Incidence Of *Brucella Abortus* And *Brucella Melitensis* In Peshawar And Identification Of Active And Passive Infection

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Abstract: Brucellosis is an infectious zoonotic disease caused by bacteria from the genus *Brucella*. It is an infection that affects mainly animals and transfer to humans through their contact with contaminated products of animals. Human brucellosis is a worldwide disease which has an annual occurrence rate of more than 500,000 cases and it is increasing with passage of time. The present study was designed to identify the incidence of Brucellosis in Peshawar District and its active (acute) & passive (chronic) situation in the population. In this study 1250 sera samples collected along with their histories through a proforma from seven different renowned hospitals of Peshawar. As a whole incidence rate of 16/100,000 was observed in Peshawar i.e. 6/100,000 incidence rate for *Brucella Abortus* and 10/100,000 for *Brucella Melitensis*. Through slide agglutination test, 455 samples out of 1250 were screened for the presence of *Brucella* Antibodies. It was observed that out of total positive samples, 157 (35%) cases were positive for *Brucella Abortus* while 298 (65%) cases were positve for *Brucella Melitensis*. The status of Acute and Chronic cases was studied through Isotype specific-Enzyme Linked Immunosorbant Assay (ELISA). Through ELISA it was found that 273 (60%) of samples had IgM antibodies while 182 (40%) samples were positive for IgG antibodies that were indication of Acute (Active) and Chronic (passive) cases, respectively. In acute cases (273), 206 (75%) and 67 (25%) samples were positive for *Brucella Melitensis* and *Brucella Abortus*, respectively while in chronic cases, 110 (60%) and 72 (40%) samples were found positive for *Brucella Melitensis* and *Brucella Abortus*, respectively. In addition, in acute cases, had active infection, were suffered with fever and arthralgia while chronic patients exhibited clinical signs related with skeletal and gastrointestinal organs but other signs such as fever, loss of weight, headache and vomiting were observed in both acute and chronic cases of brucellosis. These signs are obscure and can not be linked with active or chronic infection of brucellosis.

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Introduction:

Brucellosis is a zoonotic infection caused by the bacterial genus *Brucella.* The bacteria are transmitted from animals to humans by ingestion through direct contact with an infected animal, infected food products, or inhalation of aerosols. The disease is an old one that has been known by various names, including Malta fever, Mediterranean fever, undulant fever, gastric and remittent fever. Humans are considered to be accidental hosts, but brucellosis continues to be a major public health concern worldwide and is the most common zoonotic infection (Wafa Al-Nassir, 2013). *Brucella spp*. are usually small, non-motile, non-spore-forming, rod shaped (coccobacilli), Gram-negative, bacteria. These cause chronic disease, which usually persists for life; therefore they are also termed as facultative intracellular parasites. In human, the symptoms of brucellosis include malaise, chills, fever (about102.2-104 ^{O}F), weakness, headache, backache, anorexia and weight loss, undulant fever can continue for weeks to years (Chamberlain, 2003). Most commonly *B. abortus*, *B. melitensis*, *B. suis* biovars 1-4 and, rarely, *B. canis* cause brucellosis in humans.

Brucellosis is considered to be an occupational disease from public health view point, that mainly affects slaughter-house workers, farm labor, veterinarians and butchers (Yagupsky and Baron, 2005). Brucellosis is considered to be one of the most widespread zoonoses in the world (Schelling *et al,* 2003). According to OIE, it is the second most important zoonotic disease in the world after rabies. Human brucellosis is a worldwide disease which has an annual occurrence rate of more than 500,000 cases (Nasir WA, 2011). Brucellosis tends to occur more commonly in regions with less established animal-disease-control programs and in areas where public-health initiatives may be less effective. High-risk areas include the Mediterranean Basin (Portugal, Spain, Southern France, Italy, Greece, Turkey, and North Africa), South and Central America, Eastern Europe, Africa, Asia, the Caribbean, and the Middle East. Brucellosis is much less common in the United States,

where only 100-200 human cases are reported each year. In the Eastern Mediterranean Region, the incidence of disease ranges from 1 per 100,000 to 20 per 100,000 populations. Brucellosis is endemic in Saudi Arabia, where the national sero-prevalence occurs to be 15% (Memish, 2001).

