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Prevalence of Human Brucellosis in District Dera Ismail Khan

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Abstract
Human Brucellosis is a zoonotic disease, also called undulant fever or Malta fever. Brucellosis is caused by bacteria of the Genus Brucella, which is a neglected disease. Humans always remain an accidental host and get infected from both wild animals as well as those that are kept at home. The status of brucellosis is unknown in various parts of the country; therefore, this study aims to find the prevalence and risk factors of human brucellosis in the population of District Dera Ismail Khan. In this study, a total of 270 samples were collected over two months (May and June 2022) from the population occupationally exposed to animals in District Dera Ismail Khan and screened through ELISA and SPAT. The gender-wise high prevalence was found in females at 27.52% and 24.83% by ELISA and SPAT, respectively. The age-wise high prevalence rate was reported as 50.00% and 47.73% by ELISA and SPAT in age groups above forty. The prevalence rate was found high as 27.52% and 24.83% in housewives by ELISA and SPAT. The current study proved that the prevalence rate was found high in the population aged above 40 and in housewives who keep animals domestically than in other occupationally exposed populations.

Introduction

Brucellosis

Brucellosis is a zoonotic disease and is very contagious. Brucellosis is caused by bacteria of the genus Brucella (Batashev et al., 1998). David Bruce was the first who came to know about Brucella, and gave name after him (Whatmore et al., 2014)

Worldwide Distribution

The distribution of brucellosis has been found in 86 different countries globally and is danger for both human health and livestock. (Tadesse et al., 2016). Brucellosis is at top of list in zoonotic bacterial diseases, and annually 500, 00 cases are recorded in disease-endemic areas (Johansen, et al., 2017). Brucellosis has significant public health challenges, particularly in the Mediterranean basin and in developing countries of the Middle East. (Nikokar et al., 2011). Most of the brucellosis infection are present in Near East and North African countries (Jennings et al.,2007). In

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most Middle east countries both B. abortus and B. melitensis existence has been reported and African and Asian continents are also not secured (Musallam et al.,2016). B. suis and B. abortus disease is present throughout Central America (McDermott et al., 2013). In Europe, travellers and immigrants from the Middle East is thought to be responsible for human brucellosis or dairy products that come from endemic areas can spread brucellosis. (Georgi et al., 2017). South Asian countries including Pakistan, China and Sri Lanka are also not secured from human brucellosis infection. (Norman et al., 2016). Due to lack of awareness the prevalence of brucellosis is increasing day by day in Pakistan (Arif et al., 2017). Brucellosis has been eradicated in many parts of developed countries, but it is still not properly controlled in developing countries like Pakistan (McDermott et al 2013; Gul & Khan 2007).

Brucellosis Transmission

Human brucellosis is mainly caused through three routes, direct contact with secretions from Brucella-infected animals, indirect consumption of contaminated products, or inhalation of aerosols (Massis et al., 2005). Humans always remained an accidental host (Dautovics et al., 2006). The brucellosis infection is present globally. Brucellosis is an occupational disease therefore people that perform services in livestock, have animals farm, sell meat and have animals at home may have an infection. People that have a connection in some way with animals or their products may be at a high risk of getting infection. (Chauhan et al., 2000). The most susceptible to brucellosis are slaughters because the slaughterers are in contact with different parts of animals, internal organs and can get infection through injury or cut. (Ramos et al., 2008). Animals like cattle, sheep, goats and pigs are considered sources of brucellosis to humans. (Corbel et al., 2006). Different secretions of animals and aerosol particles can also transmit brucellosis infection (Lapaque et al., 2006).

Diagnosis

Standard Tube Agglutination Test (STAT), Milk Ring Test (MRT), Test (SAT), and Enzyme- linked immunosorbent Assay (ELISA) are frequently used (Godfroid et al., 2010). When the detection of IgM, IgG antibodies are used with ELISA, it becomes more sensitive and cheaper (Mantecón et al., 2006). The isolation and diagnosis of pathogens can be done by methods like molecular methods, blood test and microbiological methods. ELISA and agglutination test are faster and give proper diagnosis (Dokuzoguz et al., 2005). In recent decades the diagnosis that is done on Polymerase chain reaction (PCR)-based has been removed the conventional assays used for diagnosis in clinical laboratories. PCR is useful method which not only confirms brucellosis but also differentiates between subacute, chronic and acute stage of brucellosis. Polymerase chain reaction (PCR)-based screening of brucellosis was firstly recorded in early 1990s (Zerva et al., 2001).

