Epidemiology of Brucellosis Among Farm Animals

H.A. Kaoud[1], Manal.M. Zaki[1], A.R. El-Dahshan[1] , Shimaa[1] .A. Nasr[1]

Department of Veterinary Hygiene, Environmental Pollution and Management, Faculty of Veterinary Medicine, Cairo University, Giza 11221, Egypt

*:Correspondence to: Dr. H. A. Kaoud , Dept. of Veterinary Hygiene ,Environmental Pollution and Management ,Faculty of Veterinary Medicine ,Cairo University ,Giza ,Egypt. *Postal Code: 1221, Fax: 202-5725240,* Email: ka-oud@link.net

Abstract: : In this article we studied the epidemiology and the role of risk factors of *Brucella* infection in ruminants, besides the methods concerning the evaluation of biosecurity measures which are taken against the disease in farms. A cross sectional study was carried out on different Governorates representing allover Egypt to evaluate the potential major risk factors, mal- biosecurity practices and their role in the maintenance of the disease among farm animals. Serum samples (1670) were collected from 126 Herds / Flocks of sheep, goats and cattle and analyzed using Rose Bengal Plate test and iELISA test. A structured questionnaire was designed to identify and evaluate the role of risk factors for Brucellosis. .The results pointed out that, prevalence of brucellosis among herds/flocks of sheep, goats and cattle were; 26.66%, 18.88% and 17.22% respectively. And the seropositive percentages in blood samples were 21.20%, 14.5 % and 2.16% respectively. Major risk factors play a very important role in the prevention and maintenance of the disease among farm animals. The role and magnitude of risk factors varied but the presence of good sanitary measures in farms are considered as a protective factor, where R.R was less than 1 and the attributable risk was -0.01. [Nature and Science 2010;8(5):190-197]. (ISSN: 1545-0740).

Keywords: Seroprevalence, Questionnaires, Role and magnitude of risk factors, Relative and Attributable risk, Brucellosis. Abbreviations: R.R: Relative risk A.R: Attributable risk: Omega.

1. Introduction

Brucellosis is a contagions disease caused by bacteria of genus *Brucella;* it is an animal disease in origin and is a disease of the sexually mature animals with predilection of placenta, fetal fluids and tests of male animals. It has been recognized as a global problem of wild and domestic animals, especially cattle, sheep and goats (Rijpens, 1996).

Brucellosis is the most important zoonosis in terms of human suffering and is a true zoonosis in that almost all human cases are acquired from animals , in particular goats and sheep . In Egypt, brucellosis is still remaining one of the major disease problems that affect animal industry as well as human health and is still an endemic serious disease among domestic animals and humans in spite of attempts that were implemented to control the disease through bilateral projects with some agencies or international organization (Şahin et al., 2008). It has been recorded in Egypt since 1939 (Ahmed, 1939), and the estimated annual economic losses due to brucellosis were about 60 million Egyptian pounds yearly (AOAD, 1995).

In one hand, the annual incidence of brucellosis in people in the Mediterranean and Middle East countries varies from 1 to 78 cases per 100.000 (OIE, 2000, El Sherbini et al., 2007). In Egypt, many authors had reported the incidence of brucellosis among animals (Refai, 1994, and Abdel Hafeez et al., 2001) but only few reports concerning the role of risk factors and the epidemiologic evaluation of the disease (Al-Majali et al.,2007)

On the other hand animal brucellosis is well established in the Middle East and affect both cattle and small ruminants (Abdel- Ghani et al., 1983, Ismaily et al., 1988, Aldomy et al., 1992 and Darwish & Benkirane 2001) .Infection with *Brucella spp*. continues to pose a human health risk globally despite strides in eradicating the disease from domestic animals (Mantur et al.; 2007).

The main objectives of this study were to declare the role of risk factors in the maintenance of the disease among farm animals and to suggest a model of control of brucellosis in Egypt.

2. Materials and Methods

The study was carried out through the period from

March, 2006 to March, 2008 on different Governorates representing allover Egypt. A total of 126 herds/flocks of different species included 45 flocks of sheep, 55 flocks of goats and 26 herds of cattle were selected for this study. In each region, blood samples were taken from herds /flocks with no previous history of vaccination against *Brucella*. The number of samples was collected according to Thrusfield (1995) and Kaoud (2001) in simple and\or systemic random sampling as follows: Animals from each herd were randomly selected using a table of random digits. Only female cows older than 6 months of age were sampled. The herds were stratified into three herd sizes: small herds (≤ 50), medium herds (50-150) and large herds (> 150).

1-Questionnaire

A questionnaire, for each farm, was completed by the farm owner or manager to identify possible independent variables associated with the presence of seropositive cattle on the farm:

a- Precautions taken to visitors.
b- Addition of new animals
C-Multiple raising of animal species
d- Exogenous fertilizing system.
e- Lack of common sanitary measures which include routine or regularity of disinfection program, disposal of carcasses & wastes, control of traffic & vehicles and regular veterinary services).

