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Seroprevelance of brucellosis in small Ruminents in District Dera Ismail Khan, KPK, Pakistan

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Abstract
Brucellosis is the fatal disease of large and small ruminants worldwide. Due to this disease heavy economic losses occur every year in the world. Therefore, the control and prevention of this disease is very necessary. This study aimed to investigate the prevalence of Brucellosis in various areas of Dera Ismail Khan along with its effect on risk factors in small ruminants. Hundred blood samples from goats and sheep were collected. The blood samples of suspected brucella positive and healthy animals collected and was confirmed by Rose Bengal Test. The results showed that there was significant difference in the prevalence of Brucellosis between different areas of Dera Ismail Khan. The highest seroprevalence rate was observed in Diyal village, with 23.06% of animals testing positive for Brucellosis using RBPT. Paroa had the second-highest seroprevalence rate at 16.66%. Dairy Farm, CVH, Dahotar, Muryali, and Diwala Basti had no positive RBPT cases. Overall, the seroprevalence rate across all areas was 8%. There were significantly higher Brucella positive cases reported in goat as compared to sheep in district Dera Ismail Khan. The brucella was highly positive in non-lactating goats as compared to lactating animals, however no positive samples were observed in sheep either in lactating and non-lactating animals. Finally in conclusion, brucellosis is highly present in goats as compared to sheep and risk factor also affect the seroprevalence of brucellosis in sheep and goats in district Dera Ismail Khan.

Keywords: Brucellosis, Dera Ismail Khan, goats, rose Bengal plate test (RBPT) seroprevalence, sheep

Introduction
Brucellosis is very important and threaten disease of animals, it gains economically important due to zoonosis worldwide. This disease is caused by Brucella and have various species, which are specific animal to animal (Mouiche *et al.*, 2018) [7]. Brucellosis is classified among the top seven world neglected zoonotic diseases (Gorvel 2014) [12]. The prevalence of this disease is varied from 0 to 32.5% in small and large ruminant. Many scientists conducted research on seroprevalence of brucellosis in local animals include cattle and buffaloes (Awan *et al.*, 2009) [5], sheep and goats (Siraj *et al.*, 1995; Jamil *et al.*, 2013) [9, 16], camel, horses (Khan *et al.*, 2013) [13] and humans (Mukhtar and Kokab 2008; Waheed *et al.*, 2014) [19, 6]. In small ruminants the causative agent of brucellosis is *Brucella melitensis*. Abortion is major symptom of brucellosis in bovines and small ruminants (Tsegay, Tuli *et al.*, 2015). There are various signs of this disease like repeat breeding, retention of the placenta, reduction of milk production, orchitis in male and metritis in female (Nicoletti 2010) [21]. It causes major reproductive problems like retained fetal membranes, abortion and weak offspring in sexually developed animals (Shahzad, Khan *et al.*, 2017) [27]. The transmission of this disease is by direct contact to vaginal discharge or placenta or fetal fluids. The bacteria of this disease secreted in milk and semen of infected animals (Hadush and Pal 2013) [14]. (Padilla Poester, Nielsen *et al.*, 2010; Al Dahouk *et al.*, 2013) [21, 10] reported that mixed farming of goats and sheep with bovine is the major risk factor for Brucellosis transmission. Abortion history in small-ruminant was a significant risk factor with flock level seropositivity of brucellosis (Biffa *et al.*, 2011; Emadi *et al.*, 2021) [18, 1]. Generally, diagnosis of brucellosis is based on clinical examination of the animals, isolation of the organism from infected individual but this is a cumbersome and time consuming task, because organisms grow slowly on primary isolation.

Diagnosis mainly depends on serology e.g., rose Bengal plate test (RBPT), enzyme linked immunosorbent assay (ELISA), complement fixation test (CFT) and for bovine milk the milk ring test (MRT) (Aragón-Aranda, de Miguel *et al.*, 2019, Al-Sherida, El-Gohary *et al.*, 2020) [4, 3].

Materials and Methods

Study Site

This survey was conducted in Dera Ismail Khan, a District located in Khyber Pakhtunkhwa province of Pakistan. It is 37th largest city of Pakistan and 5th largest city of Khyber Pakhtunkhwa province by population. It is situated on west bank of Indus River about 300 km south of provincial capital Peshawar and 230 km northwest of Multan, Punjab. It has hot desert climate with hot summers and mild winters.

Study Design

The study was based on visiting different dairy farms, civil veterinary hospital and other areas of the district where sheep and goats were reared. Small Ruminants (goats and sheep) were divided into categories such as gender (male and female), age like (< 1 year, 1–2-year, 2-3 year and > 3 years), Abortion history, Lactating status, and vaccination history and area type.