Treatment of Brucellosis

Brucellosis has a complex nature which makes it difficult to treat, but treatment done for long time with antibiotics is useful. Antibiotics in combinations are found to be more effective in many cases against brucellosis (Falagas et al., 2006). Tetracycline, rifampicin, quinolones are mostly recommended in clinics

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(Geyik et al., 2002). Doxycycline and rifampicin (600 mg for six weeks) can be used for treatment of acute brucellosis cases according to World Health Organization (Ersoy et al.,2005). Monotherapy through rifampicin for treatment of pregnant women is mostly used while for children different antibiotics in combination are used (Karabay et al., 2004).
Due to this high prevalence of brucellosis this study aims to find the prevalence of brucellosis in the people occupationally exposed to animals in the district Dera Ismail khan, Khyber Pakhtunkhwa and to know the risk factors associated with brucellosis in these people.

Material and Methods

Study Area

This research work was done in districts Dara Ismail khan, Khyber Pakhtunkhwa, Pakistan. Area of Dera Ismail khan is 9,334 sq km. Total population of district Dera Ismail khan is 1,695,688. Saraiki and Pashto are the main languages. There is a total of five tehsil in Dera Ismail khan. It is plan and warm area situated south of Khyber Pakhtunkhwa. It is situated at 31°49' North latitude and 70°54' East longitudes. It is surrounded by district tank from west, district Laki Marwat on northern side and Dera ghazi khan is present on south of Dera Ismail khan.

Data Collection

The data was collected through a stratified random sampling technique. A total of 270 blood samples were collected from May to August from people who are occupationally exposed to animals. The strata of human blood samples were from dairy farm workers, from women whose rearing animals domestically, butchers and veterinarians. A pre designed questionnaire was also used for collecting information about the risk factors of disease. The blood samples were processed in Mubarak labs near Lady reading hospital (LRH) Peshawar.

Lab Testing

The blood samples were processed by the serum plate agglutination test (SPAT) and enzyme linked immunosorbent assay (ELISA).

Enzyme Linked Immunosorbent Assay

The serum samples collected were screened for brucellosis infection by IgM-specific immunoglobulin through Enzyme Linked immunosorbent Assay (ELISA) method by ELISA kit that was found commercially. The serum was treated under normal ELISA protocols.

Sample Preparation

The blood samples were centrifuged for 3-4 minutes at 40k rpm to separate serum from blood cells. To increase the sensitivity and specificity of the IgM, given sample diluent has been prepared to remove rheumatoid (RF) and IgG interferences. For dilution process 200µl of diluent were added to 10µl of the blood serum then mixed. The serum turned turbid after diluting with sample diluent. Before ASSAY all the samples and reagents were brought to room temperature for 30 minutes. Now the samples are ready for processing.

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ELISA Protocol

1. The required number of costed strips were placed into the container.
2. Calibrator, Positive control and Negative control were used.
3. 100µl of diluted serum, calibrator and controls were dispensed into the appropriate wells. 100µl sample diluent were added to the blank well. The bubbles were removed from the liquid and mixed properly. Then I reared for 20 minutes at room temperature.
4. All the liquid were aspirated after 20 minutes incubation, and each well is washed three times with 300µl of 1X wash buffer. The wells were blotted on paper towels.
5. In each well 100µl of enzyme conjugate were dispensed and reared for 20 minutes at room temperature.
6. The enzyme conjugate was replaced after 20 minutes, and each well were washed with 300µl of 1X wash buffer. The wells were blotted on paper towels.
7. In each well 100µl TMB substrate were dispensed and were incubated for 10 minutes at room temperature.
8. After 10 minutes 100µl of stop solution were added.
9. The O.D. was read at 450 nm using ELISA reader within 15 minutes.

Calculation

The Calibrator Factor (CF) value = 0.41
Cut-off value = Calibrator OD × Calibrator factor (CF) = 1.72 × 0.41 = 0.7052
Antibody (Ab) index = OD value of each sample/ cut-off value = OD value / 0.7052

The results of all the samples were taken in comparison with the mean cut-off value manually.

Negative Result: samples having absorbance value lower than cut –off value.
Positive Results: samples having absorbance value higher than cut-off value.

Serum Plate Agglutination Test (SPAT)

All the samples of serum were subjected to serum plate agglutination test (SPAT) for finding anti-Brucella antibodies. A slide was marked for B. melitensis and Brucella abortus as M and A. A drop of B. melitensis and B. abortus antigens were added with help of puppet to each serum drop on glass slide and mixed gently with tooth stick to examine under light microscope. Any agglutinations on the slide were considered as positive test Figure 1.

