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Monitoring of Ruminant Sera for the Presence of Brucella Antibodies in Alexandria Province

Yasser N. Haggag, Hamed A. Samaha, Mohamed A. Nossair, Hend S. Mohammad

Department of Animal Hygiene and Zoonoses, Faculty of veterinary medicine, Alexandria University

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Correspondence to:
Mohamed Nossair,
mohammadnossair @yahoo.com

Abstract

A serological investigation was carried out in some ruminant species including cattle (100), sheep (200) and goats (200) in Alexandria Province. Serum samples were randomly selected then tested for the presence of antibodies against Brucella by using the Rose Bengal test (RBT) supplied by Veterinary Vaccine Institute, Abbasia, Egypt and only positive samples were confirmed by Complement Fixation test (CFT). It was found that the seroprevalence of Brucella antibodies in the examined serum samples of cattle, sheep and goats was 6, 6 and 7 %, respectively. The effect of some epidemiological factors including seasons of the year and sex groups on the occurrence of infection in ruminant animals were studied. The obtained results clarified that occurrence of Brucella infection was higher in females than in males with highest seasonal incidence occurring in spring season. Our study revealed that brucellosis is endemic at high levels in all ruminant species in the study area and questions the efficacy of the control measures in that place. The high intensity of infection transmission among ruminants combined with high livestock and human density and widespread marketing of unpasteurized milk and dairy products may explain the increasing rates of human brucellosis. The public health importance of brucellosis was discussed and an effective integrated human-animal control strategy is urgently needed.

1. INTRODUCTION

Brucellosis is neglected tropical zoonoses allegedly reemerging in Middle Eastern countries. Infected ruminants are the primary source of human infection; consequently, estimates of the frequency of ruminant infection are useful elements for building effective control strategies. Unfortunately, these estimates are lacking in most Middle East countries including Egypt. Brucellosis is a highly contagious and important zoonotic disease caused by different species of the genus Brucella, small, Gram negative, non-motile, non-spore forming, rod shaped (coccobacilli) bacteria (Baek et al., 2003; Kakoma et al., 2003) that are pathogenic for a wide variety of animals and also for humans (Mathur, 1971). In animals, brucellosis mainly affects reproduction and fertility, reduces the survival of newborns, and diminishes milk yield. The mortality of adult animals is insignificant (Sewel and Blocklesby, 1990). In human beings, the symptoms of disease are weakness, joint and muscle pain, headache, undulant fever, hepatomegaly, splenomegaly, night sweats and chills, marked asthenia and anorexia (Hugh-Jones, 2000). The WHO considers brucellosis to be a neglected zoonosis because, despite its widespread distribution and effects on multiple species, it is not prioritized by national and international health systems. The major route of human infection in endemic areas is ingestion of unpasteurized milk or its products. In non-endemic areas, occupational exposure through direct contact with infected livestock, or Brucella culture, via the respiratory tract, conjunctiva and skin abrasion (Young, 1991 and Hartmut et al., 2003). The importance of brucellosis is not known precisely, but it can have a considerable impact on human and animal health, as well as on socioeconomic factors, as rural income relies largely on livestock breeding and dairy

products and people usually live in very close proximity with their livestock. There are a lot of undiagnosed cases of abortion, stillbirth and retained placenta which are thought to be down to brucellosis and these have a significant impact on the development of livestock (Rahman et al., 2011) so, diagnosis of brucellosis is considered the corner stone of any control program and is based on immunological and bacteriological finding. It is recommended to use the serological tests as a means of indirect diagnosis of the disease in different animals as the occurrence of the disease is largely dependent on the animal reservoir (Alton et al., 1988). Numerous reports had previously described the situation of brucellosis among ruminants and human being e.g. Seddek (1999), Khoudier (2000), Abd El-Hafeez et al. (2001), Montasser et al. (2002), Habib et al. (2003), Cetinkaya et al. (2005), Krkic-Dautovic et al. (2006), Hegazy et al., (2011) and Rahman et al., (2011).