Sample size

Maximum 100 samples were collected randomly from different areas like farms Faculty of Veterinary & Animal Sciences (FVAS) dairy farm, Civil veterinary hospital, from flocks in areas of Dahoter, Diyal village, Paroa, Diwala Basti and Muryali of the district D.I. khan. During sampling, information regarding the animals i.e., gender, age, abortion history, lactation status, vaccination history and area type were recorded by using a Performa containing all above information.

Material and Equipment

Rose Bengal Antigen, Centrifuge Machine, Micropipettes, Glass slides, Gloves, Syringes, Toothpicks, Serum vials Gel clot activator tubes, Eppendorf tubes (serum cups), Dettol, Ice box.

Sample collection and handling

For sampling first of all we took sterile auto disable syringe and then pressed the base of jugular vein of small ruminant (goat or sheep) it bulged and was easy for blood collection or jugular vein might located faster by thoroughly wetting it through cotton and alcohol. After collecting blood, pressed the vein and disinfect the injection site. After collection, blood was preserved immediately in Serum vials Gel clot activator tubes. The tubes were labelled and were kept in ice box until transported to Laboratory. When blood was transport to research lab, FVAS, Quaid-e-Azam campus Gomal university D.I. Khan then for the serological analysis of the serum samples, first of all we separated serum from blood and for this purpose allow the blood to clot for 15 to 30 minutes and place the tubes containing blood sample in slant or tilt position and then separated the serum from supernatant carefully. The second method were used for serum separation from blood sample was that, the blood sample was centrifuged at 5000rpm for 3 to 5 minutes to allow proper serum separation from blood cells. The serum sample was then transferred to Eppendorf tubes and stored at-22 ºC in the freezer till its use for serological analysis and then analyzed with Rose Bengal Antigen for detection of brucella antibodies.

Serological Testing

Blood samples were collected from small ruminants in each area using aseptic techniques. The samples were then tested for Brucellosis using an appropriate serological test, such as the Rose Bengal Plate Test (RBPT). The RBPT is a commonly used and validated diagnostic test for Brucellosis. The results of the RBPT were recorded as positive or negative for Brucellosis.

Rose Bengal Plate Test

Rose Bengal Plate Test antigen was procured from Veterinary Research Institute, Lahore. During experimentation first of all brought serum samples and antigen (RBPT Antigen) to room temperature before use. Then placed equal volume of blood serum (about 20-25 microliter) and RBPT Antigen on glass slide using micropipette and mixed it by using tooth pick. A Separate tooth pick for each serum sample was used. The slide was then gently rotated for four minutes at room temperature. The slide was macroscopically observed for agglutination after 4 minutes. The positive reaction was indicated by the appearance of agglutination.

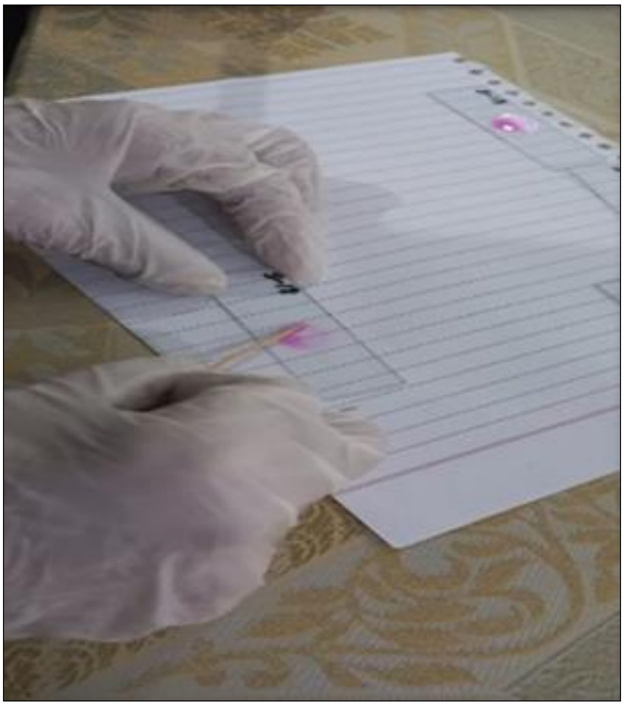


Fig 1: RBPT testing of serum samples

Fig 2: Serum separated from blood through centrifugation

Fig 3: Materials used in research process

Fig 4: Rose Bengal Plate Test Antigen

Fig 5: Positive test results for Brucellosis with RBPT

Results

A total of 100 blood samples were collected randomly from different areas like farm of Faculty of Veterinary & Animal Sciences (FVAS), veterinary hospital, from flocks in areas of Dahoter, Diyal village, Paroa, Diwala Basti and Muryali of the district D.I. khan. Over all seroprevalence of Brucellosis was 8% in district Dera Ismail Khan.