A

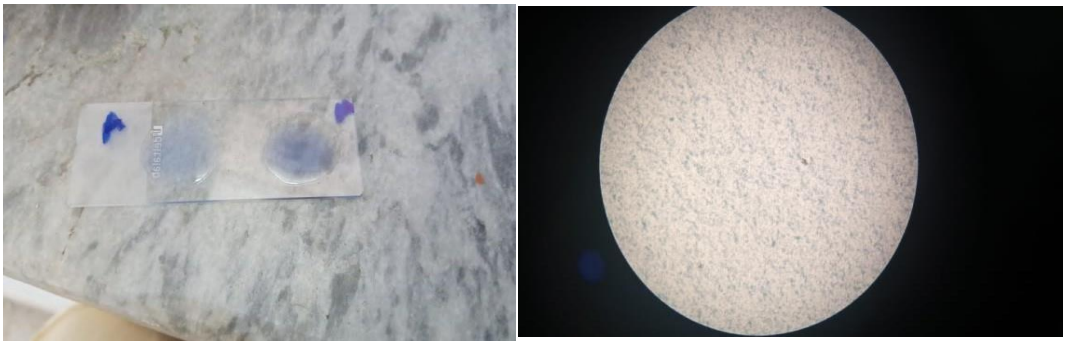

Fig 1. (A). Result for SPAT (B). Prepared slide for SPAT

B

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Analysis

The data collected through questionnaire and results by ELISA and SPAT was entered to Microsoft Excel spreadsheet and statistical analysis was performed through STATA of version 14 software. Significant value was set as $p<0.05$ statistically.

Results

Prevalence

The gender wise prevalence of brucellosis was 19.83% in male and 27.52% in female by ELISA and 14.88% in male and 24.83% in female by SPAT test respectively. Brucellosis prevalence in females is found higher than males. Table 3.1

Table: 3.1 Gender based prevalence of brucellosis in Dera Ismail khan.

S. no.	gender	Sample size	Positive cases	
			ELISA (0.142)	SPAT (0.043)
1	Male	121	24(19.83%)	18(14.88%)
2	Female	149	41(27.52%)	37(24.83%)

To find age wise prevalence in population the whole data is categorized in four different class like 10-20,21-30,31-40, and above than 40 having class interval 10. The prevalence rate found by ELISA is 13.64%,14.02%,35.85%,50.00% in groups 10-20,21-30,31-40, and above 40 respectively. Similarly, result shown by SPAT is 13.64%,9.35%,28.38%,47.73% in different age groups. Table: 3.2

Table: 3.2 Age wise prevalence of brucellosis in Dera Ismail khan.

S.no	AGE Group	Sample size	Positive cases	
			ELISA (0.000)	SPAT (0.000)
1	10-20	66	9(13.64%)	9(13.64%)
2	21-30	107	15(14.02%)	10(9.35%)
3	31-40	53	19(35.85%)	15(28.30%)
4	40 -above	44	22(50.00%)	21(47.73%)
5	Total	270	65(24.07%)	55(20.37%)

Dera Ismail khan is a big district with respect to population so whole district is divided geographically into city 1 and city 2. Human brucellosis infection was found here, like 39.39% in city 1 and 9.42% in city 2 by ELISA and 32.58% in city 1 and 8.70% in city 2 by SPAT test. Table: 3.3

Table: 3.3 Area wise prevalence of brucellosis in Dera Ismail khan.

S#	Area	Sample size	Positive cases	
			ELISA (0.000)	SPAT (0.000)
1	City 1	132	52(39.39%)	43(32.58%)
2	City 2	138	13(9.42%)	12(8.70%)
3	Total	270	24.07%	20.37%

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The table is formulated as to show prevalence of human brucellosis on basis of Marital Status. Prevalence found by ELISA was 26.76% and 23.12% in single and married people respectively. Similarly SPAT test showed prevalence 25.35% in single and 18.59% in married people. Table: 3.4

Table: 3.4 marital status wise prevalence of brucellosis in Dera Ismail khan

S.No	M. Status	Sample Size	Positive Cases	
			ELISA (0.537)	***SPAT (0.225)***
1	Single	71	***19(26.76%)***	***18(25.35%)***
2	Married	199	***46(23.12%)***	***37(18.59%)***

The result of positive cases given by ELISA is 23.23% of the population having no education, 27.45% of the population having matric level education, 23.96%in population having secondary education and 20.83% in population having graduation. Similarly, result shown by SPAT is 21.21% of population having no education ,21.57% at matric level, 18.75% at secondary level and 20.37% at graduate level. Table showed that population that are having high education have less prevalence of brucellosis and vice versa. Table: 3.5

Table: 3.5 Education based prevalence of Brucellosis in Dera Ismail khan.