Brucellosis is now endemic in Pakistan like many countries of the world. According to a research done on adults as well as on children for finding out brucellosis incidence in Khyber PakhtunKhwa, it was concluded from the results that the incidence rate amongst adult population was 14.8% while amongst children was about 10.4 % (Abdul Qadar Khan Mohmand *et al*, 2012). Previous studies performed in Peshawar was not that much satisfactory. Further inflow of immigrant from surrounding war affected areas of FATA and Afghanistan has changed the scenario of Brucellosis. This situation demanded for re-evaluation of current scenario of Brucellosis in Peshawar. Consequently, this study was designed to evaluate the current situation of Brucellosis in Peshawar and further to have an idea regarding the Active (acute) and Passive (chronic) status of infection.

Materials And Methods:

A specific proforma was designed to have data regarding the history and clinical signs of the patients. Different hospitals were visited and among these seven hospitals were selected (Table-1) for blood sample collection. Special permissions were taken from head of these hospitals to work on Brucellosis. Blood samples were collected from all suspected patients and brought back to Centre of Biotechnology And Microbiology (COBAM) for further processing. The initial screening was made through slide agglutination test. The following procedure was followed.

Slide Agglutination Test(SAT):

For performance of the test, the blood samples were firstly centrifuged at 3000 rpm, to obtain the sera. The sera samples and antigens were brought to room temperature to obtain a normal physiological state. Using a suitable pipette, one drop serum sample was added on a clean transparent glass slide. The antigen was stirred well and one drop of antigen suspension was added to the serum and mixed it gently with the help of a toothpick. For each test, separate toothpick was used. The slide was then rotated by hand and agglutination observed with an indirect light against a dark background (Spink *et al,* 1952). The test was performed for each serum sample and with both antigens of *Brucella Abortus* and *Brucella Militensis*. All positive samples were further subjected to isotype specific ELISA to have an idea about chronic and acute infection.

Enzyme Linked Immunosorbant Assay(ELISA):

As a second part of study, to know the type of antibodies in these samples, Immunoglobulin G and Immunoglobulin M Enzyme Linked Immunosorbant Assay was performed for further confirmation of isotype of antibodies produced against *Brucella*. The Following method was followed:

First, an incubator was set to 37 (±) $1C^0$ and all reagents were brought to room temperature before use. The number of wells to be employed was determined and four wells were allocated for controls: two for the cut off serum and one each for the negative and positive sera respectively, during the ELISA for IgM, 25 microliter of VIRCELL IgG sorbant was added to each of the required wells, except for the wells where controls were to be dispensed while for performing ELISA IgG, VIRCELL IgG sorbent was not added. Five microliter of sample was added and then 75 microliter of the diluent to each well (Fig.2).

The control wells were prepared by adding first 100 microliter of the serum diluent to each well and then 5 microliter of the positive control, 5 microlitter of the cut off control (in duplicate) and 5 microliter of the negative control to the corresponding wells. The wells were covered with a sealing sheet and incubated at 37 C^0 for 45 minutes. Then the seal was removed, aspirated the liquid from all wells and washed 5 times with 0.3 ml of washing solution per well and drained off any remaining liquid. Immediately 100 microliter of IgM or IgG conjugate solution was added into each well and after covering with a sealing sheet, incubation was repeated at $37C^0$ for 30 minutes. The seal was then removed, aspirated liquid from all wells and washed five times with 0.3 ml of washing solution per well. Consequently, 100 microliter of substrate solution was added into each well and incubated at room temperature for 20 minutes in dark and then 50 ul of stopping solution was added into all wells. With a spectrophotometer, the results were recorded at 450 nm filter within one hour of stopping (Magee *et al* 1980), (Fig.3). The sera samples with indexes below 9 were considered as not having IgG or IgM specific antibodies against *brucella* while those with indexes above 11 were considered as having IgG or IgM specific antibodies against *brucella.* Samples with equivocal results (9-11) were retested.