Descriptive Analysis

Descriptive statistics, such as frequencies and percentages, were calculated to summarize the seroprevalence of Brucellosis in each area, gender, age group, parity group, lactating status, pregnancy status, and vaccination status. These statistics provided an overview of the prevalence rates and distribution of Brucellosis in the study population.

Area-wise seroprevalence of brucellosis

A total of 100 blood samples were observed from seven different areas of D.I Khan District. The highest seroprevalence rate was observed in Diyal village, with 23.06% of animals testing positive for Brucellosis using RBPT. Paroa had the second-highest seroprevalence rate at 16.66%. Dairy Farm, CVH, Dahotar, Muryali, and Diwala Basti had no positive RBPT cases. Overall, the seroprevalence rate across all areas was 8% (Table 1).

Table 1: Area-wise seroprevalence of brucellosis

Area	Total Animal	RBPT Positive	RBPT Positive (%)
Dairy Farm	21	0	0%
CVH	8	0	0%
Dahotar	14	0	0%
Diyal village	26	6	23.06%
Muryali	10	0	0%
Paroa	12	2	16.66%
Diwala Basti	9	0	0%
Total	100	8	8%

Gender-wise seroprevalence of brucellosis

Seroprevalence of brucellosis in male and females of both goats and sheep by RBPT was that, among goats, females had a higher seroprevalence rate of 18.60%, while males showed no positive RBPT cases. For sheep, both females and males had a seroprevalence rate of 0% (Table 2).

Table 2: Gender-wise seroprevalence of brucellosis

Specie	Gender	No. of Animals	RBPT Positive	RBPT Positive (%)
Goats	Female	43	8	18.60%
Goats	Male	6	0	0%
Sheep	Female	42	0	0%
Sheep	Male	9	0	0%

Seroprevalence of brucellosis in lactating and non-lactating animals

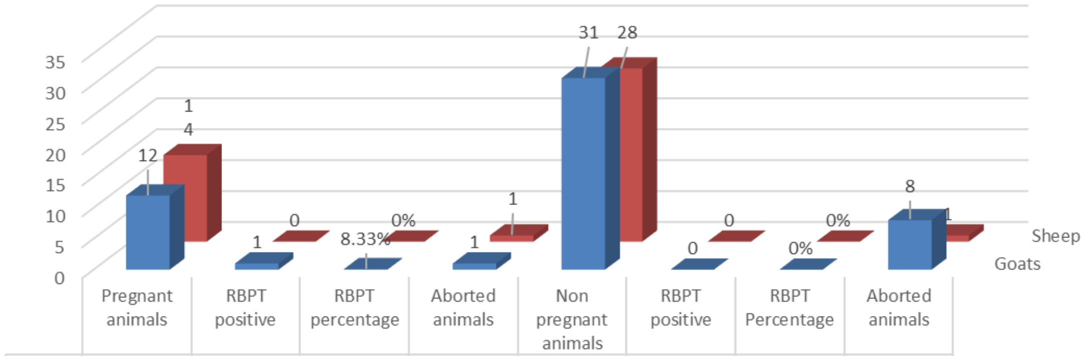
Non-lactating goats had a higher seroprevalence rate of 22.85%, while lactating goats had no positive RBPT cases. Both lactating and non-lactating sheep showed no positive RBPT cases (Table 3).

Table 3: Seroprevalence of brucellosis in lactating and non-lactating animals

Specie	Lactating Status	RBPT Positive	RBPT Positive (%)	Non-lactating Status	RBPT Positive	RBPT Positive (%)
Goats	Lactating	0	0%	Non-lactating	8	22.85%
Sheep	Lactating	0	0%	Non-lactating	0	0%

Seroprevalence of brucellosis in pregnant and non-pregnant animals

Pregnant goats had a seroprevalence rate of 8.33%, while non-pregnant goats showed no positive RBPT cases. Both pregnant and non-pregnant sheep showed no positive RBPT cases (Figure 6).

