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Seroepidemiological Analysis of Brucellosis in Goat and Household Animal Keepers of District Swat

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BRUCELLOSIS is a chronic bacterial infection that can infect a variety of animal species and humans. The disease has been studied in many parts of the world including Pakistan, however, caprine brucellosis has not been well reported and very few studies are published at the national level. This study was conducted to investigate the seroprevalence of brucellosis in goat and household animal keepers of district Swat Pakistan. A total of 235 blood samples were collected from goats and 53 blood samples from animal keepers following the standard protocols. All the serum samples were analyzed by the Rose Bengal Plate Test (RBPT) and Serum Plate Agglutination Test (SPAT) for the detection of anti-brucella antibodies. All the goat serum samples were also subjected to Enzyme-Linked Immunosorbent Assay (ELISA) for the comparison of two commonly used serological tests. The overall seroprevalence of brucellosis in the goat population of district Swat was recorded as 13.61% and 16.17% while in the human population it was recorded as 3.77% and 5.66% by RBPT and SPAT respectively. A significant correlation was found between abortion and seropositivity of brucellosis in goats with a $p<0.05$. By comparing RBPT and SPAT considering ELISA as a gold standard, the SPAT test showed a greater sensitivity than RBPT recorded as 82% and 80 % by SPAT and RBPT respectively. It is concluded that brucellosis is prevailing in both the goat as well as in animal keepers of district Swat. Screening of animals and awareness of the general population through mass media is recommended.

Keywords: *Brucellosis, Seroprevalence, RBPT, SPAT, Swat Pakistan.*

Introduction

Brucellosis is a chronic granulomatous infection causing a significant economic and health loss in animal husbandry. Goat, cattle, sheep, dog, and swine are the natural animal hosts [1]. The causative agent for brucellosis is the genus *Brucella*; nine different species of *Brucella* are currently known, the main causative species for the animal as well as human brucellosis are *B. melitensis, B. suis* and *B. abortus*. *B. melitensis* causes disease in small ruminants especially in goats [2]. Other species are known to cause disease in many other organisms [3]. Brucellosis in sheep, cattle, goats, and pigs is characterized by the retained placenta, abortion, epididymitis, orchitis, and temporary impair fertility [4]. Acute metritis followed by retained fetal membranes may lead

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to death [5]. Brucellosis is considered by the Office International des Epizooties (OIE) and World Health Organization (WHO) as the 2nd most prevalent zoonosis after rabies [6]. More than 500,000 human cases are reported annually [7]. The infection in humans has a diverse range of clinical signs and symptoms in which the most important is undulant fever.

The diagnostic method for brucellosis should be selected based on the biological sample, species to be diagnosed, or according to local regulations [8,9]. At present Rose Bengal Plate Test (RBPT) and Serum Plate Agglutination Test (SPAT) is the most broadly used tests for the initial diagnosis of *Brucella* infection [10]. As these are the screening tests so the positive results should be confirmed by a more specific confirmative test like Polymerase Chain Reaction (PCR) or ELISA [11]. Geographically brucellosis occurs throughout the world. The disease has been eradicated in many developed countries however in developing countries including Pakistan it remains an unrestrained dilemma [6].

The Swat district is situated at 350 North Latitude and 720 and 300 East longitude in the north of KPK province of Pakistan. The district is a mountainous area and the rural income of the people is greatly dependent on livestock as well as the people are in close contact with goats on daily basis. The goat is the 2nd animal domesticated after the dog [12]. The up-to-date picture is unavailable however in the year 2010 Swat had a total of 236229 goats [13]. Brucellosis has been extensively studied in buffaloes, cattle, dogs, and horses but Caprine brucellosis has not been focused on in previous studies and limited data is available at the national level [6]. Another issue that requires consideration is the prevalence of brucellosis in occupationally exposed humans, the current project was therefore designed to find the seroprevalence of brucellosis in goat and household animal keepers of district Swat, Pakistan similarly it was also planned to compare the sensitivity and specificity of RBPT and SPAT for the diagnosis of caprine brucellosis.

