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PREDICTORS OF BRUCELLOSIS SEROPOSITIVITY AMONG EXPOSED WORKERS

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Abstract

Introduction: Brucellosis is a zoonotic infection which is responsible for substantial economic losses along with human morbidities. In Egypt, it is a definite cause of more than 3% of acute febrile illnesses. Brucellosis is principally affecting animals however it can be transmitted from animals to human. Many workers are at risk of infection with brucellosis as herders, hunters, agriculturalists, dairy workers, veterinarians, and slaughterhouse workers. **Aim of work:** to determine the prevalence of brucellosis seropositivity among occupationally exposed workers, to identify risk factors and to assess the predictors of seropositivity among the studied group. **Material and Methods:** Seventy five workers occupationally exposed to livestock animals were included and were subjected to an interview questionnaire about Brucellosis risk factors and blood samples were collected and analyzed by Rose Bengal plate test for B. Abortus and B. Melitensis. **Results:** Seropositivity for Brucella among studied workers was 43(57.3%). It was highest among high risk work activities including veterinarians 19 (44.2%) followed by animal service workers 10 (23.3%), butchers and veterinary assistants 6 (14% each) (p=0.626). The predictors of seropositivity for Brucellosis were dealing with unvaccinated animals (p=0.012) and high risk work activities (p=0.037). **Conclusion:** Dealing with unvaccinated animals and high risk work activities are the main predictors of seropositivity of Brucellosis among occupationally exposed group.

Key words: Brucellosis, High risk activities, Occupations, Seropositivity and Cattle.

Introduction

Brucellosis is a major zoonotic infection which is responsible for substantial economic losses along with human morbidities (Pappas et al., 2006). In 1887, David Bruce discovered Brucella (B.) Melitensis that infects sheep and goats followed by identification of other several species such as B. Abortus (which infects cattle), B. Neotomae, B. Ovis, B. Suis, B. Canis (Corbel, 1997). Strains of Brucella have been isolated from marine mammals named B. Ceti and B. Pinnipedialis (Foster et al., 2007 and OIE -World Organization for Animal Health 2016). Also, B. Microti species, was isolated from foxes and soil in Europe (Scholz et al., 2008), the last species B. Inopinata and B. Papionis respectively isolated from human breast implants and baboons (Whatmore et al., 2014 and OIE -World Organization for Animal Health, 2016).

Genus brucella is gram-negative coccobacilli that affect cattle, sheep, goats and other livestock. Brucellosis can be transmitted from animals to human or by consumption of raw milk or raw meat infected with Brucella organisms or by direct contact (Corbel, 1997). Brucellosis was endemic in the Middle East countries, causing thousands of new cases every year in spite of continuous measures to control the disease (WHO, 2006; Pappas and Memish 2007 and Dean et al., 2012). Although Brucellosis has been controlled in majority of developed countries, still it is a big problem in the Mediterranean area, western Asia, in Africa, and South America (Pappas et al., 2006; El-Okda and Hamed, 2010). At 1939 brucellosis was the first time to be reported in Egypt. It is a definite cause of more than 3% of acute febrile illness conditions among humans (Afifi et al., 2005 and Pappas et al., 2006). It is a major public health problem in Egypt particularly in the Nile Delta area (Hegazy et al., 2011). This might be explained by the fact that more than half of Egyptians residents in rural areas are in close contact with animals like cattle, sheep, and goat (Holt et al., 2011). Significant risk factors for brucellosis infection were the presence of other cases of Brucellosis at home, presence of unimmunized animals, contact with infected animal's tissues and body fluids like blood, urine, vaginal

secretions, aborted animal placenta and fetus, consumption of contaminated animal products including meat, raw or unpasteurized milk and milk products (Mantur et al., 2007; Sofian et al., 2008 and Haque et al., 2011). Moreover, many workers are at risk of infection with brucellosis as shepherds, dairy workers, farmers, veterinarians, abattoir workers, so brucellosis is an occupational disease (Bossi et al., 2004 ; Ajay Kumar and Nanu, 2005;Sahin et al., 2008 and Stringer et al., 2008). Animal services workers exhibited the highest prevalence of seropositivity compared to other groups, followed by assistant of veterinarians. The most important predictors of seropoisitivity were working more than five hours a day in addition to age less than thirty years old (El-Okda and Hamed, 2010).