Therefore, the following study was carried out for the detection of brucellosis in ruminants (cattle, sheep and goats) with these objectives: estimate the frequency of ruminant brucellosis using RBT as a screening test; the epidemiological study of brucellosis in various livestock species, throw a beam of light upon the public health significance of the disease and preventive measures to its control in ruminants.

2. MATERIAL AND METHODS

2.1. Collection of samples

The study was conducted for a period of 12 months from March 2014 to February 2015 in the Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, Alexandria University. A total of 500 serum samples (in different seasons) were randomly collected from individually owned ruminant animals from different localities in Alexandria Province; 100 from cattle, 200 from sheep and 200 serum samples from goats. This was carried out according to **Alton et al. (1988)** by allowing 10 ml of blood to flow freely from jugular vein of ruminant animals by using sterile dry special double ended needle into a sterilized vacutainer tube in which the blood samples were left at room temperature for 30 minutes, then placed in the refrigerator for 24 hours and when the colt retracted, serum was removed by Pasteur pipette, then centrifuged at 3000 rpm for 10 minutes. They were stored at -20 ^{0}C in the deep freezer till examined serologically in laboratory of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, Alexandria University.

Table (1): Description of serum samples collected from different ruminant animals.

Ruminant species					
Cattle	Sex group		Age Group (Y)		
(25/ Season)	Male	Female	< 2	2 - 4	> 4
	9	91	16	25	59
Sheep	Sex group		Age Group (Y)		
(50/ Season)	Male	Female	<4	4 - 6	> 6
	14	186	68	83	49
Goats	Sex group		Age Group (Y)		
(50/ Season)	Male	Female	<1	1 - 4	> 4
	29	171	64	70	66

2.2. Serological Examination:

All the ruminant serum samples were subjected to RBT as a screening test in order to identify animals infected with brucellosis using 8 % Rose Bengal stained Brucella abortus strain 99 cells in lactate buffer (pH 3.65 $\pm$ 0.05). RBT was performed according to the procedure described by the OIE (2008). The test serum samples and Rose-Bengal antigen were kept for one hour at room temperature before the beginning of the test. A result was considered positive when there was any degree of agglutination noticeable and the absence of agglutination was considered as negative. The positive reactors were confirmed by CFT.

3. RESULTS

Table (2): Prevalence of brucellosis in examined serum samples of ruminants

Ruminant species	No. of examined samples	Positive reactors	%
Cattle	100	6	6.0
Sheep	200	12	6.0
Goats	200	14	7.0
Total	500	32	6.4
Chi-square	0.49 NS		

NS= Non-significant at (P<0.05)

Table (3): Prevalence of brucellosis in examined serum samples of cattle with regard to the season of the year

Cattle	No. of examined samples	Positive reactors	%
Spring	25	0	0.0
Summer	25	4	16.0
Autumn	25	0	0.0
Winter	25	2	8.0
Total	100	6	6.0
Chi-square	3.78 NS		

Table (4): Prevalence of brucellosis in examined serum samples of cattle with regard to the sex group

Cattle	No. of examined samples	Positive reactors	%
Females	91	5	5.5
Males	9	1	11.1
Total	100	6	6.0
Chi-square	0.31 NS		

NS= Non-significant at (P<0.05)

Table (5): Prevalence of brucellosis in examined serum samples of sheep with regard to the season of the year

Sheep	No. of examined samples	Positive reactors	%
Spring	50	3	6.0
Summer	50	5	10.0
Autumn	50	0	0.0
Winter	50	4	8.0
Total	200	12	6.0
Chi-square	28.27 **		

**= Significant at (P<0.0001)

Table (6): Prevalence of brucellosis in examined serum samples of sheep with regard to the sex group

Sheep	No. of examined samples	Positive reactors	%
Females	186	10	5.38
Males	14	2	14.3
Total	200	12	6.0
Chi-square	3.35 NS		

