BRUCELLA SEROLOGY IN ABATTOIR WORKERS

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Background: Brucellosis is an occupational hazard with those particularly at risk either living in close proximity with animals or handling them. It is a public health problem in developing countries with adverse health implications both for animals and human beings as well as economic implications for individuals and communities. The Objectives were to estimate the seroprevalence of brucellosis among abattoir workers of Lahore District and to determine the association of brucellosis with nature of job of the workers. **Methods:** Data was collected in April 2008. It was a cross-sectional study in which four main slaughterhouses in Lahore were included. The slaughterhouse workers were divided into seven strata based on their nature of job: meat sellers, slaughterers, animal keepers, drivers, cleaners, loaders and vets/paravets. A total of 360 such workers were selected using stratified random sampling technique. Sampling frames for different strata were prepared and from each frame, proportionate numbers, were selected through simple random method using random number tables. Data was obtained using a questionnaire. Additionally blood samples were collected and analyzed for anti-*Brucella* Immunoglobulin G (IgG) using enzyme-linked immunosorbent assay (ELISA) technique. **Results:** The seroprevalence of anti-*Brucella* IgG was found to be 21.7%. A statistically significant difference was observed between the immune status of the respondents and their nature of job ($p=0.005$), age groups ($p=0.013$), and duration of job ($p=0.003$). **Conclusion:** The disease is an important public health problem in Pakistan. The disease can be prevented in the slaughterhouse workers through the use of personal protective devices. Public health authorities should educate the general public regarding prevention of the disease with specific emphasis on people working in slaughterhouses.
Keywords: Abattoir workers, slaughterhouse, *Brucella* IgG, brucellosis, seroprevalence

INTRODUCTION

Brucellosis is a zoonotic disease of wild and domestic animals in which man is an accidental host.[1] It has a worldwide distribution, affecting humans and animals both in the developed and developing countries. The disease burden is more profound in the developing countries due to lack of: effective public health measures, domestic animal health programs and appropriate diagnostic facilities. The situation is compounded by the resemblance of the disease with other diseases leading to incorrect diagnoses and under-reporting of the disease.[2] There has been a rebirth of interest in brucellosis of the developed world, due to the growing phenomenon of international tourism, leading to importation of the disease. There is also a danger of the *Brucella* bacteria being used as a biological weapon.[3,4]

Every year 100 to 200 cases of brucellosis are reported in the United States.[5] The Mediterranean Basin, south and Central America, Eastern Europe, Asia, Africa, the Caribbean and the Middle East are considered as high-risk countries.[6] In the Eastern Mediterranean Region, the incidence of disease ranges from 1 per 100,000 to 20 per 100,000 population. However, the actual figure is thought to be 20 to 25 times greater than the official figures.[7] Brucellosis is endemic in Saudi Arabia, where the national seroprevalence is 15%.[8] The incidence of brucellosis in the general population of Peshawar, Pakistan, was reported as 27.65%.[9]

The disease is primarily an occupational disease of those working with infected animals or their tissues. These occupations can be listed as: farmers, shepherds, butchers, abattoir workers, veterinarians and laboratory workers.[10] The slaughterhouse workers are more prone to acquire infection as compared to other occupations because they are exposed to carcasses and viscera of infected animals and get infected through cuts and wounds and splashing of infected blood and other fluid in the conjunctiva.[11] A study conducted in Iran cited the seroprevalence of brucellosis among slaughterhouse workers in Saudi Arabia, as 35.7%.[12]

In humans the intracellular bacteria, gives rise to a chronic granulomatous infection, causing clinical morbidity that requires combined prolonged antibiotic treatment.[13,14] The cost of treatment and work days lost to ill health, combined with a loss of productivity in the animal husbandry, leads to decreased availability of food that adversely affects the health and economic well being of the population.[15,10] In Pakistan, humans and animals live in close proximity. The rural population is dependent on agriculture and livestock farming to earn a living.[16] Hence in order to plan meaningful interventions, there is a need to estimate the burden of disease in the population. Keeping this in mind

this particular study was conducted on a high risk occupational group of abattoir workers.

The objectives of the study were to estimate the seroprevalence of brucellosis in abattoir workers of Lahore District and also to determine association of brucellosis with nature of job of workers.