Two methods were used in Egypt as a control program for the disease: vaccination of all animals and killing of animals with positive serologic results. The difficulty of precisely detecting all infected animals, particularly shedders, is the main limitation (Samaha et al., 2008).

Accordingly, there is a need for ascertaining potential risk factors for Brucellosis seropositivity, also, to define the predictors of sereopositivity with comparable occupational exposure, which will permit the achievement of our ultimate goal of its control and eradication.

Aim of work

To determine the prevalence of Brucellosis seropositivity among occupationally exposed workers, to identify risk factors, to assess the predictors of seropositivity among the studied group.

Material and Methods

Study design: It is a cross-sectional study.

Place and duration of study: The study was carried out among workers in farm of Faculty of Veterinary Medicine, Suez Canal University, Ismailia Veterinary directorate, El Tal El Keber veterinary unit, and El Tal El Keber slaughter house, Ismailia governorate, Egypt; between May 2014 and February 2015.

The farm contained both educational and commercial activities. It consisted of

several playgrounds classified according to the ages of the animals bred inside them. In addition to the playground, there was a dairy barn where milking process took place. Besides, there was a warehouse where food processing and storage of animals' food. Animals bred were mainly cows and buffalos (cattle) in addition to a horse and a camel for educational purposes only. The offices of veterinarians, veterinary supervisors, and administrators were located adjacent to the play grounds. There were also fields planted with corn and alfalfa for feeding the animals. A slaughterhouse was present in the place where slaughtering processes took place from time to time but not regularly. Ismailia Veterinary directorate consisted of a building containing different offices for the different departments of the directorate (e.g slaughtering, leathers, poultry, fish, etc.). In addition, there was two laboratories one for preparation and simple analytic procedures of animal samples.

El Tal El Keber veterinary unit consisted of a clinic with a pharmacy included, an area for preparation of medications instantly used by the veterinarians and a place for surgical procedures. The unit also included several offices for the working veterinarians and veterinary assistants.

At El Tal El Keber slaughter house, there was a small office for administrative work and an outdoor area for keeping the animals to be slaughtered, along with slaughtering process and meat processing activities.

Study sample: Seventy five workers (which were the whole working population in the setting) occupationally exposed to animals were included , working as veterinarians (performed examination of animals and meats, vaccinations and medications administration and delivering), animal service workers, veterinary assistants (aided veterinarians in animal sampling, vaccination and giving medications), veterinary supervisors (checking of food composition and supervision of working activities performed by the workers as milking, cleaning and feeding), and butchers (performing slaughtering and meat processing activities, some butchers were involved only in leather preparation). Risk of exposure to infection was considered

high for veterinarians, butchers, animal service workers (Bossi et al., 2004). Veterinary supervisors were considered to have low risk of exposure to infection. Those who performed lab work were considered at low risk due to absence of risky procedures in their laboratory as it depends on simple analytic techniques on open bench with no cultures performed (CDC, 2012).

Study methods:

- **Pre-designed Questionnaire:**

The questionnaire included personal and occupational histories (nature of work, duration of occupation, hours worked per day, hours worked per week, and questions about history of Brucellosis).

- **Laboratory Investigation**

Blood samples (3 ml.) were collected in a sterile coded tubes from all workers and analyzed by Rose Bengal plate test (RBPT) to confirm the presence of seropositivity for B. Abortus and B. Melitensis which were common in Egypt as well as population under the study working with cows (which can be infected with B. Abortus) and could be exposed environmentally to goats or sheep (infected with B. Melitensis), in addition to, the antibodies against B. Abortus sometimes cross react with B. Melitensis antigen and vice versa. So, we used a serological test with two reagents to determine Brucella Abortus antigen and Brucella Melitensis antigen (Micropath® Brucella abortus, Omega Diagnostics, Scotland, United Kingdom).

Consent

All subjects participated in the study gave informed consents after appropriate clarification regarding confidentiality of data and aim of the study.

Ethical approval

The approval of medical research committee of Faculty of Medicine, Suez Canal University was obtained and complied with local legislation and the Helsinki Declaration.