S.no.	Education	Sample size	Positive cases	
			ELISA (0.920)	*SPAT (0.970)*
1	Illiterate	99	*23(23.23%)*	*21(21.21%)*
2	*Matric*	*51*	*14(27.45%)*	*11(21.57%)*
3	*Secondary*	*96*	*23(23.96%)*	*18(18.75%)*
4	*Graduation*	*24*	*5(20.83%)*	*5(20.37%)*

Prevalence of brucellosis in population of Dara Ismail khan on occupation base is as 27.52% in Housewives,25.49% in Farm workers ,22.50% in Butchers and 6.67% in Veterinary doctors, similarly result shown by SPAT is 24.83% in Housewives,19.61% in Form workers ,17.50% in Butchers and 3.33% in Veterinary doctors. We found here in this table that prevalence of brucellosis is higher in Housewives than others. Table: 3.6

Table: 3.6 Occupation based prevalence of Brucellosis in Dera Ismail khan

S.no.	Occupation	Sample size	Positive cases	
			ELISA (0.109)	***SPAT (0.060)***
1	***Housewives***	***149***	***41(27.52%)***	***37(24.83%)***
2	***Form Workers***	***51***	***13(25.49%)***	***10(19.61%)***
3	***Butchers***	***40***	***9(22.50%)***	***7(17.50%)***

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4	***Veterinary professionals***	***30***	***2(6.67%)***	***1(3.33%)***
5	***Total***	***270***		

This table is formulated to show the prevalence of brucellosis in populations having exposer to different types of animals. Population having exposer to Goat, Sheep, and Cattle have prevalence rate 20.00%, 40.00%, 29.89% respectively with 10.75% prevalence rate in population exposed to all type of animals by ELISA. Similarly result shown by SPAT is 20%, 37.14%, 24.14% of people exposed to goat, sheep, and cattle with 10.75% in people having exposer to all types of animals. Table: 3.7.

Table: 3.7 Types of animals kept by people in Dera Ismail khan.

s.no.	Occupation	Sample size	Positive cases	
			ELISA (0.011)	*SPAT (0.063)*
1	*Goat*	*55*	*11(20.00%)*	*11(20%)*
2	*Sheep*	*35*	*14(40.00%)*	*13(37.14%)*
3	*Cattle*	*87*	*26(29.89%)*	*21(24.14%)*
4	*All*	*93*	*14(15.05%)*	*10(10.75%)*

This table 3.8 shows that rate of brucellosis infection is higher in population have milking the animals than slaughtering, treatment and others type of cares by both ELISA and SPAT tests. Table: 3.8

Table: 3.8 Type of exposure of people to animals

S.no.	Occupation	Sample size	Positive cases	
			ELISA (0.063)	SPAT (0.063)
1	*Milking*	*120*	*36(30.00%)*	*31(25.83%)*
2	*Slaughtering*	*40*	*9(22.50%)*	*7(17.50%)*
3	*Treatment*	*74*	*17(22.97%)*	*15(20.27%)*
4	*Others*	*36*	*3(8.33%)*	*2(5.56%)*

People spend time with animals of 1 hr, 2-4 hrs, 4-8hrs, and above this have prevalence rate 11.11%, 25.00%, 23.77%, 35.71% by ELISA and 5.56%,22.41%,18.85%,35.71% by SPAT test respectively. Table: 3.9

Table: 3.9 Time spent with animals by people.

S.No.	Occupation	Sample size	Positive cases	
			ELISA (0.431)	*SPAT (0.176)*
1	*1 hr*	*18*	*2(11.11%)*	*1(5.56%)*
2	*2-4 hrs*	*116*	*29(25.00%)*	*26(22.41%)*
3	*4-8 hrs*	*122*	*2(23.77%)*	*23(18.85%)*
4	*Above*	*14*	*5(35.71%)*	*5(35.71%)*

Only 6.25% and 3.13% peoples tested positive by ELISA and SPAT respectively have knowledge about zoonotic diseases while 26.47% and 22.69% tested positive by ELISA and SPAT have no knowledge about zoonotic diseases respectively. Table: 3.10

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Table: 3.10 People having Knowledge about Zoonosis.

s.no.	Knowledge about Zoonosis	Sample size	Positive cases	
			ELISA (0.012)	***SPAT (0.010)***
1	***No***	***238***	***63(26.47%)***	***54(22.69%)***
2	***Yes***	***32***	***2(6.25%)***	***1(3.13%)***

Risk factors of Brucellosis

Our present study showed that the use of dairy products and unpasteurized milk are the risk factors of brucellosis. Exposure to animals for a long time by caring, treatment and slaughtering is also among the risk factors that cause brucellosis. It was also found that people are following not following precautionary measures while handling animals.