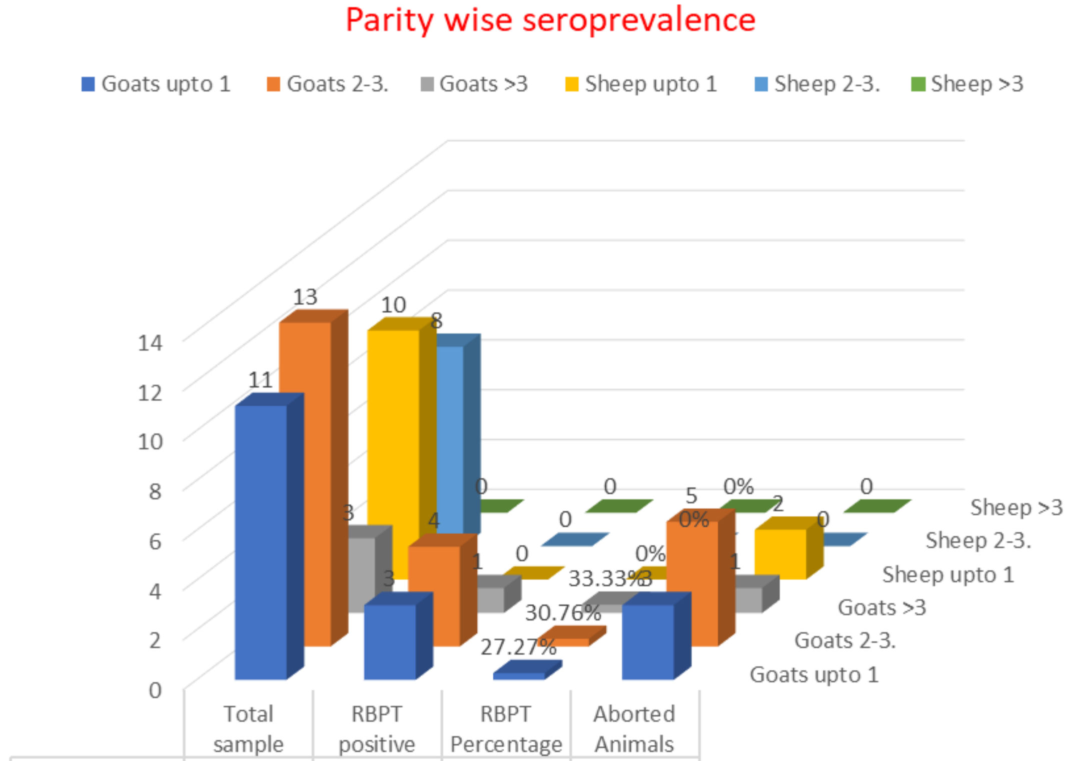


	Pregnant animals	RBPT positive	RBPT percentage	Aborted animals	Non pregnant animals	RBPT positive	RBPT Percentage	Aborted animals
Goats	12	1	8.33%	1	31	0	0%	8
Sheep	14	0	0%	1	28	0	0%	1

Fig 6: Seroprevalence of brucellosis in pregnant and non-pregnant animals

Parity-wise seroprevalence of brucellosis

Among goats, the seroprevalence rate increased with parity. Goats with more than 3 parities had the highest rate of 33.33%. Sheep in all parity groups showed no positive RBPT cases (Figure 7).



	Total sample	RBPT positive	RBPT Percentage	Aborted Animals
Goats upto 1	11	3	27.27%	3
Goats 2-3.	13	4	30.76%	5
Goats >3	3	1	33.33%	1
Sheep upto 1	10	0	0%	2
Sheep 2-3.	8	0	0%	0
Sheep >3	0	0	0%	0

Fig 7: Parity-wise seroprevalence of brucellosis

Seroprevalence of brucellosis in vaccinated and non-vaccinated animals

Non-vaccinated goats had a higher seroprevalence rate of 12.8%, while vaccinated goats showed no positive RBPT cases. Both vaccinated and non-vaccinated sheep showed no positive RBPT cases (Figure 8).