SUBJECTS AND METHODS

This cross-sectional study was conducted in 2008. The four main slaughterhouses of Lahore were used in the study. The total study population of 4448 slaughterhouse workers consisted of Yateem Khana Chowk slaughterhouse (4173 workers), the Baghbanpura slaughterhouse (131 workers), Shahdarah slaughterhouse (115 workers), and Army slaughterhouse (29 workers). Epi-Info version 3.3.2 was used for sample size calculation, based on the total population of 4448 workers, with an expected proportion of 35% and a margin of error of 5%, using confidence level of 95%. The calculated sample size was 324, but making an allowance for non-response, a sample size of 360 was drawn for the study.

Stratified sampling technique on proportional basis was used for selection of study subjects. The slaughterhouse workers were divided into seven strata based on their nature of jobs: meat sellers, slaughterers, animal keepers, drivers, cleaners, loaders and vets/paravets. Sampling frames for different strata were prepared and from each frame, proportionate numbers were selected through simple random method using random number tables. Based on the above technique we calculated the proportionate number of workers from each job category to be included in our sample. The proportionate numbers calculated and included in the study were as follows: meat sellers 166 (total 2100), 85 slaughterers (total 1075), 40 Animal keepers (total 506), 28 drivers (total 354), 20 cleaners (total 253), 12 loaders (total 151). The last category of vets/paravets was included as a whole owing to their small numbers (total 9). The study population thus selected was interviewed using a pre-tested structured questionnaire and blood samples were obtained. Before collection of data and blood specimens the purpose of the study and blood collection procedure was explained to the workers and a written informed consent was obtained. Using aseptic techniques, 5ml of blood was drawn. Each syringe was marked with a permanent marker recording the questionnaire number of the respective study subject. The blood samples were analyzed at the Bacteriology Department of the Institute of Public Health, Lahore. After centrifugation of the blood at 4000 rpm for 5 min, the sera were analyzed using Enzyme Linked Immunosorbent Assay Technique for anti-*Brucella* IgG. The kit used was of Nova Tec

Immunodiagnostica GmbH (Germany). This kit was used for the qualitative determination of anti-*Brucella* IgG.

RESULTS

There were 360 male respondents with age ranging from 13 to 78 years, with a mean of 34.36±11.91 years. Majority of the respondents were illiterate numbering 209 (58%). Out of total 217, 60% were residents of urban localities. A large proportion 351 (97%) of the respondents belonged to the slaughterhouses managed by the government. The analysis of their blood samples using ELISA technique showed that 78 (21.7%) of the respondents tested positive for *Brucella* specific IgG, whereas, 282 specimens (78.3%) tested negative for anti-*Brucella* IgG. (Figure-1)
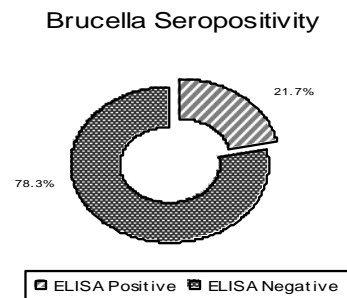

Figure-1: Immune status of respondents

As shown in Table-1, the age group of 51–60 years had maximum proportion (38.5%) of individuals with positive immune status, followed by 41–50 years (33%). The smallest proportion of seropositives was in the youngest age group i.e. up to 20 years (9.3%). There was a statistically significant difference among the age groups with regard to immune status. The respondent's duration in present job ranged from a minimum of 7 days to a maximum of 50 years, with a mean of 15±9.6 years. Respondents with duration of job less than a year, and those with job durations exceeding 20 years had a seropositivity of 33.3% and 32.6% respectively. A rise in seropositivity is observed as the duration of occupational exposure increases, with the exception of less than a year's job duration. There is significant statistical association between the immune status and duration of job of the respondents (p=0.003). Of the respondents belonging to urban localities, a proportion of 22.6% tested positive on ELISA, whereas, 20.3% of those residing in rural localities were found seropositive. The proportion of seropositivity ranged from 20.6% in primary level educated, to 22.2% in those workers having education of matriculation and above. In the case of slaughterhouses administered by the government, 76 out of 351 (21.7%), and in the case of slaughterhouse under army

administration, 2 out of 9 (22.2%) of the workers tested positive respectively.