NS= Non-significant at (P<0.05)

Table (7): Prevalence of brucellosis in examined serum samples of goats with regard to the season of the year

Goats	No. of examined samples	Positive reactors	%
Spring	50	6	12.0
Summer	50	4	8.0
Autumn	50	1	2.0
Winter	50	3	6.0
Total	200	14	7.0
Chi-square	1.63 NS		

NS= Non-significant at (P<0.05)

Table (8): Prevalence of brucellosis in examined serum samples of goats with regard to the sex group

Goats	No. of examined samples	Positive reactors	%
Females	171	10	5.85
Males	29	4	13.79
Total	200	14	7.0
Chi-square	0.0003 NS		

NS= Non-significant at (P<0.05)

4. DISCUSSION:

Although many countries have eradication programs for controlling brucellosis, economic losses can be heavy due to abortion and infertility and subsequent culling so herds should be monitored for the presence of infection. Despite eradication programs, including vaccination, testing and slaughter, brucellosis remains a major zoonosis worldwide (Baek et al., 2003; Kakoma et al., 2003) and the disease has remained prevalent in many areas in the world. Each year half a million cases of brucellosis are reported worldwide but according to WHO, these numbers are greatly underestimated. Even so, brucellosis is distributed throughout the world wherever livestock are being raised. Likewise, in many less developed countries and in developing countries brucellosis continues to cause major losses in livestock and poses a serious threat to people (Crawford et al., 1990).

The obtained results as shown in Table (2) revealed that the overall seroprevalence of brucellosis in ruminant species in the current study was 6.4 % where, the frequency of antibody detection against brucellosis in cattle, sheep and goats was 6, 6 and 7%, respectively and the statistical analysis of the previously obtained results showed no significant association between the recorded prevalence in cattle, sheep and goats.

The recorded data in Table (2) firstly revealed that the overall sero-prevalence of brucellosis in cattle was 6 %. This result was higher than that obtained by Seddek (1999) (2.81%), Abd El-Hafeez et al. (2001) (2.0%) Montasser et al. (2002) (1.29%) and Rahman et al., (2011) (2.66 %) while was lower than that reported by Khoudier (2000) (10.92 %) and El-Gamel (2004) (11.0 %), Aggad and Boukraa (2006) (15.7%), Moawad and Osman (2006) (29.8%), Haggag and Samaha (2007) (9.6 %), Hegazy et al., (2011) (12.2 %) and Megersa e t al (2011) (8 %). The variation in the prevalence of brucellosis in cattle may be due to animal population, their susceptibility, vaccination status and the hygienic measures applied in each locality. Data tabulated in Table (2) also clarified that the presence of neutralizing antibodies against Brucella in examined samples of sheep was 6 %. This result was nearly equal to that obtained by Abd El-Hafeez et al. (2001) (6.2 %) while, it was higher than that obtained by Seddek (1999) (4.8 %), Montasser et al. (2002) (4 %), El-Bassiony et al. (2007) (2 %) and Rahman et al., (2011) (2.3 %). On the other hand, it was lower than that recorded by Nossair (2005) (14.8 %) and Hegazy et al., (2011) (12.2 %). In addition, the presented data in Table (2) showed that the prevalence of brucellosis in goats was 7 %. The obtained result in the current study was in agreement with Radwan and El- Shabrawy (2005) who examined 300 serum samples of goats by RBT and found that 7.7 % was positive and it was higher than obtained by Rahman et al., (2011) who recorded a prevalence of 3.2 %. On contrary, it was lower than that obtained by and Hegazy et al., (2011) who recorded a prevalence of 11.3 %. These obtained results confirmed the endimicity of brucellosis in ruminants in the examined region that may constitute human health hazards.