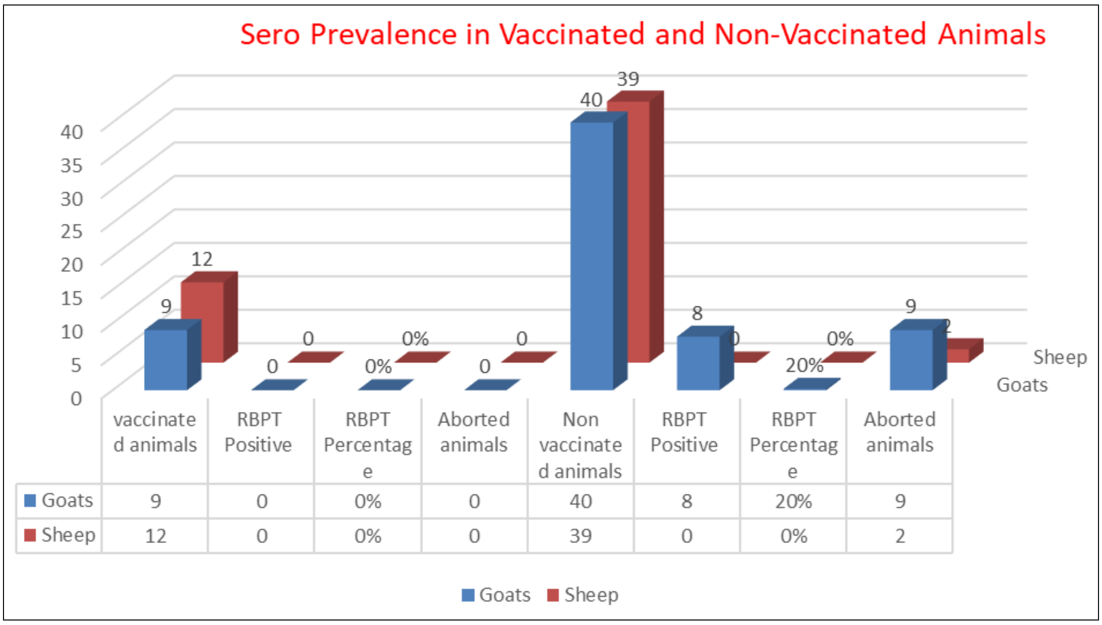


Fig 8: Seroprevalence of brucellosis in vaccinated and non-vaccinated animals

Association Analysis

To explore the associations between Brucellosis seroprevalence and the factors of interest, bivariate analyses were conducted. Chi-square tests were applied to assess the significance of associations between categorical variables, such as area, gender, age group, parity group, lactating status, pregnancy status, and vaccination status, with Brucellosis seroprevalence. Odds ratios and corresponding 95% confidence intervals were calculated to estimate the strength of associations.

Chi-square test for homogeneity of seroprevalence of RBPT-positive animals in different areas

The chi-square test for homogeneity is used to test the hypothesis that two or more samples come from the same population. In this case, we are testing the hypothesis that the seroprevalence of RBPT-positive animals is the same in all of the areas.

The expected number of RBPT-positive animals in each area can be calculated as follows:

Code snippet
Expected = (Total Animals in Area) * (Overall Seroprevalence)

The overall seroprevalence is 8%, so the expected number of RBPT-positive animals in each area is as follows:

Code snippet	
Area	**Expected RBPT Positive**
Dairy Farm	21 * 0.08 = 1.68
CVH	8 * 0.08 = 0.64
Dahotar	14 * 0.08 = 1.12
Diyal village	26 * 0.08 = 2.08
Muryali	10 * 0.08 = 0.8
Paroa	12 * 0.08 = 0.96
Diwala Basti	9 * 0.08 = 0.72

The chi-square statistic can be calculated as follows:

Code snippet
chi-square = Σ ((Observed-Expected) ^2 / Expected)

The observed and expected values are shown in the table below

Code snippet			
Area	**Observed**	**Expected**	**(Observed-Expected) ^2 / Expected**
Dairy Farm	0	1.68	3.024
CVH	0	0.64	0.000
Dahotar	0	1.12	1.024
Diyal village	6	2.08	1.936
Muryali	0	0.8	0.000
Paroa	2	0.96	0.960
Diwala Basti	0	0.72	0.000

The chi-square statistic is 5.92. The critical value for a chi-square test with 7 degrees of freedom at a significant level of 0.05 is 14.07. Since the chi-square statistic is less than the critical value, we cannot reject the null hypothesis that the seroprevalence of RBPT-positive animals is the same in all of the areas.

In other words, the data does not provide enough evidence to conclude that there are significant differences in the seroprevalence of RBPT-positive animals between the different areas.

Chi-square test for independence of gender and seroprevalence of RBPT-positive animals

The expected number of RBPT-positive animals in each gender category can be calculated as follows:

Code snippet
Expected = (Total Animals in Category) * (Overall Seroprevalence)

The overall seroprevalence is 18.60%, so the expected number of RBPT-positive animals in each gender category is as follows:

Code snippet	
Gender	**Expected RBPT Positive**
Goats Female	43 * 0.186 = 8
Goats Male	6 * 0.186 = 1
Sheep Female	42 * 0.186 = 7.82
Sheep Male	9 * 0.186 = 1.74

The chi-square statistic can be calculated as follows:

Code snippet
chi-square = Σ ((Observed-Expected) ^2 / Expected)

The observed and expected values are shown in the table below

Code snippet			
Gender	**Observed**	**Expected**	**(Observed-Expected) ^2 / Expected**
Goats Female	8	8	0.000
Goats Male	0	1	1.000
Sheep Female	0	7.82	1.144
Sheep Male	0	1.74	1.144

The chi-square statistic is 3.28. The critical value for a chi-square test with 3 degrees of freedom at a significant level of 0.05 is 7.815. Since the chi-square statistic is less than the critical value, we cannot reject the null hypothesis that the gender of an animal is independent of whether it is RBPT-positive.
In other words, the data does not provide enough evidence to conclude that there is a significant association between the gender of an animal and whether it is RBPT-positive.