Results:

The study was performed in District Peshawar over year period 2013 i.e. January to December 2013. During the study, a total of 1250 sera samples were collected from seven tertiary hospitals of Peshawar (Table-1) to test for *Brucella Abortus* and *Brucella Melitensis*. It was found that 455 samples were positive for *Brucella* Antibodies, which indicated that *brucella* incidence rate in Peshawar is 16/100,000 persons. Furthermore, incidence rate of 6/100,000 and 10/100,000 were calculated for *Brucella Abortus* and

Brucella Melitensis, respectively. Out of total positive samples, 65% (298) and 35% (157) cases were positive for *Brucella Melitensis* and *Brucella Abortus*, respectively. All positive (455) sera samples when made subjected to ELISA (Enzyme Linked Immunosorbant Assay) for specific Isotype of Antibodies, these resulted into 273 (60%) and 182 (40%) positive for IgM (Acute cases/active infection) and IgG (Chronic case/passive infection) antibodies, respectively. In acute cases, 206 (75%) and 67 (25%) samples were positive for *Brucella Melitensis* and *Brucella Abortus*, correspondingly while in chronic cases, 110 (60%) and 72 (40%) samples were found positive for *Brucella Melitensis* and *Brucella Abortus*, separately. In addition, in acute cases, had active infection, were suffered with fever and arthralgia while chronic patients exhibited skeletal and gastrointestinal problems. Other common signs were found in both groups (Table-3).

Discussion:

Brucellosis is considered to be one of the most widespread zoonotic disease in the world. It is is one of the most devastating trans-boundary animal diseases and also a major trade barrier and economically very important (Gul and Khan, 2007). In Pakistan, few studies have been carried out to estimate its incidence rate reflecting that Brucellosis is a neglected disease in Pakistan.

The results showed that 455 sera samples were positive for Human Brucellosis (298 for *Brucella Melitensis* and 157 for *Brucella Abortus*). The statistical analysis revealed that the incident rate of Brucellosis cases in Peshawar is 16/100,000 individuals. Bokaie S *et al* 2009 detected the incidence rate as 175/100,000 of human brucellosis in Khoy District of Iran. Similarly in Mid-Anatolia, Turkey, Haldun *et al*, 2003 reported incidence of brucellosis as 3.2/750 subjects. Further, the incident rate for *Brucella Melitensis* and *Brucella Abortus* was recorded in this study as 10/100,000 and 06/100,000, respectively. These results indicating a 40% higher surge of *Brucella Melitensis* in Peshawar as compared with *Brucella Abortus* as former is very contagious to humans and contains many biovars (Centre for food security and public health, 2009).

The results were further confirmed through more reliable test, ELISA, (Araj GF, 2010, Brodly JA *et al,* 1966 and Lucero *et al* 1999). The active infection was recorded in 60% cases while in remaining cases the nature of disease was chronic. A. R. Lulu *et al* 1987 in Kuwait reported the similar results while using IgG, IgM and IgA isotype specific ELISA. He described in his survey that 77% patients had acute while 10% patients had chronic brucellosis. Araj. G. F *et al* in 1986 observed the analogous scenario while recording 53% and 5% of patients as acute and chronic, respectively. The acute cases look to be on higher surge as compared to chronic cases. It might be either due to higher number of new cases or body immune system control over the infection that not allowing the infection to localize and take shape of chronic disease.

