss in animal husbandry [6]. The annual incidence of human brucellosis are estimated half a million worldwide. However, *B. suis*, *B. canis*, *B. abortus* and *B. melitensis* causes these human infections [7]. Moreover, in humans the common serological tests are mostly used for bovine brucellosis screening are Rose Bengal Precipitation and ELISA tests however the best and easy method for detection is RBPT. It is purely based on agglutination of color particulate antigen (killed brucella organism) by antibodies present in the patient's serum [8]. Brucellosis is a significant health problem in an agricultural country like Pakistan where majority of its public is involved in land cultivation and livestock farming [9]. This study aims to investigate the prevalence of brucellosis in small ruminants and butchers in Quetta, Pakistan.

Materials and methods

Study site

Balochistan is one of the province of Pakistan and Quetta is the provincial capital of Balochistan. It is situated at an elevation of 1676-1900 meters above the sea level. The weather remains moderate (dry and cold) in Quetta. In the month of January and

February the maximum rain and snowfall occurs. Summer season is variable, while the month of June and July is usually hot where the temperature remains in between 20 and 30 °C respectively [10].

Collection and preparation of samples

A total of 530 blood samples (Sheep= 250; Goat= 250 and butchers= 30) from each male and female population were collected randomly and aseptically using disposable pre-sterile plastic syringes. The blood was then left at 45 °C at least for 2 hour to clot, followed by separation of serum collected in separate sterile Eppendorf tubes. All the serum samples were stored at -20°C in deep freezer till further use.

Rose bengal plate agglutination test (RBPT)

The test was carried out in Pathobiology Laboratory of Center for Advance Studies in Vaccinology and Biotechnology, University of Balochistan, Quetta. The antigen used was procured from Veterinary Research Institute, Lahore.

Before conducting the test both sera and antigen were kept at room temperature for one hour. One drop of serum sample and a drop of Rose Bengal antigen were added on a clear glass slide. The contents of both drops were mixed thoroughly but gently by using a wooden sterile stick. The reaction was observed after 3 minutes using known positive and known negative serum control during each batch of test minimized the chances of false positive and false negative results. Complete agglutination was recorded as positive and no agglutination as negative [9].

Serum agglutination test (SAT)

Preparation of phenol saline

100 ml of phenol saline was prepared for the serial dilution. For this purpose 0.85 g of sodium chloride was mixed in 100 ml of distil water (DW). Later, 0.5 ml of phenol was added in this mixture. The mixture was autoclaved for (20) minutes at 121 °C and

the solution was kept for examination of antibody titer for serum agglutination test. About 0.8 ml of phenol saline was added in labeled tubes followed by the addition of 0.2 ml serum sample and was mixed thoroughly but gently (1/5 dilution). An amount 0.2 ml was drawn from the first test tube, mixed and transferred into the next tube. After gentle mixing, 0.2 ml was transferred to the next and so on up to the last tube. 0.2 ml was drawn and discarded from the last tube. Later, 0.2 ml of the standardized *B. abortus* concentrated antigen dilution (1:2) were added to each tube containing serum dilution giving a series of final dilutions. The antigen and serum was mixed gently and incubated at 37 °C overnight. After overnight incubation tubes were examined against a dark background and antibody titer were recorded [11].

Questionnaire survey

A questionnaire for each animal was completed from butchers through an interview to identify the sero-positive animals. Potential individual risk factors and their categories were as follow: age (<1 and >1 years), sex (male and female), breed of goats (*Barbari*, *Lehri*, *kajali*, and *Khurasani*) and sheep (*Balochi*, *Shinwari*, *Bibrik* and *Mengali*) and body conditions (good and poor) [12].

Hygiene and hematological count of butchers

Brucellosis is a contagious zoonotic disease and can seriously infect humans. The general health, hygiene and hematological count of the butchers working in the facilities were evaluated in this study. The slaughtering, handling, and chopping of meat were observed. Upon the approval of bioethical committee and consent of the respondents and taking care of ethical issues blood samples (n=30) were collected aseptically from butchers and subjected to complete blood count, rose bengal test and serum agglutination test [13].

Results

Rose bengal precipitation test (RBPT)

Serum samples of sheep, goats and humans were analyzed using Rose Bengal Plate Test. The results showed that only female animals were affected by *Brucella* as (3.4 %) while no human brucellosis was found in the study. In this study 10 goats (Females) and 8 Sheep (Females) were found positive for brucellosis via RBPT. Moreover, results indicated that brucellosis is more prevalent in goats than sheep population (Table 1).