Discussion

Being a zoonotic disease brucellosis is transmittable to humans from animals at home and from infected wild animals. Bacteria of Brucella genus caused this disease (Purwar et al., 2007). People who are occupational disease are selling meats, managing livestock, busy farming, keeping animals and laboratory workers have high risk of getting brucellosis. People who have physical contact with animals or use products of animals have high chances of infection (Chauhan et al., 2000)

The aim of study was to find the sero-prevalence and risk factors associated with brucellosis in district Dera Ismail khan. Data is collected only from occupationally and domestically exposed people to animals i.e. the Farmer, Housewives, Butchers and veterinary doctors' overall prevalence was recoded 24.07% by ELISA and 20.3% by SPAT.

Seroprevalence of 32.90% was reported by (Shahid et al., 2014) in hospitalized patients in district Peshawar. Similarly, prevalence rate reported in animal handlers was 6.84% by RBPT, 12.3% by PCR and 24% by SPAT while prevalence in veterinary professionals was 18% by PCR, 4% by RBPT and 30% by SPAT in district Bannu (khan et al., 2018).

The sero-positivity of brucellosis was also recorded by (Perveen et al., 2015) in District Charsadda of Khyber Pakhtunkhwa. In male the prevalence rate was found 9% by PCR while 12% by SPAT in female 6% by PCR while 8% by SPAT. The present study gave high prevalence rate than recorded by perveen et al.The difference in prevalence rate may be due to area difference or environmental conditions.

Similarly, prevalence rate reported by (Ali et al. 2018) in Punjab was 16% by using RBPT and ELISA. In district Lahore (Yousef et al. 2021) performed a study on human brucellosis and overall prevalence reported as 17% by using RBPT and q RT-PCR. Among all, the present study shows high prevalence conducted in 2021, it may be due to area difference or the use of different diagnostic tests.

The brucellosis effects all age group of humans. The prevalence rate was found high among age group 31-40(35.85%) and above 40 age is (50.00%) by ELISA and 28.30%, 47.73% by SPAT respectively. (Yousef et al.2021) reported in their study high prevalence rate of brucellosis between age of 31-50 years. The findings of this study are like present study which shows that prevalence rate is high in people whose age range is above 31 years. The people having age above

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than 31 are more in direct contact with animals and their products.

Among epidemiological factors, area is also an effective factor. In present study it was found that city 1 has high prevalence of 39.39% and 32.58% by ELISA and SPAT and city 2 has prevalence rate of 9.42% and 8.70% by ELISA and SPAT.

Shahid et al also found 37.06% prevalence rate in female patient which is higher among other occupational groups in his study in district Peshawar. These two studies are resembled to our present study which show that prevalence rate is high in housewives than other occupational groups (Shahid et al.,2014). This show that housewives keeping cattle at home are more in contact with animals than other occupational housewives fore high prevalence rate was found in housewives.

Only 6.25% and 3.13% peoples tested positive by ELISA and SPAT respectively have knowledge about zoonotic diseases. Most of the people have no knowledge about zoonotic diseases. In order to know more about the prevalence rate of brucellosis in district Dera Ismail khan, further investigation is needed. Awareness among people and increased prevention and control measures are also needed.

Conclusion

Dera Ismail khan is in the southern side of Khyber Pakhtunkhwa, Pakistan. Brucellosis is a zoonotic disease which is highly contagious. It was concluded from present study that females were found more effected from brucellosis than males because they are mostly in direct contacts with their animals due to their daily activities. Besides this prevalence rate was found high in rural areas in comparison with urban areas because in rural areas people keep animals which enhance the risk of getting infection. The prevalence rate was found high in housewives that keep animals domestically because of they try to handle animals to born and even remove dead fetus from animal's wombs by hand. The present study also reports that people who have age above 40 showed high prevalence rate than other age groups due to prolonged exposure to animals which increase the risk of gaining infection. The high rate of Brucella infection was found in population have milking and slaughtering the animals it might be the reason that they were not using any precautionary measures. Therefore, people need to follow all precautionary measures while handling the animals to reduce the chance of getting infection. The use of unpasteurized milk and dairy products should be avoided, and awareness program should be started among people to know about zoonotic diseases.

Ethical Statement

This study proposal was evaluated and Approved by the Advanced Study Research Board (ASRB /Dir/A&R/AWKUM/2021/ 5635) committee members of Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa, Pakistan. All ethical standards regarding human subject use, informed consent from participants and following the ethical guidelines outlined in the Declaration of Helsinki to protect human rights and welfare are ensured.

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