Table-1: Socio-demographic profile of respondents by serology (n=360)

VARIABLE	Total	ELISA POSITIVE		*p*-value
		No.	%	
Age group (in years)				
Up to 20	43	4	9.3	
21–30	118	19	16.1	
31–40	112	26	23.2	*p*=0.013
41–50	54	18	33.3	
51–60	26	10	38.5	
Above 60	7	1	14.3	
Duration of job (in years)				
<1	6	2	33.3	
1–5	48	2	4.2	
6–10	90	16	17.8	*p*=0.003
11–20	130	30	23.1	
>20	86	28	32.6	
Residential background				
Urban	217	49	22.6	*p*>0.05
Rural	143	29	20.3	
Educational status				
Illiterate	209	46	22	
Primary	97	20	20.6	*p*>0.05
Matriculation	45	10	22.2	
Graduate and above	9	2	22.2	
Management of slaughterhouse				
Government	351	76	21.7	*p*>0.05
Army	9	2	22.2	

The distribution of *Brucella* positive workers in various job categories is highlighted in Table-2. The proportion of seropositives in the animal keepers was the highest (37.5%) followed by loaders and vets/paravets having an equal proportion of 33.3%. The least proportion of seropositives were in the category of cleaners (15%) with none of the drivers testing positive. There is a statistically significant difference between the immune status of the respondents and their nature of job (*p*=0.005).

Table-2: Sero-positivity by nature of job

NATURE OF JOB	Total	ELISA positive	
		No.	%
Animal keeper	40	15	37.5
Loader	12	4	33.3
Vet/paravet	9	3	33.3
Slaughterer	85	23	27.1
Meat seller	166	30	18.1
Cleaner	20	3	15
Driver	28	0	0
Total:	360	78	21.7

Chi-square value of 18.58, df = 6, *p* = 0.005

DISCUSSION

The most widespread zoonotic disease, brucellosis, is a source of major public health concern globally with negative implications for the economic prosperity of nations through its effect on humans, and the animal industry.[17] The disease is contagious and chronic in animals, leading to abortions, infertility and loss of livestock. The pattern of disease in humans is determined by the geographical distribution it follows in animals. Humans get infected by direct contact with infected animal products, ingestion of contaminated food, and inhalation of contaminated aerosols.[18] Brucellosis is an occupational disease, of those who come in contact with infected animals and their products such as abattoir workers, veterinarian, slaughterers, laboratory workers, shepherds and farmers.[19]

The present study was conducted to determine the seroprevalence of brucellosis in one such high risk group, i.e., the abattoir workers. In this study the seroprevalence estimated using ELISA technique was found to be 21.7%. In Pakistan very few studies have been carried out on brucellosis especially on slaughterhouse workers and the only study retrieved from literature on the same occupational group as in the present study, was conducted by Masoumi *et al* in 1992. The prevalence found by the study was 8.33%.[20] The results differ from our study due to the less sensitive test used, which was serum agglutination test and not ELISA.[21] Studies performed on other occupational groups, include one carried out by Sohaila *et al* on milkmen, reporting the seroprevalence of brucellosis as 6.1%.[22]

The prevalence of brucellosis shows marked variation between countries. Our neighbouring country India has reported many studies on the prevalence of brucellosis. The seroprevalence of 25.45% and 25.5% determined by Kumar *et al* and Barbuddhe *et al* respectively, among abattoir workers are comparable with the present study.[23,24] The comparable results of Indian studies to Pakistani studies may be attributed to both countries being agrarian societies, with close contact between animals and humans increasing their vulnerability to the disease. The seroprevalence of brucellosis among slaughterhouse workers in Saudi Arabia was reported to be 35%,[25] in Algeria it was 37.6% among abattoir workers, breeders, butchers and veterinarians[26] and in Brazil the seroprevalence of brucellosis was 4.1% among slaughterhouse workers.[11]

Within a slaughterhouse, individuals have different job descriptions, altering their exposure to the disease. Therefore, in the present study workers were selected according to their nature of job and their seropositivity determined. A statistically significant difference was observed between the immune status of the respondents and their nature of job (*p*=0.005). Highest seropositivity was found in the category of animal keeper (37.5%), followed by the loaders (33.3%) and the vets/paravets (33.3%).

The high seropositivity observed in the animal keepers could probably be attributed to greater amount of time they spend with the animal, catering to the needs of the animal and even sleeping beside them. Whereas, vets/paravets look after the parturient animal and at times have to remove the retained uterine products probably giving rise to the high seropositivities among this category.