Data management

All analyses were conducted using the SPSS for Windows Statistical Package, version 22.0. The distribution of variables was compared with the normal distribution by means of the Kolmogorov–Smirnov goodness-of-

fit test. The analysis of all normally distributed data was performed using student's-t test. The differences between groups in non-parametric quantitative data were assessed by Mann–Whitney U-test. Chi- square test was used for testing significant differences of qualitative variables. For cross-tables where the number of cells whose expected count less than 5 were >25% of cells, Fisher's Exact tests and Exact Chi-square test were used. Odds ratios were calculated for estimation of magnitude of risk of different risk factors. Backward conditional logistic regression analysis was done for risk factors of brucellosis seropositivity. The level of significance was considered less than 0.05.

Results

The mean age of the studied workers was 44.7±10.1. Males were represented more than females (56, 74.7% and 19, (25.3%) respectively). Urban residents were slightly higher than rural ones (42, 56% and 33, 44%) respectively. Those with high education represented 48% of the studied workers.

All the studied workers were occupationally exposed to cattle while 72 (96.0%) of workers were occupationally exposed to animals other than cattle (sheep, goat and camels). Concerning non-occupational exposures, 29 (38.7%) of the workers were exposed to cattle and 5 (6.7%) were exposed to other animals (sheep, goat, camels, and dogs).

Table 1: Seropositivity and occupational characteristics.

Title		Seropositive (43) Freq. (%)	Seronegative (32) Freq. (%)	p-value	Total (75) Freq. (%)
Occupation	Animal service worker	10 (23.3)	12 (37.5)	0.626	22 (29.3)
	Veterinary supervisor	2 (4.7)	2 (6.3)		4 (5.3)
	Veterinary assistant	6 (14.0)	3 (9.4)		9 (12.0)
	Veterinarian	19 (44.2)	13 (40.6)		32 (42.7)
	Butcher	6 (14.0)	2 (6.3)		8 (10.7)
Work places #	Farm	23 (53.5)	19 (59.4)	0.611	42 (56.0)
	Veterinary directory	10 (23.3)	11 (34.4)	0.289	21 (28.0)
	Veterinary unit	10 (23.3)	5 (15.6)	0.386	15 (20.0)
	Slaughter house	8 (18.6)	6 (18.8)	0.987	14 (18.7)
	Laboratory	4 (9.3)	9 (28.1)	**0.033***	13 (17.3)
		(Mean±SD)	**(Mean±SD)**		**(Mean±SD)**
Occupation duration in years		17.2±9.8	20.0±11.3	0.260	18.4±10.5
Working hours per week		38.9±9.7	40.8±11.4	0.525	39.8±10.5

#: It's to be noted that there were cases where workers worked in more than one place,

*: Statistically significant at $p<0.05$

As presented in Table (1) the mean duration of occupation of the studied workers was 18.4±10.5 years with mean working hours per week of 39.8±10.5 hours. Veterinarians represented the highest proportion (32, 42.7%) followed by animal service workers 22 (29.3%) , the least represented occupations were veterinary supervisors being 4 (5.3%). As regard their workplaces, 42 (56%) of the studied workers worked in farms while the least represented group was those worked in laboratory being 13 (17.3%). It's to be noted that there were cases where workers worked in more than one place.

Seropositivity for Brucellosis was highest among veterinarians (19, 44.2%) while the least frequency was detected among veterinary supervisors (p=0.626) . The highest frequency of seropositivity was detected among workers working in farms (23, 53.5%) (p=0.611), veterinary units (10, 23.3%) (p=0.386) and veterinary directorate (10, 23.3%) (p=0.386). This was followed by slaughter house workers (8, 18.6%) (p=0.987). Workers in laboratories were 4(9.3%) of seropositives versus 9 (28.1%) of seronegatives and this difference was statistically significant (p=0.033). The mean duration of occupation was lower among seropositive workers than seronegative ones (17.2±9.8 and 20.0±11.3, respectively). There was no significant difference in mean duration of work and working hours per week in relation to seropositivity (p= 0.260, 0.525 respectively).