Materials and Methods

Ethical approval

The current study was approved by the ethical committee of the Hazara University, Mansehra Khyber Pakhtunkhwa Pakistan. Written consent was signed with the animal owner before the sample collection.

Study area and design

The study was performed between November 2017 to May 2018 at the district Swat of KPK Pakistan to find the seroprevalence of brucellosis in goats and household animal keepers.

Collection of blood samples

A total of 235 blood samples were collected from goats and 53 blood samples from animal keepers by using a simple random sampling method. A questionnaire was also designed in which the animal information like, sex, herd size, abortion history, vaccination, disability, and other symptoms were recorded. After proper labeling, the samples were shifted to the Microbiology laboratory at Veterinary Research and Disease Investigation Centre, Balogram, Swat. The blood samples were refrigerated at 4 °C overnight. The next morning the serum was separated by centrifuging the blood at 4000 RPM and was stored at -20 °C till further processing.

Laboratory analysis

All the serum samples were screened by RBPT, SPAT and ELISA. The serological tests were performed by using commercially available reagents.

Rose Bengal Plate Test: RBPT was performed according to the procedure provided by Alton [14] using the antigen supplied by Veterinary Research Institute (VRI) Lahore. Simply a 30µl of serum was taken through a pipette on a clean glass slide then an equal amount of the antigen suspension was added near to the serum spot on the glass slide. Both the serum sample and antigen were mixed immediately with an applicator stick, in such a way that it forms a round area of ½ inch. The slide was then rotated for four minutes by hand. According to the level of agglutination, the agglutinations were recorded as 0, +, ++, and +++. Serums with 'score 0' were recorded as a negative result, with + were equivocal and those with ++, +++ were recorded as positive tests.

Serum Plate Agglutination Test: SPAT was performed according to the protocol described by Alton[14]. In short with a wax pencil a clean glass slide was divided into two parts having 1.5-inch squares. Through a pipette, 20µl serum was added to each square on the glass slide. One drop of *B. abortus* antigen was added to 20 µl serum at one square while one drop of *B.melitensis* suspension to the other. The slide was then rotated for 2-3 minutes by hand. Any visible agglutination was recorded as a positive result.

ELISA: All the serum samples were also subjected to ELISA for the comparative evaluation of RBPT and SPAT. ELISA was performed according to the procedure provided by manufacturers (ID. vet, ID Screen® Brucellosis Serum Indirect Multi-species, France). In short, all the reagents were brought to room temperature and homogenized. 190µl of dilution buffer and 10 µl of serum were added to the antigen-coated well. After 45 minutes of incubation, the wells were washed out three times with washing buffer. By adding 100µl of the conjugate, the wells were re-incubated for 30 minutes and washed 3 times. The 100 µl of substrate solution was then added to the wells and incubated for 15 minutes at dark. Finally, 100 µl of stop solution was added. The wells were then measured by PR 4100 Microplate Reader at 450nm to record OD.

Data Analysis: The data were analyzed statistically by using SPSS version 25. A chi-square test was performed to find the P-value among the different factors. The performance characteristics of RBPT and SPAT were evaluated by using "TWO by TWO" tables with ELISA provided by smith [15].

Results

In the current study, the overall seroprevalence of brucellosis in the goat population of district Swat was recorded as 32/235 (13.61%) and 38/235 (16.17%) by RBPT and SPAT respectively. The seroprevalence of brucellosis in humans was recorded at 3.77% and 5.66% by RBPT and SPAT respectively. Data is presented in **Table 1**.