Further it was observed that fever and arthralgia were common signs in acute brucellosis while in chronic cases skeletal and gastrointestinal signs were prominent but other signs such as fever, loss of weight, headache and vomiting were observed in both acute and chronic cases of brucellosis. Similar observations were seen by Tamar *et al* in 2010. He recorded that fever, arthralgia and sweats were the most frequently symptoms for acute brucellosis patients. Among chronic brucellosis patients, arthralgia was the most frequently noted symptom. Neuropsychiatric symptoms such as depression, difficulty in concentration and sleep disturbance, were observed rarely. Mustafa Ertek *et al* reported in 2006 that skeletal complications were the most frequent, followed by nervous system, cutaneous, genitourinary system, cardiovascular system, gastrointestinal system and hematological system complications. It is quite difficult to correlate the chronic and acute conditions with clinical signs as these are commonly seen in both conditions and similarly in so many other diseases. To find a final conclusion regarding brucellosis the clinical signs do not provide enough information and it is required to consult different diagnostic techniques for final confirmation of brucellosis.

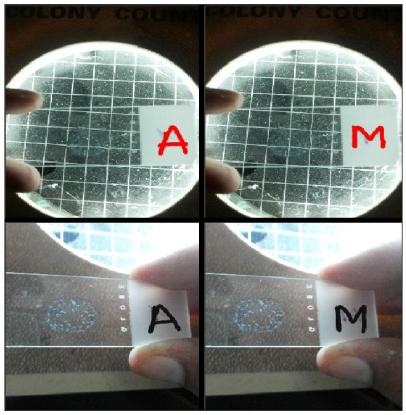

Figure1: Positive Results for B.melitensis And B.abortus During SAT

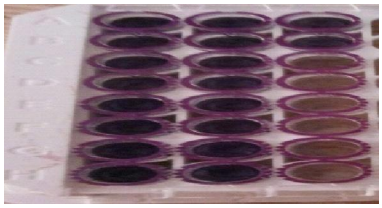

Figure 2. Diluent In Each Well

TABLE-1. Different No. Of Samples Collected From Different No. Of Hospitals Of Peshawar During -September 2013

S.NO	List Of Hospitals Visited In Peshawar	No. Of Patients /Samples
1	Khyber Teaching Hospital	250
2	Hayatabad Medical Complex	230
3	Lady Reading Hospital	197
4	Hashtnagri Government Maternity Hospital	180
5	Johar Khatoon Hospital	176
6	Rehman Medical Institute Peshawar	138
7	North West General Hospital	79

Total Number of Samples Collected = 1250
Total Number Of Positive Samples = 455

TABLE-2. Immunoglobulin M (IgM) & Immunoglobulin G (IgG) ELISA Results

NO. Of +ve SAMPLES	IgM ELISA (ACUTE)		IgG ELISA (CHRONIC)	
	Positive	%	Positive	%
455	273	60%	182	40%

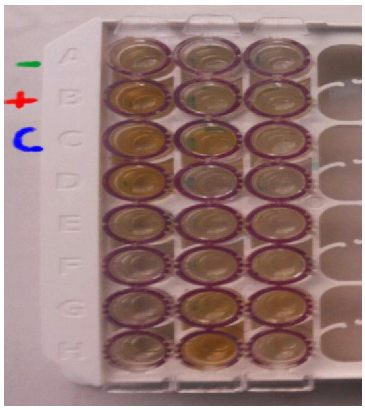

Figure.3: ELIZA for iso-typing of IgG in raised sera

TABLE-3. Presenting Signs in Brucellosis Patients

Signs of Brucellosis	Signs of Acute Infection	Signs of Chronic Infection	Signs Common in Both Infections
Arthralgia	•		
Sweating			
Fever	•		•
Chills			
Myalgia			
Headache			•
Loss of weight			•
Diarrhea			
Vomiting			•
Skeletal complications(joint pain)		•	
Genital complications(miscarriage)		•	
Respiratory Tract Infections			
Hepatospleenomegaly			
Orchitis			

Conclusion:

The incidence of Brucellosis is on increase and in near future it will further go higher due to increase in population and incursion of people and animals from surrounding war affected areas of FATA and Afghanistan into Peshawar. *Brucella Melitensis* is more prevalent in district Peshawar as compared to *Brucella Abortus*. The determination of isotype of antibodies can help in confirmation of Brucellosis and treatment but the status of active (acute) and passive (chronic) infection cannot be linked with clinical signs as these are quite complicated.

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