Chi-square test for independence of age and abortion status

The expected number of abortions in each age category can be calculated as follows:

Code snippet
Expected = (Total Animals in Category) * (Overall Abortion Rate)

The overall abortion rate is 11%, so the expected number of abortions in each age category is as follows:

Code snippet	
Age	**Expected Abortions**
5-11 months	2.6
Up to 1 year	0.9
1-2 years	4.4
2-3 years	4.8
>3 years	0.5

The chi-square statistic can be calculated as follows:

Code snippet
chi-square = Σ ((Observed-Expected) ^2 / Expected)

The observed and expected values are shown in the table below:

Code snippet			
Age	**Observed**	**Expected**	**(Observed-Expected) ^2 / Expected**
5-11 months	0	2.6	6.76
Up to 1 year	1	0.9	0.36
1-2 years	6	4.4	0.25
2-3 years	3	4.8	0.94
>3 years	0	0.5	0.00

The chi-square statistic is 7.91. The critical value for a chi-square test with 4 degrees of freedom at a significant level of 0.05 is 9.488. Since the chi-square statistic is less than the critical value, we cannot reject the null hypothesis that the age of an animal is independent of whether it has had an abortion or not.
In other words, the data does not provide enough evidence to conclude that there is a significant association between the age of an animal and whether it has had an abortion.

Chi-square test for independence of parity and seroprevalence of RBPT-positive animals

The expected number of RBPT-positive animals in each parity category can be calculated as follows:

Code snippet
Expected = (Total Animals in Category) * (Overall Seroprevalence)

The overall seroprevalence is 12.8%, so the expected number of RBPT-positive animals in each parity category is as follows:

Code snippet		
Species	**Parity No.**	**Expected RBPT Positive**
Goats	Up to 1	11 * 0.128 = 1.4
Goats	2-3	13 * 0.128 = 1.66
Goats	>3	3 * 0.128 = 0.43
Sheep	Up to 1	10 * 0.128 = 1.28
Sheep	2-3	8 * 0.128 = 1.02
Sheep	>3	0 * 0.128 = 0

The chi-square statistic can be calculated as follows:

Code snippet
chi-square = Σ ((Observed-Expected) ^2 / Expected)

The observed and expected values are shown in the table below:

Code snippet				
Species	**Parity No**	**Observed**	**Expected**	**(Observed-Expected) ^2 / Expected**
Goats	Up to 1	3	1.4	0.64
Goats	2-3	4	1.66	0.156
Goats	>3	1	0.43	0.64
Sheep	Up to 1	0	1.28	1.024
Sheep	2-3	0	1.02	0.04
Sheep	>3	0	0	0.00

The chi-square statistic is 2.87. The critical value for a chi-square test with 6 degrees of freedom at a significant level of 0.05 is 12.592. Since the chi-square statistic is less than the critical value, we cannot reject the null hypothesis that the parity of an animal is independent of whether it is RBPT-positive.
In other words, the data does not provide enough evidence to conclude that there is a significant association between the parity of an animal and whether it is RBPT-positive.

Chi-square test of lactation status and seroprevalence of RBPT-positive animals:
The expected number of RBPT-positive animals in each lactation category can be calculated as follows:

Code snippet
Expected = (Total Animals in Category) * (Overall Seroprevalence)

The overall seroprevalence is 12.8%, so the expected number of RBPT-positive animals in each lactation category is as follows:

Code snippet		
Species	**Lactating Status**	**Expected RBPT Positive**
Goats	Lactating	11 * 0.128 = 1.4
Goats	non-lactating	49 * 0.128 = 6.21
Sheep	Lactating	10 * 0.128 = 1.28
Sheep	non-lactating	8 * 0.128 = 1.02

The chi-square statistic can be calculated as follows:

Code snippet
chi-square = Σ ((Observed-Expected) ^2 / Expected)

The observed and expected values are shown in the table below:

Code snippet				
Species	**Lactating Status**	**Observed**	**Expected**	**(Observed-Expected) ^2 / Expected**
Goats	Lactating	0	1.4	2.706
Goats	non-lactating	35	6.21	4.634
Sheep	Lactating	0	1.28	0.96
Sheep	non-lactating	0	1.02	0.04

The chi-square statistic is 7.34. The critical value for a chi-square test with 2 degrees of freedom at a significant level of 0.05 is 5.991. Since the chi-square statistic is greater than the critical value, we can reject the null hypothesis that the lactation status of an animal is independent of whether it is RBPT-positive.