Calculation:

•Biosecurity practices and mal- practices as a risk factor concerning seropositives:

Risk factors	Seroprevalence		
	Positive	Negative	
Exposed	A:Number of seroprevalence	B:Number of seroprevalence	A + B
Not-Exposed	C:Number of seroprevalence	D:Number of seroprevalence	C + D
	A + C	B + D	

$$RR = \frac{\text{Incidence in the exposed}}{\text{Incidence in the nonexposed}} = \frac{a/a + b}{c/c + d}$$

AR= Incidence of exposed – Incidence of non exposed

OR= a×d / c×b

Omega Risk Measure= a+c/b+d

2-Collection of blood samples

Approximately 7- 10 ml of blood was down from Jugular vein of apparently healthy animals using plain vacutainer tubes and needles. Samples were kept overnight at 4◦C to allow the separation of serum then centrifuged at 3000 rpm for 10 minutes. The collected sera were coded and kept at -20◦C up to the time of the test.

3-Serologial examination ofthe samples

The collected sera were screened for the presence of antibodies against *Brucella* antigens (Alton *et al.*, 1988) by using the Rose Bengal plate test "RBPT" and a commercially available indirect enzyme linked immunosorbent assay (iELISA)

3. Results

Prevalence of Brucellosis among herds/flocks of sheep, goats and cattle

Prevalence of *Brucella* among herds/flocks of sheep, goats and cattle in the studied Governorates using RBPT are shown in Figure (1a).

The results of RBPT pointed out that, prevalence of brucellosis among herds/flocks of sheep, goats and cattle were 26.66%, 18.88% and 21.6%, respectively. When RBPT positive samples were subjected to iELISA test, 21.20%, 14.5 % and 17.22%, respectively showed positive reactions to brucellosis (Figure 1-b).

Prevalence of Brucellosis of the seropositive blood samples in animals (sheep, goats and cattle).

The seropositive percentages in blood samples (subjected to iELISA test) of sheep, goats and cattle were 21.20%, 14.5 % and 2.16% respectively.

Risk factors and their role in the introduction and maintenance of *Brucella* infection in animal farms.

Figure(2) indicated that there was an association concerning the applied biosecurity practices in farms and seropositive reactors of brucellosis, where the relative risk of: farm visitors, addition of new animals, multiple raising of animal species, exogenous fertilizing system and presence of common sanitary measures were: 1.14 , 5 , 8.33 , 2.27 and 0.96, respectively. The presence of good sanitary measures in farms considered as a protective factor where R.R was less than 1 (negative association) and the attributable risk was - 0.01.

The magnitude of the risk factors.

Figures (3) declared the magnitude of the risk factors (Farm visitors, addition of new animals, multiple species rearing and exogenous fertilizing system) were 0.03, 0.32, 0.66 and 0.28,

respectively; in other meaning these risk factors are responsible for the introduction of *Brucella* infection in farms in percentages of 3 %, 32%, 66% and 28%, respectively. Multiple raising of different animal species is considered as highly risk factors.

Odds in favor of acquiring *Brucella* infection in animal farms and the risk of having risk factors.

The degree of association between risk factors and *Brucella* occurrence was calculated by omega measure as shown in Figure (4). According to this measure, risk factors were arranged as following: visitors, exogenous fertilization & lack of sanitary measures (0.31) addition of new animals (0.26) and rearing of multi-species (0.25).

Odds ratio (Psi) and the relative frequency of risk factors for Brucellosis.

Odds ratio (Psi) measures the relative frequency of risk factors for brucellosis to be occurred in farms. According to Odds ratio (Psi) measure, the frequency of risk factors was arranged as following: visitors (1.20), addition of new animals (7.77), rearing of multi-species (28.80) exogenous fertilization (3.2) and lack of sanitary measures (1.7) as shown in Figure (5).

Table 1. Distribution of the *Brucella* seropositive and seronegative herds/flocks of sheep, goats and cattle and the relevance with different investigated variables

		Brucella result		
Variable	Category	Number	Positive No. (%)	Negative No. (%)
Visitors biosecurity	Yes No	80 46	20 (25) 10 (21.74)	60 (75) 36 (78.26)
Addition of New animals	Yes No	50 76	20 (40) 6 (7.89)	30 (60) 70 (92.11)
Rearing of multiple-species	Yes No	20 106	15 (75) 10 (9.43)	5 (25) 96 (90.57)
Exogenous fertilizing	Yes No	10 116	5 (50) 25 (21.55)	5 (50) 91 (78.45)
Sanitary measures	Yes No	26 100	6 (23.08) 24 (24)	20 (76.92) 76 (76)