By comparing the seroprevalence of *B. abortus* and *B. melitensis* in goat population, 25 out of 235 serum samples were positive for *B. abortus,* which is 10.63% of the total examined samples. This was the overall prevalence of *B. abortus* recorded in this study either in separate form or in mixed form with *B. melitensis*. In a total of 235, 33(14.04%) samples were found positive for *B. melitensis* either in separate or in mixed form with *B. abortus*. 20 serum samples gave positive reactions for both the *B. abortus* and *B. melitensis* that are 8.51% of the total analyzed samples. In the human population, 03 out of 53(1.88%) serum samples were found positive for *B. abortus*. This was the overall prevalence of *B. abortus* recorded in this study either in separate form or in mixed form with *B. melitensis*. While 03 out of 53 (5.66%) were positive for *B. melitensis* in separate or mixed form with *B. abortus*. One out of 53 human serum samples gave a positive reaction against both the *B. abortus* and *B. melitensis* examined by SPAT. Data are shown in Table 2.

In the current study, different risk factors were evaluated to find their association with the positivity of brucellosis. In these factors, abortion was found to have a significant relationship with the seropositivity of brucellosis by giving 69% positive results with a p-value <0.05. Other variables like herd size and animal sex didn't show statistically significant results. The data are shown in Table 3.

By evaluation of serological tests used in this study RBPT showed sensitivity 80%, specificity 98%, Positive Predictive Value 87% and Negative Predictive Value 96% as compare to SPAT which showed 82% sensitivity, 95% specificity, 76% Positive Predictive Value, and 93 % Negative Predictive Value. The data are shown in Table 4.

TABLE 1. The Sero-Prevalence of Brucellosis in Goat and Human Population of District Swat Examined by Various Diagnostic Techniques

	Goats			Humans		
Diagnostic Technique	**Total samples**	**positive samples**	**Positive percentage**	**Total samples**	**Positive samples**	**Positive percentage**
RBPT	235	32	13.61%	53	2	3.77%
SPAT	235	38	16.17%	53	3	5.66%

Note. RBPT: Rose Bengal Plate Test and SPAT: Serum Plate Agglutination Test.

TABLE 2. Comparative prevalence of *B. abortus* and *B. melitensis* in goats and humans.

Examined spp	Total samples N	*B. abortus* N (%)	*B. melitensis* N (%)	Positive for both *B. abortus*&*B. melitensis*N (%)
Goat	235	25 (10.63)	33 (14.04)	20 (8.51)
Human	53	01 (1.88)	03 (5.66)	01 (1.88)

TABLE 3. Association of different factors with Seropositivity of Brucellosis.

Characteristics	Animal condition	No	Positive N	Percentage	P-Value
Abortion	Aborted	23	16	69.56	<0.05
	No record	19	07	3.55	
Herd size	Small	94	11	11.70	0.177
	Medium	60	06	10	
	Large	81	15	18.51	
Sex	Male	41	05	12.2	0.863
	Female	194	27	13.9	

Note. A chi-square test was performed to find the P-value

TABLE 4. The Performance Characteristics of RBPT and SPAT using I-Elisa as a Gold Standard.

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
RBPT	80.00	98.00	87.50	96.55	95.31
SPAT	82.85	95.50	76.31	96.95	93.61

Note. RBPT Rose Bengal Plate Test, SPAT: Serum Plate Agglutination Test, PPV: Positive Predictive Value, NPV: Negative Predictive Value.

Discussion

Brucellosis has great importance as an infection of animals where it causes substantial economic harm due to loss of milk yield and progeny, in addition, it's the second most prevailing zoonosis that greatly affects human health. This is the first study that comprehensively reports the seroprevalence of brucellosis in goat as well as in animal keepers from district Swat of KPK Pakistan. The seropositivity in this study was considered due to the natural infections because no vaccination record was found in the district. Regardless of the diagnostic techniques, the overall prevalence of brucellosis in the goat population recorded in this study varies from previous data as Khan reported the seroprevalence of brucellosis in the goat population of Jhang and Okara districts to be 76% [16], the reason for the higher incidence rate in their study could be attributed to the diagnostic technique, as they have performed milk I-ELISA for the screening of milk samples, another study conducted by Hamidullah show 32% prevalence in goats of Kohat district, both of these results are higher than our current study [17]. While the recorded results are in agreement with the study conducted by Din, their findings show the prevalence of brucellosis as 13.33%, in the goat population of district Bhimber, Azad Jammu, and Kashmir [18]. Other studies conducted in different areas of Pakistan have lower findings