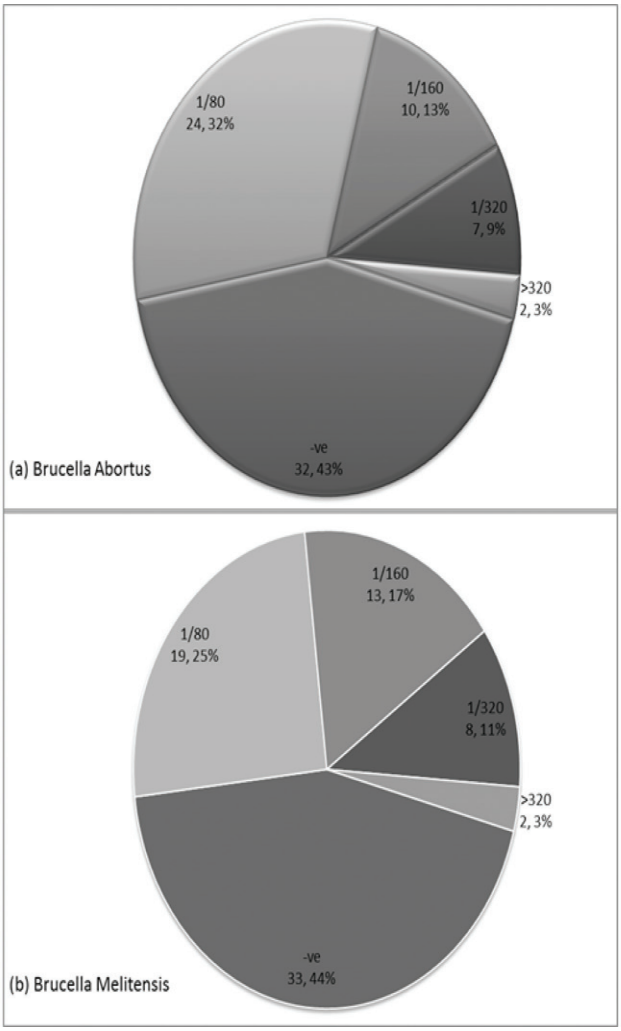

Fig. (1): Results of Rose Bengal Antibody titer among the studied workers, for (a) Brucella Abortus, (b) Brucella Melitensis.

Figure 1 showed that the majority of positive cases 24 (32%) had1/80 antibody titer towards Brucella Abortus while 2 (3%) had >1/320 antibody titer (Figure 1a). Regarding Brucella Melitensis, 19 (25%) had antibody titer 1/80 and only 2 (3%) had >1/320 antibody titer (Figure 1b).

Table 2: Seropositivity for Brucella Abortus and Melitensis among the studied group.

Type of Brucella	Study group (75) Freq. (%)	95% CI
Combined (Abortus and Melitensis)	42 (56.0)	67.2- 44.7
Abortus only	1 (1.3)	5.6-2.5
Total cases of seropositivity	43 (57.3)	68.5- 46.1

Table 2 showed that seropositivity for combined Brucella Abortus and Melitensis among the studied workers was 56% (42) and 1.3% (1 case) for Brucella Abortus.

Table 3: Relation between brucellosis seropositivity and different exposures, injuries encountered at work and risk of work activities.

Title	Seropositive (43) Freq. (%)	Seronegative (32) Freq. (%)	p-value	OR(95%CI)
Occupational exposures				
Dealing with unvaccinated animal	26 (60.5)	10 (31.3)	0.012*	3.4 (1.3-8.8)
Exposure to Abortus	15 (34.9)	10 (31.3)	0.741	1.2 (0.4-3.1)
Exposure to animal leathers	14 (32.6)	10 (31.3)	0.904	1.1 (0.4-2.8)
Exposure to animal wastes	20 (46.5)	15 (46.9)	0.975	1.0 (0.4-2.4)
Exposure to animal samples	17 (39.5)	18 (56.3)	0.151	0.5 (0.2-1.3)
Handling Raw meats	18 (41.9)	11 (34.4)	0.510	1.4 (0.5-3.5)
Work Injuries (kicking, biting)	34 (79.1)	24 (75.0)	0.677	1.3 (0.4-3.7)
Needle stick Injury on Brucella vaccine administration	14 (32.6)	12 (37.5)	0.656	0.8 (0.3-2.0)
Activities risk level				
High risk	37 (86.0)	21 (65.6)	**0.037***	3.2 (1.0-10.0)
Low risk	6 (14.0)	11 (34.4)		

*: Statistically significant .