The study was analyzed by the sero-prevalence of brucellosis in different breeds of both goats and sheep. The results indicated that in local breeds of goats (*Barabari* and *Khurasani*), in sheep (*Mengali* and *Shinwari*) were more affected for brucellosis (Table 2). Inferior health were noted in majority of animals (Table 3).

Table 1. Sex wise prevalence of bovine brucellosis in serum samples of target goats and sheep by using rose bengal precipitation test (RBPT)

Category		Positive by RBPT (%)	
Sheep		+ve	%
Male	202	0	0
Female	48	8	16
Total	250	8	16
Goat			
Male	195	0	0
Female	55	10	18
Total	250	10	18
Human	30	0	0
Grand total	530	18	3

Note: +ve = Positive, % = Percentage

Table 2. Sero-prevalence of brucellosis in target breeds of goats and sheep by using rose bengal precipitation test (RBPT)

Category	Breeds	Negative	Positive	Total No	Prevalence (%)
Goat	<i>Barbari</i>	121	5	126	3.96
	<i>Khurasani</i>	61	3	64	4.68
	<i>Lehri</i>	38	2	40	5
	<i>Kajali</i>	20	0	20	0
	Total	240	10	250	5.45
Sheep	<i>Mengali</i>	91	5	96	5.20
	<i>Shinwari</i>	63	2	65	3.07
	<i>Bibrik</i>	49	1	50	2
	<i>Balochi</i>	39	0	39	0
	Total	242	8	250	4.1

Goats (*Barbari*, *Lehri*, *kajali*, *Khurasani*), **Sheep** (*Balochi*, *Shinwari*, *Bibrik*, *Mengali*)

*insignificant association of brucellosis in sheep and goat breeds ($P < 0.05$)

Table 3. Animals, breeds, ages and their body conditions analysis obtained via questionnaire

Category	Breeds	Samples examined	Age		Body condition	
			<1 (%)	>1 (%)	Good (%)	Poor (%)
Goat	<i>Barbari</i>	126	19	80	38	62
	<i>Khurasani</i>	64	15	84	18	81
	<i>Lehri</i>	40	12	87	30	70
	<i>Kajali</i>	20	10	90	40	60
Sheep	<i>Mengali</i>	96	12	87	60	39
	<i>Shinwari</i>	65	18	81	24	75
	<i>Bibrik</i>	50	8	92	76	24
	<i>Balochi</i>	52	19	80	75	25

Serum agglutination test (SAT)

Serum agglutination test for the RBPT positive samples in (*Barbari*) goats were found 1:525, for positive samples of sheep (*Mengali*) were 1:125 in 1: 5 dilution.

Health hygiene status of the worker

Under observations 70% of butchers and slaughter house workers were observed as performing their job in totally unhygienic manner. The workers were examined during slaughtering animals their location of the shops, hanging meet on open roads without covering it. The water for washing the meet and handling were totally unhygienic. The workers right after slaughtering the animals did not wash their hands and used to start chopping up meet. No personnel protective

equipment's (PPE's) were seen to be used. The butchers were directly handling meet without wearing gloves and long boots.

Complete blood count

Complete Blood Count was performed for the blood samples of butchers and slaughter house workers. Leukopenia was detected ($WBC < 4300/mm^3$) in 70% of the butchers working in the facilities, Anemia in 70% and Thrombocytopenia (Platelets $< 150.000/mm^3$) within samples of butchers. Hemoglobin deficiency ($HGB < 12mg/dl$) were found in 80% of the workers. Reports indicated that butchers and slaughter house workers are at high risk due to their weak immunological profile.

Discussion

Brucellosis is recognized as a significant public health challenge, with a major economic and financial burden in countries where the disease remains endemic [14]. It is caused by genus *Brucella*, which are Gram -ve, aerobic, non-spore forming and facultative intracellular coccobacilli. Brucellosis in bovine is mainly caused by *Brucella abortus* and less commonly by *Brucella melitensis*. Human brucellosis is caused by *B. abortus*, *B. suis*, *B. melitensis* and *B. canis* [15]. *B. abortus* and *B. suis* have been isolated worldwide from a wide variety of wild life species. The risk of transmission increases by wildlife overabundance, movements of wild and domestic animals and fomites [16]. The proper control of ovine brucellosis in livestock has always received great attention in almost all countries of the world. It has been primarily carried out by screening the herd and detecting infected animals or carriers those could be eliminated to free the stock from menace [9]. Brucellosis has been reported in different parts of the world as affecting domestic animals and humans. This disease is important as major causes of economic losses in the livestock and as a great public health significance [17]. A number of rapid, reliable and more sensitive diagnostic techniques are in vogue including RBPT milk ring test (MRT), complement fixation test (CFT), rivanol test (RT), along with isolation and identification of the organism, enzyme linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) techniques [9].