like 1.93% in Punjab, 1.76% in Baluchistan, and 3.13% from Tribal-Kurram Agency reported by Nasir, Jamil and Khan respectively [19,20,27]. Including the current study, the reported data show variability which could be attributed to geographical variations altered management system, diagnostic techniques, and vaccinations of animals. However, many studies have concluded that brucellosis is prevalent in both the animals and human population of Pakistan [30].

In the current study, the seropre valence of the human population was 5.66% and 3.66% by SPAT and RBPT respectively while Ahmad has presented 2.66% positive results of human brucellosis in district Swat [21]. The current results are slightly higher than reported data, which may be due to the targeted population as we diagnosed the animal keepers who are at high risk (humans with close contact with animals) while the referenced study shows the prevalence of the general human population of district Swat. Our findings support the study performed by Khan who gave the seropre valence of *Brucella* from Tribal-Kurram Agency of Pakistan as 4.39% in high-risk individuals [20]. By questionnaire record, all the positive human individuals were the consumer of unpasteurized milk which could be the reason for infection. Consumption of raw milk has been identified as a risk factor for brucellosis by many researchers like Almuneef, Ahmed, and many others [22, 23,29].

In both the goat as well as in human individuals the prevalence of *B. melitensis* was higher than *B. abortus.* Our reports are supported by previously published data as Din reported a comparatively higher prevalence of *B. melitensis* than that of *B. abortus* in both the goat as well as in humans [18]. According to OIE the main causative agent for brucellosis in goat is *B. melitensis* [7,9].

By considering abortion, herd size, and animal sex, a significant association was found between brucellosis and abortion. The prevalence of disease was higher (89%) in goats having abortion history than the animals with no clinical signs (3.55%), a ($P < 0.001$) was observed. A significant association between brucellosis and abortion is also reported by Al-Majali, Khan, and many others [24, 28]. A higher incidence of the disease was recorded in goats with larger herd sizes but the results were not statistically significant ($P \geq 0.05$). Similarly, the sex of the animal also didn't show any significant association with the seropositivity of brucellosis by giving a $P \geq 0.05$.

By comparing the results of serological tests used in this study with that of ELISA to find the sensitivity, specificity, and other parameters, no such great difference was found in diagnostic parameters of RBPT and SPAT. With a slightly higher result, the SPAT test showed greater sensitivity than RBPT. Some studies show higher results of RBPT than SPAT, as Din reported that RBPT is a more sensitive test than SPAT for the screening of brucellosis [18]. While in agreement with our study, Ghodasara reported that RBPT is less sensitive among the ELISA, SPAT, and Serum tube agglutination test (STAT) [25]. In a study on the comparative evaluation of RBPT and SPAT, Khan reported the higher sensitivity and specificity of RBPT than that of SPAT [20]. In the current study, RBPT and SPAT showed some false positive and false negative results which are in agreement with Hussain and Khan [20,26].

Conclusions

From the current study we conclude that brucellosis is prevailing in the goat and human population of district Swat which confirms the previously reported data from different areas of the country. Both the RBPT and SPAT can be used for the screening of *Brucella* suspected animals but not as confirmatory tests because these tests showed some false-positive and false-negative results.

Recommendations

Swat is one of the mountainous regions and most people keep dairy goats because it gives self-sufficiency and home supply of fresh milk to the families. As brucellosis is prevailing in the area and the goat herds are mostly moveable that can transmit the disease to other healthy animals. The health authorities should take preventive measures immediately that can effectively reduce the spread of disease in animals. The awareness of the general population is also required that will control the disease transmission to humans.

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Conflict of interest

The authors declare that they have no conflicts of interests

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