Yes: means the presence of the factor No: Means the absence of the factor

Table 2. The magnitude and association between different risk factors and seropositive reactors of brucellosis in Egypt

Biosecurity practices	Relative risk	Attributable risk	Omega measure	Odds ratio
-Farm visitors	1.14	0.03	0.31	1.20
-Addition of new animal	5.00	0.32	0.26	7.77
-Multiple raising of species	8.33	0.66	0.25	28.80
-Exogenous fertilizing system	2.27	0.28	0.31	3.2
-Lack of sanitary measures	0.96	- 0.01	0.31	1.7

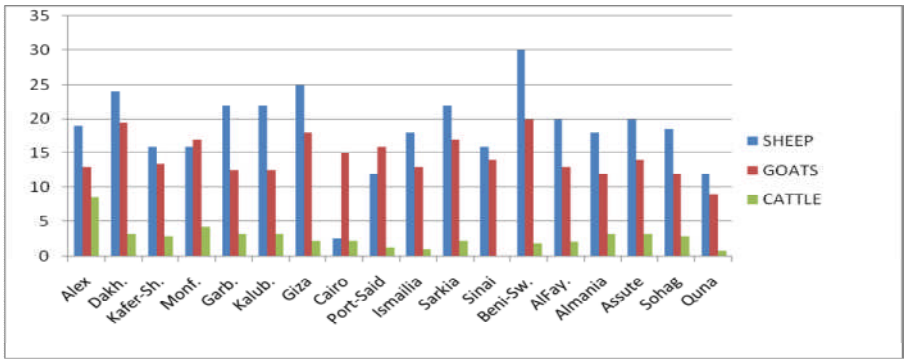

Figure (1-a)

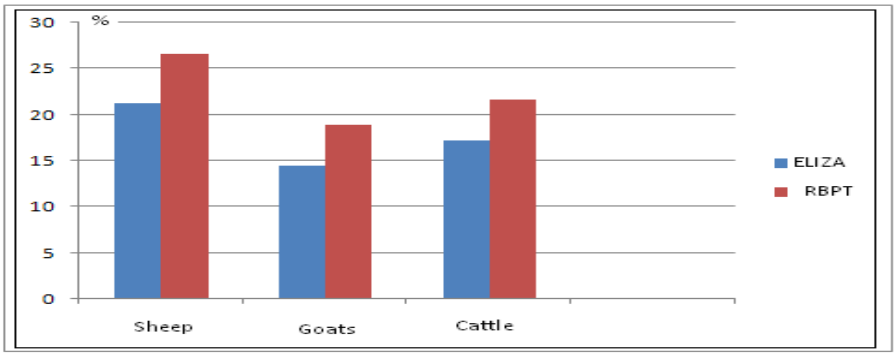

Figure (1-b)

Figure (1-a&1-b) Prevalence of *Brucella in Governorates and* among herds/flocks using iELISA and RBPT