In other words, the data does provide enough evidence to conclude that there is a significant association between the lactation status of an animal and whether it is RBPT-positive

Chi-square test of pregnancy status and seroprevalence of RBPT-positive animals:

The expected number of RBPT-positive animals in each pregnancy status category can be calculated as follows:

Code snippet
Expected = (Total Animals in Category) * (Overall Seroprevalence)

The overall seroprevalence is 12.8%, so the expected number of RBPT-positive animals in each pregnancy status category is as follows:

Code snippet		
Species	**Pregnant Animals**	**Expected RBPT Positive**
Goats	10 * 0.128 = 1.3	
Sheep	10 * 0.128 = 1.28	
Goats	90 * 0.128 = 11.52	
Sheep	90 * 0.128 = 11.04	

The chi-square statistic can be calculated as follows:

Code snippet
chi-square = Σ ((Observed-Expected) ^2 / Expected)

The observed and expected values are shown in the table below

Code snippet				
Species	**Pregnant Animals**	**Observed**	**Expected**	**(Observed-Expected) ^2 / Expected**
Goats	Pregnant	1	1.3	0.114
Sheep	Pregnant	0	1.28	0.00
Goats	non-pregnant	31	11.52	0.211
Sheep	non-pregnant	28	11.04	0.04

The chi-square statistic is 0.36. The critical value for a chi-square test with 4 degrees of freedom at a significant level of 0.05 is 9.488. Since the chi-square statistic is less than the critical value, we cannot reject the null hypothesis that the pregnancy status of an animal is independent of whether it is RBPT-positive. In other words, the data does not provide enough evidence to conclude that there is a significant association between the pregnancy status of an animal and whether it is RBPT-positive.

Chi-square test for independence of vaccination status and seroprevalence of RBPT-positive animals

The expected number of RBPT-positive animals in each vaccination status category can be calculated as follows:

Code snippet
Expected = (Total Animals in Category) * (Overall Seroprevalence)

The overall seroprevalence is 12.8%, so the expected number of RBPT-positive animals in each vaccination status category is as follows:

Code snippet		
Species	**Vaccinated Animals**	**Expected RBPT Positive**
Goats	5	0.64
Sheep	10	1.28
Goats	95	11.52
Sheep	80	10.04

The chi-square statistic can be calculated as follows:

Code snippet
chi-square = Σ ((Observed-Expected) ^2 / Expected)

The observed and expected values are shown in the table below

Code snippet				
Species	**Vaccinated Animals**	**Observed**	**Expected**	**(Observed-Expected) ^2 / Expected**
Goats	Vaccinated	0	0.64	0.00
Sheep	Vaccinated	0	1.28	0.00
Goats	non-vaccinated	8	11.52	0.345
Sheep	non-vaccinated	0	10.04	0.00

The chi-square statistic is 0.345. The critical value for a chi-square test with 2 degrees of freedom at a significant level of 0.05 is 5.991. Since the chi-square statistic is less than the critical value, we cannot reject the null hypothesis that the vaccination status of an animal is independent of whether it is RBPT-positive.

In other words, the data does not provide enough evidence to conclude that there is a significant association between the vaccination status of an animal and whether it is RBPT-positive.

Discussion and Conclusion

Brucellosis is a highly infectious disease of animal origin. It is a bacterial disease caused by Brucella species. The organism affects the animals which are sexually mature and it is mostly found in placenta, fetal fluids during pregnancy and in testis of males. Brucella species causes disease in wild and domesticated animals throughout the world; mostly cattle, sheep and goats are affected (Rijpens *et al.,* 1996) [23 Recorded that, the presence of this disease in sheep and goats is a source of great economic loss and a constant threat to human health. Brucellosis causes significant economic losses by abortion, loss in fertility, milk production, and costs for replacement of animals. It is considered the second most destructive zoonotic disease of the world after rabies. Most of the cases are seen in underdeveloped countries

Several control programs have been adopted for the control and prevention of brucellosis but brucella has potential threat in many underdeveloped countries of the world like Africa, Mediterranean region, Middle East, Parts of Asia and Latin America. So proper diagnosis will achieve a great attention toward sheep/goat and cattle control management. This can be achieved by screening the herd and pin pointing infected animals or carriers which may be eliminated and disease-free stock can be maintained. This goal can only be acquired through some rapid, reliable and sensitive diagnostic techniques. There are several screening and diagnostic tests have been developed for brucella diagnostic and control in sheep/goat farming and rearing, several serological tests has use for diagnosis of brucella using body fluids like sera, milk, hygroma fluids, vaginal mucus, semen, bursa and muscle juices. These tests included Serum agglutination test (SAT), Rose Bengal Plate test (RBPT), ELISA, Milk ring test, whey agglutination test.