Table (3) showed the risk of work activities as assessed in relation to seropositivity. Those dealing with unvaccinated livestock had significantly higher seropositives titre (26, 60.5%) and were 3.4 more liable to be seropositive than those who didn't deal with unvaccinated livestock as denoted by OR 3.4 95% CI 1.3-8.8). High prevalence of seropositivity was also detected among workers exposed to animal wastes (20, 46.5%), raw meats (18, 41.9%) and animal samples (17, 39.5%). The least frequency was reported among those exposed to leathers (14, 32.6%). There was a significantly higher frequency seropositive among high risk group (37, 86.0%) than low risk one (6, 14.4%) (p=0.037, OR 3.2, 95%CI 1.0-10.0).

Table 4: Predictors of Brucellosis seropositivity among studied workers.

Predictors#	**β coefficient**	**p-value**	**OR(95%CI)**
Dealing with unvaccinated animals	1.143	**0.023***	3.1 (1.2-8.4)
High risk work activities	1.107	0.074	2.9 (0.9-9.3)
Constant	0.295	0.206	1.3

*: Statistically significant at p <0.05

#: Dependent variable was Serological status: seropositive=1, seronegative=0;independent variables include Age in years (excluded to avoid collinearity with work years), Occupational duration in years, Working hours per week, Dealing with unvaccinated livestock (dealing=1, no dealing=0), Consumption of unpasteurized milk and milk products (yes=1, no=0), work activities (high risk= 1, low risk=0), work injuries (yes=1, no=0).

Constant: it is used in regression line equation to determine for every independent variable how the change of the dependant variable is.

Table 4 showed a conditional logistic regression analysis for occupational risk factors of Brucellosis seropositivity among studied workers. Variables entered into the model were occupational duration, work hours, dealing with unvaccinated animals, work injuries, work activities risk level, consumption of unpasteurized milk and milk products (some variables were excluded from the equation because they were non-significant). Predictors of Brucellosis seropositivity detected by logistic regression were dealing with unvaccinated animals (p=0.023, OR 3.1, 95%CI 1.2-8.4) and high risk work activities (p=0.074, OR 2.9, 95%CI 0.9-9.3) .

Discussion

In the present study seropositivity for Brucellosis was highest among veterinarians 19 (44.2%) followed by animal service workers 10 (23.3%), then butchers and veterinary assistants (6, 14% each), while the least frequency was detected among veterinary supervisors (p=0.626) (Table 1). This was consistent with Karadzinska-Bislimovska et al., 2010 who stated that the occupational risk among veterinarians was the highest during parturition or abortion of animals, in addition to examination, vaccination, insemination, and management of animal diseases. They also indicated that workers involved in the processing of animal products, such as slaughter men, meat packers, collectors of fetal calf serum, processors of skins and wool, renderers besides dairy workers could be exposed to Brucella species.

Beheshti and his colleagues, 2010 conducted a cross-sectional study in Iran to identify the prevalence of Brucellosis and its risk factors in a high risk group (No=141) and a contrast control group (No =44). The results revealed that eleven (7.8%) subjects from high risk group and none of the contrast control group were seropositive for Brucella (Beheshti et al., 2010).

A study achieved in Saudi Arabia for ten years cases of brucellosis presented to King Fahd University Hospital; they detected that from 84 patients with brucellosis, 64% provided history of contact with animals, 27% were farmers and 16% were sheppard, 6% were engaged in slaughtering animal, 4% working in laboratory (Boukary et al., 2013).

Seropositivity for Brucella among the studied workers was 57.3% (43), 56.0% (42) for combined Brucella Melitensis and Abortus infection and 1.3% (1 case) for Abortus infection only (Table 2). This is markedly higher than what was reported by previous studies that have been performed to assess the seroprevalence of Brucella in different countries. In a study conducted in Italy, the overall incidence of human brucellosis in Italy during the period between1997–2002 was 15.6 cases / 100 000 males and 9.5 cases / 100 000 females (62% vs. 38%), the data confirmed that 25% due to occupational exposure which was closest to the

surveillance data (De Massis et al., 2005). According to sociodemographic data, the peak incidence of disease subsequent to occupational exposure should be between March and April, and a next peak between November and January. However, the seasonal peak noted from April to June is consistent with the consumption of fresh cheese, lamb slaughtering, milking of Ewes, indicating that Brucellosis in Italy is principally a foodborne zoonosis, rather than an occupational disease (De Massis et al., 2005).