The seasonal distribution of positive reactors of cattle brucellosis was tabulated in Table (3). Chi square analysis of the obtained result of the effect of season on the frequency of detection of Brucella antibodies in cattle serum samples showed non-significant relationship although the numerical values revealed that the highest prevalence was observed in summer season (16 %) followed by winter season (8%). These results were not in agreement with Haggag and Samaha (2007) who found that the highest prevalence was in winter season and Nossair (2005) who recorded that the majority of cases occurred in spring season due to the effect of moderate atmospheric temperature that permits the survival of Brucella organisms in the environment.

Sex-related sero-prevalence of brucellosis in cattle was shown in Table (4); it was found that 5 out of 91 females tested positive (5.5%), while only one sample of the 9 tested males was positive (11.1%). Moreover, Chi square analysis of the obtained result of the effect of sex on the frequency of detection of Brucella antibodies in cattle serum samples showed non-significant relationship although the numerical values revealed that the prevalence of brucellosis was higher in males than females. This result was in

agreement with Lavsen et al. (1988) and Rahman et al., (2011) who found that the prevalence was relatively higher in females than that in males. The higher rate of infection in females will be due to infection within the female reproductive tract providing a potential reservoir for the organism to propagate.

The effect of seasonal changes on the detection rate of brucellosis in examined serum samples of sheep was tabulated in Table (5). It was found that among 200 serum samples examined throughout the year, only 12 samples were found to be positive and the highest prevalence was observed in summer season (10 %) followed by winter season (8%) then the spring season (6%). Chi square analysis of the obtained result showed significant association between the season of the year and the detection rate of brucellosis in sheep. The higher detection rate of brucellosis in summer season in sheep was in agreement with cattle where higher detection rate was also observed in summer season confirming that the disease may be transmitted from sheep to cattle and vice versa so multi species rearing should be avoided in order to minimize the risks of inter species transmission of brucellosis.

The sexual distribution of the positive samples of sheep brucellosis was found in Table (6) where Chi square analysis of the obtained result of the effect of sex on the frequency of detection of Brucella antibodies in sheep serum samples showed non-significant relationship that was in agreement with Ashenafi et al., (2007) who noticed that no statistically significant difference was found between males and females ($chi2 = 2.57$, $P > 0.05$). On contrary, the numerical values revealed that the prevalence of brucellosis was higher in males (14.3 %) (2 out of 14) than females (5.38 %) (10 out of 186 examined samples).

Moreover, the seasonal distribution of positive reactors of goats' brucellosis was tabulated in Table (7); the highest prevalence was observed in spring season (12 %) followed by summer season (8%) then winter season (6%) and lastly autumn season (2%). Chi square analysis of the obtained result of the effect of season on the frequency of detection of brucella antibodies in goats' serum samples showed non-significant relationship. The obtained result was in agreement with that obtained by Megersa et al., (2011) who stated that wet season was among the risk factors associated with brucellosis infection in goats. Serological evidence of brucellosis in goats may throw the light upon the dangerous role played by goats in continuous spreading of brucellosis to cattle and sheep as well as human being throughout the year so strict control measures must be followed to avoid risks attributed to rearing of goats.

Finally, Chi square analysis of the obtained result of the effect of sex on the frequency of detection of Brucella antibodies in goats' serum samples was found in Table (8) and showed non-significant relationship although the numerical values showed higher values in males (13.8 %) than females (5.85%).

From the obtained results in this study, it can be concluded that the serological examination of ruminant animals revealed a relatively higher prevalence of Brucella infection among them indicating that ruminants still play a dangerous role of potential infection to man. So, the following hygienic measures should be under taken including, efficient pasteurization or boiling of milk, vaccination of cattle against brucellosis, application of the policy of test and slaughter of all positive reactors among cattle, and those come in contact with infected cattle should wear protective clothes. In addition, the interchange of information and surveillance data between veterinary and public health service is mostly essential for prevention of the disease in man and animals. In the light of the results here reported and other concordant published evidence, we recommend that serious consideration should be given to an integrated human-animal brucellosis control program in the investigated region and that surveys aimed at estimating the frequency of ruminant brucellosis are carried out in other parts of the country.

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