In the present study two of the tests viz RBPT, SAT have been compared. Out of 500 serum samples randomly collected from goats and sheep population of Quetta city 18 were found positive for brucellosis. Current study showed all positive cases were found in female animals and this interlink our study with [18], who believed on the

controversial reports regarding the prevalence of brucellosis in relation to sex of animals, their study also ensures the presence of brucellosis in female animals. The prevalence of brucellosis is ensured in native city by Shafee *et al.* [9], they examined 200 milk samples of ovine brucellosis in organized dairy forms of Quetta city. The prevalence of brucellosis in milk were examined by MRT and indirect ELISA. They found brucellosis 4.6 % in cattle while 1.7% in buffaloes by (MRT).

[12] Used the same RBPT and SAT for brucellosis in goats and sheep. They reported that out of 318 serum samples 2.5% and 22% prevalence's were parallel estimated. Apart these results, as our research was based on questionnaire regarding status of slaughtering animals. They investigated the potential risk factors which included breeds, age groups, localities, body conditions and having aborted animals in herds. Our questionnaire is related with their work, we have found brucellosis in poor animals including only females, the results have the comparison with their study where their investigation showed the maximum positive cases in female animals and same was found in our study.

For the confirmatory tests we can compare our results of RBPT and SAT with the results of [19]. They reported the RBPT and SAT as standard methods. Detection of brucellosis, according to them RBPT and SAT are quantitative measurements of antibodies. While we also found both of the methods effective in detection of brucellosis. They briefed the RBPT as officially introduced in Britain where these tests were found rapid, simple, sensitive and in comparison, we also found the same as a moderate specificity.

Brucellosis is responsible for considerable public health issues involving economic losses due to loss of milk production and

infertility in adult male, as well as in man. One of the main objective of our study was to collect serum samples from butchers as well. The aim was to check the prevalence of brucellosis in man. Serum samples 30 of slaughterhouse workers and butchers were subjected for RBPT and CBC. The present study was a cross-sectional study on brucellosis among goats and sheep population verses humans (butchers) our findings do not greatly differ with those reported in other countries. As [20] studied sero-prevalence of brucellosis in Afghanistan in humans and livestock, for this investigation They examined (1,017) humans (1,143) sheep and (876) goats serologically. Their results were found about one in four (24.5%) had either seropositive animals or humans. However, we detected 18 positive cases among 530 serum samples drawn by both animals and humans while fortunately no positive human brucellosis was examined by RBPT (Table 1). Their sample size was huge and the results were found positive in 1 out of four animals like wise about one in six households (15.7 %) had at least one brucella sero-positive person these findings could be due to large sample size. Our interview based study is similar with [21] who had an interview based research and used a verbal consent from the respondents. The objectives of their survey were explained to the people before start of interview who used (Ormmoro or Amharic) local languages to conduct the interview. The questionnaire focused of demographic characteristics of the interviewee. The questionnaire was based on animal husbandry, product consumption and handling of dead and disposable practices. The interview was comprised on of total 98 persons from rural and urban areas of Southern Ethiopia from where the animals tested for brucellosis. The objective of our study was same as [21]. We interviewed the

butchers and slaughterhouse workers and the questions were particularly resembled with theirs as, past experiences of working as a butcher, purchasing and keeping animals in slaughter houses. The marital life and number of children, disease history regarding their job. We observed the animal keeping practices, slaughtering and chopping up the meat. We also analyzed those practices through which the slaughter house workers and butchers were purchasing and importing animals.

Conclusion

The sero-prevalence of *Brucella* was found 3.39 % in sheep, goats and butchers. It was also found that the immune level of the butchers working in the slaughterhouses was very low and they were prone to the infectious agents. The overall practice of the slaughtering and handling of animals in the slaughterhouses were found unhygienic. It was concluded in our study that brucellosis is a risk factor for zoonosis particularly in the farmers, butchers and other worker handling the livestock. Identification and proper handling of the infected animals need more attention in order to prevent the spread of this zoonotic infection in the community.

Authors' contributions

Conceived and designed the experiments: A Akbar & M Shafee, Performed the Experiments: S Ali, Analyzed the Data: S Ali & N Ullah, Contributed reagents/materials/ analysis tools: M Shafee, A Muhammad & B Tahira, Wrote the paper: S Ali, M Shafee & A Akbar.

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