In a study performed in Tanga, Tanzania, serum samples were withdrawn from workers in different occupations and were screened for Brucella antibodies using Rose Bengal Plate Agglutination Test. The total seroprevalence of antibodies to Brucella Abortus was 5.5%; which was significantly higher ($p<0.05$) among subjects engaged in high-risk activities such as cutting throats of animals and cleaning parts of slaughtered cattle (Swai and Schoonman, 2009).

Furthermore, a cross-sectional study comprising slaughterhouse workers of Lahore district, Pakistan, was accomplished to clarify risk factors associated with Brucella seropositivity. A proportionate random sample of 360 labors from different slaughterhouses was selected. Seropositivity was found to be 21.7% (Mukhtar, 2010). In Egypt, by screening the seroprevalence of brucellosis among 220 individuals with high risk jobs from slaughter houses including veterinarians, veterinian assistants, butchers and services workers; they detected nearly 16% of the workers were seropositive for Brucellosis (El-Okda and Hamed, 2010).

Recently, Kutlu and his colleagues in 2014 performed a multicenter retrospective survey to label the risk factors of Brucellosis among veterinary workers working in Turkey. They detected that 11.8% have occupational Brucellosis. The results of the study concluded that veterinarians and veterinary technicians are at increased risk for infection with Brucellosis

These differences in seropositivity between the current study and the previous ones could be attributed to differences in the studied population. For example the duration of occupation

in El-Okda and Hamed, 2010 was 9.2±4 years while in the current study the participants had a higher work duration of 18.4±10.5 years.

In addition, there were differences in the proportions of the nature of different occupations represented in previous study from the current one. Butchers and abattoir workers were highly represented in the previous studies with a lower proportion of veterinarians, while in the current study veterinarians and their assistants who showed marked seropositivity represented 58% of the studied workers.

Regarding different exposures encountered at work, the highest seropositivity was among those dealing with unvaccinated animals (26, 60.5%) (p=0.012). High seropositivity was reported also among workers exposed to animal wastes (20, 46.5%) and raw meats (18, 41.9%). The least frequency was reported among those exposed to animal leathers (32.6%) (Table 3).

This is in consistence with WHO report; 2016 who stated that animal vaccination control the severity of Brucellosis as abortions are more common in unvaccinated animals with shedding of a large number of organisms. A study done in Alexandria, Egypt described the trend and probable risk factors for Brucellosis. The study found that contact with infected animals and animal products were the ultimate method of Brucellosis transmission (Meky et al., 2007).

Araj and Azzam, 1996 detected that high risk occupation including butchers and farmers, showed high prevalence of Brucellosis in Lebanon (60%).

Likewise, a study done in Yemen, declared that jobs dealing with animals including farmers and shepherds represent significant risk factors, adding to level of education and poor socioeconomic status (Al- shamahy et al., 2000).

Relation between high risk activities and seropositivity was statistically significant (p=0.032) (Table 3). This agrees with Bossi et al., 2004who stated that risk factors for infection included different activities that included handling of infected animal tissues, body fluids and aborted parts. In addition, WHO (2016) concluded that periods of survival of B. Abortus in animal waste of farm slurry were 7

weeks up to >8months which clarifies high infectivity of animal wastes.

The conditional logistic regression analysis for occupational risk factors of Brucellosis seropositivity among studied workers showed predictors for Brucellosis were dealing with unvaccinated animals (p=0.023, OR 3.1, 95% CI 1.2-8.4) and high risk work activities (p=0.074, OR 2.9, 95%CI 0.9-9.3) (Table 4).

This is consistent with the work done by Karadzinska-Bislimovska and his colleagues; 2010 who identified that the way of disease gaining in many exposed workers is almost impossible to determine, and reasonably often it is a result of not only one, but more risk activities (different entry portals at the same time). The occupational risk in veterinarians became the highest during parturition or abortions of animals, their checkup, artificial insemination, injection or immunization, and management of diseases.

Conclusion

Seropositivity for Brucella among studied workers was 57.3% in both Ismailia city and El Tal El Keber. Seropositivity was highest among veterinarians followed by animal service workers, then butchers and veterinary assistants. There was a statistically significant relation between seropositivity for Brucellosis and dealing with unvaccinated animals and high risk work activities. Dealing with unvaccinated animals lead to increased frequency of Brucellosis transmission from animal to human and increase seroprevalence of Brucellosis among high risk occupational groups.

Limitations of the study:

Small sample size for multivariate analysis, more elaboration about non occupational exposure as risk factors for seropositivity.

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