The literature review does not point to any study having the same job categories as in the present study. However, studies have been conducted showing the association of nature of job to seropositivity. In a study by Karimi et al in Iran, seroprevalence of brucellosis was found to be 20% in slaughterers and 4% in butchers (meat sellers).[12] Kumar et al conducted a study on abattoir workers in which, maximum seropositives of *Brucella* were found among the blood collectors group (99.77%), followed by the animal handlers (68.96%), butchers (68.00%), sweepers (57.14%) and 28.57% among the veterinarians.[23] Another study conducted on high risk group individuals by Agasthaya et al reported results which found butchers and shepherds to be least affected. Individuals belonging to the veterinary group showed maximum seropositivity to brucellosis.[27] No statistical association was found between the activity of slaughterhouse workers and seropositivity to brucellosis.[11]

Brucellosis affects all age groups and both sexes.[26,28] In this study all respondents were males. A statistically significant difference was seen among the age groups with regard to immune status ($p<0.05$). The age group of 51–60 years contained the highest number of seropositives, followed by the age group of 41–50 years. In the present study, a direct relationship was observed between the workers age groups and their immune status. A minimum percentage (9.3%) of seropositives was found in the age group of up to 20 years, whereas, a maximum percentage (38.5%) was seen in the age group of 51–60 years. The low seropositivity in the age group of above 60 (14.3%) negates the argument of a direct relationship between age groups and seropositivity, but this can be attributed to the fact that their number was far less compared to other categories (Only seven individuals out of a total of 360 belonged to the age category of above 60 years). A study by Abu-Shehada et al also showed an increase in seroprevalence with advancing age.[29] Various age groups have been identified by different studies for containing the maximum number of cases. According to Kadri et al, the age group of 21–30 years had the highest number (43%) of *Brucella* positives.[28] A study conducted in Peshawar by Ali et al in 2007 identified the age group of 25–35 years, as the most commonly involved age group.[9]

The rural population because of its involvement in animal husbandry is more prone to infection as compared to its urban counterparts.[30] Nabi et al have cited a study in which 84.2% of cases were from rural areas and 15.8% from urban areas.[31] But no significant difference was found in seroprevalence between residential backgrounds in the present study ($p>0.05$). The results of Baba's study in Nigeria are consistent with the present study showing no significant association between immune status and residential background.[15] We found no relationship between the workers educational level and their immune status. The results of the study by Sumer et al are similar to the current study.[32] However, Karimi et al have reported opposing results. They have shown a strong positive correlation between low literacy and seropositivity.[12]

It was noted that as the workers' number of years at work increased, so did their seropositivity. The seropositives were minimal (4.2%) in the job duration category of 1–5 years, and maximum (32.6%) in the category of more than 20 years. Among the workers having less than a year's experience at job, two individuals (33.3%) were found seropositive. One was a slaughterer, who had history of assistance in parturition of animals and also of keeping animals within the home premises. His seropositivity could be explained by exposure to these risk factors despite a short duration of job. A significant association between the immune status and duration of job of the respondents was observed ($p=0.003$) in the current study. Karimi et al and Sohaila et al have also highlighted a strong association between brucellosis and duration of occupational exposure.[12,22]

CONCLUSION

Seropositivity to *Brucella* has a high prevalence among slaughterhouse workers owing to their close contact with animals.

RECOMMENDATIONS

The prevention of human brucellosis is dependent on control of the disease in domestic livestock. This can be achieved by elimination of infected animals and mass vaccination of healthy ones. This will render individuals coming in contact with animals at a lower risk and help produce *Brucella* free animal products. Animal owners should be taught about the importance of vaccination of their animals.
The lack of human vaccines makes it necessary for individuals employed in high risk occupations to take protective measures. The use of protective clothing while handling stillbirths or products of conception

can reduce occupation related disease. The avoidance of unpasteurized dairy products will prevent infection in the general population. As there is no effective and organized brucellosis control program in the country, the ultimate control would be achieved through a specially designed program aimed at public health education about the disease and its risk factors, maximum cooperation between the health and veterinary authorities, and alertness of the physicians to include brucellosis in their immediate diagnosis especially in the high risk groups. The disease is treatable with prompt and proper intervention. Enforcement of laws regulating the running of slaughterhouses should be implemented and adhered to in order to minimize the spread of infection. It is also recommended that further studies be carried out in other parts of the country not only involving the occupations at risk but also the general population.

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