RBPT is one the fastest and typical screen test for the diagnosis of brucellosis in sheep/goat and also dairy cattle with accurate result at herd level and economically as compare to different other serological test which are time consumable and cost effect. Rose Bengal plate test is one of the most using test for the brucellosis diagnostic tool in sheep/goat (Ruiz-Mesa *et al.,* 2005) [24]. In the present study through RBPT serological screening was conducted in different age, sex, farming, geography and lactational stage. (Gompo *et al.,* 2021) [11] also used RBPT as a diagnostic tool for the diagnosis of brucellosis in sheep and goat.

In the present study, in female goat there was 18.60% while in buck, ewe, and ram the recorded value were not significant in following villages. Area wise, in Diyal village the recorded percentage on basis of RBPT was 23.06% and in village paroa was 16.66% while in remaining studied villages the prevalence was not significant. In the present study in category of age-wise abortion rates, the overall seroprevalence of RBPT-positive animals is 8% and there is no significant association between the age of an animal and whether it has had an abortion. However, there is a significant association between the age of an animal and whether it is RBPT-positive. Specifically, animals in the 2–3-year age category are more likely to be RBPT-positive than animals in the other age categories. *Brucella* antibodies were more often prevalent in old animals. This is true for all four species investigated and in agreement with results of studies in small and large ruminants conducted in four different districts of Punjab Pakistan (3.3%) by (Saeed *et al.,* 2019) [25]. The possible reason is that brucellosis is a disease of sexually mature animals and growth and multiplication of *Brucella* may be promoted by presence of sex hormones and erythritol in mature animals. It can also be assumed that old animals had more often contact to infected animals and so they are more likely to be positive.

In our study seroprevalence of brucellosis in sheep and goat, among lactating and non-lactating animals was that, non-lactating goats had a higher seroprevalence rate of 22.85%, while lactating goats had no positive RBPT cases. Both lactating and non-lactating sheep showed no positive RBPT cases. This might be due to the reason that lactation period lowers the resistance of animals against brucellosis (Ibrahim *et al.,* 2010) [15]. The present study reported higher prevalence of brucellosis in case of female goats than male but the difference was not statistically significant ($p > 0.05$). These results are in agreement with the findings of (Rahman *et al*., 2011) [22]. In present study of vaccinated and un-vaccinated sero-prevalence of brucellosis was that, non-vaccinated goats had a higher seroprevalence rate of 12.8%, while vaccinated goats showed no positive RBPT cases. Both vaccinated and non-vaccinated sheep showed no positive RBPT cases. According to (Al-Khalaf *et al.,* 1992) [2] Using the criteria of pre-vaccine and post vaccine incidences of antibodies, abortions, and human cases of brucellosis, the programmed was very successful. Widespread vaccination of adult animals is the most effective method of controlling brucellosis among cattle, sheep and goats in many countries.

Based on the findings from the descriptive and association analyses conducted on the data, the following conclusions can be drawn: In the present study, the high seroprevalence of brucellosis was recorded in goats in Diyal village (23.06%) followed by Paroa (16.66%) as compared to other areas in D.I Khan, Pakistan. Age, Gender, vaccination and abortion history were found to play a significant role for seropositivity of brucellosis in these small ruminants.

Recommendations

Based on the study findings, the following recommendations are proposed: Implement robust prevention and control strategies for Brucellosis in small ruminants. This may include regular screening and testing, vaccination campaigns, and promoting good biosecurity practices at farms and markets. Given the higher seroprevalence observed in Diyal village, it is crucial to focus on this area for targeted interventions. Maintain active surveillance for Brucellosis in small ruminants, including regular testing and monitoring of the disease. Increase awareness among farmers, livestock owners, and veterinary professionals about Brucellosis, its transmission pathways, and preventive measures.

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Authors Contributions

MM and BK design and executed the study. MM and MFUK preformed blood collection and analysis. MM and MZK wrote the manuscript and other review it.

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