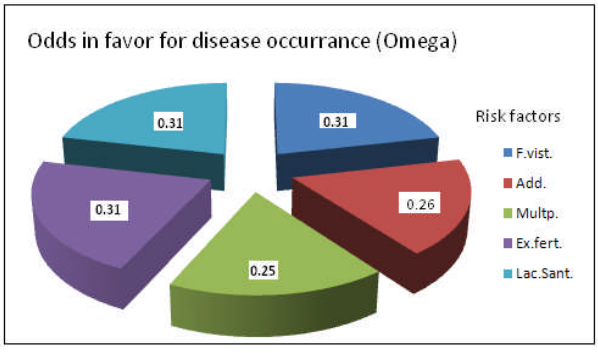

Figure (2) Relative Risk

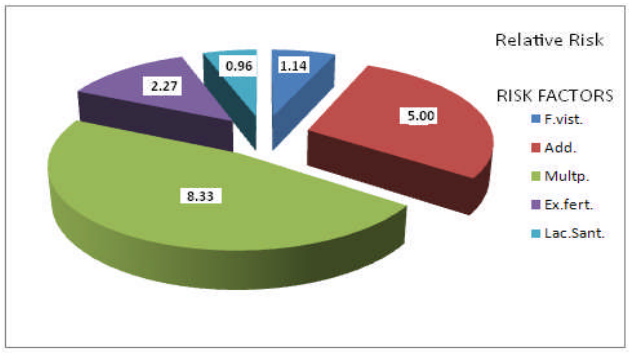

Figure (3) The magnitude of the risk factor

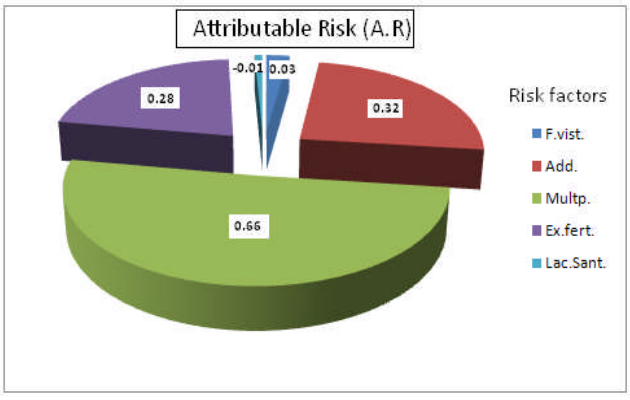

Figure (4) Odds in favor of acquiring *Brucella* infection

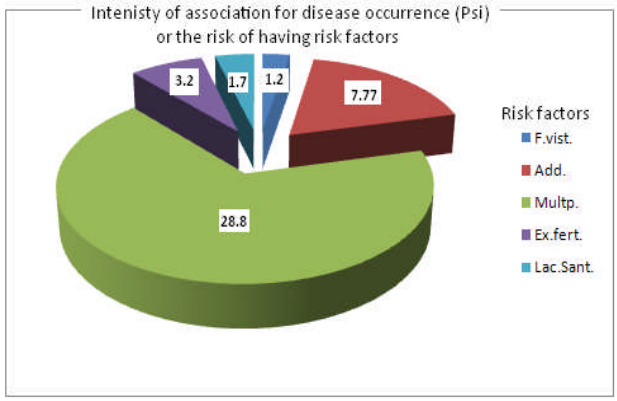

Figure (5) The association between the risk factors and occurrence of brucellosis in animal farms

4. Discussions

This study was conducted with the aim of identifying herd and biosecurity characteristics as potential risk factors associated with brucellosis seroprevalence in ruminants in Egypt. This information is required in order to outline measures to control zoonotic brucellosis (Lithg-Pereira, 2001).

Small ruminant brucellosis is still endemic in most countries of the Mediterranean basin, the Middle East and Central Asia (Aldomy *et al*., 1992 and Radostitis *et al.*, 2000). The public health and economic impact of brucellosis remains of particular concern in developing countries. The clinical manifestations of brucellosis in goats are similar to those in cattle in regard to abortion, stillbirth and reproductive failure. Thus this disease causes heavy economic losses in animal production resulting form abortions, sterility, decreased milk production, and the costs of replacer animals (Ariza *et al*., 1995 and Radostitis *et al.*, 2000). In addition, the disease is an impediment to free animal movement and export.

The prevalence of bovine brucellosis in Egypt was lower than those reported by Refai *et al*., (1990) and Ahmed *et al*., (2002) and it was higher than those reported by Darwesh and Benkiran (2001) in Syria, Amin *et al*., (2005) in Bangladesh and Silva *et al*.,(2000) in Srilanka. The prevalence of brucellosis in sheep and goats in Egypt was lower than that reported by AL-Majali (2005) in Jordan, Radwan *et al*., (1982) in Saudia Arabia, Jackson *et al*., (2004) in Kosovo and it was higher than those reported by Abdel-Hafeez (2001), Shalaby *et al*.(2002) & (2003) and Noha Oraby *et al*.*(* 2007).

The Seroprevalence of brucellosis in sheep, goats and cattle were widely but unevenly distributed throughout flocks and provinces (Jackson *et al.*, 2004, Al- Ani *et al*., 2004 and Muma *et al.*; 2006).

Risk factors and their role in the introduction and maintenance of Brucella infection in animal farms

Major risk factors play very important role in the maintenance and spreading of the disease among farm animals and their human contacts. Relative risk used as a measure to determine if an association exists, and whether there is an excess risk of the disease in populations who have been exposed to disease (Gordis, 2004). Figs (3) and (4) indicated that there was an association concerning the applied biosecurity practices in farms and seropositive reactors of brucellosis, where the relative risk of farm visitors, addition of new animals, multiple raising of animal species, exogenous fertilizing system and presence of common sanitary measures were 1.14, 5, 8.33, 2.72 and 0.96, respectively. The presence of good sanitary measures in farms considered as a protective factor where R.R was less than 1 (negative association) and the attributable risk was - 0.01, this means that the sanitary measures (routine farm disinfection, disposal of carcass & wastes, vehicle disinfection and the presence of veterinary service) play a minor role in preventing the introduction of infection, while its role in preventing the spread of the infection inside the farms or herds has a major property. These results indicated that farm visitors, addition of new animals, multiple species rearing and exogenous fertilizing system were considered as very important risk factors for the introduction and spread of *Brucella* infection among animal farms. Reviriego *et al.*, (2000),Al-Majali (2005) and Earhart *et al*.,(2009) suggested that the addition of new animals or contact with other small ruminants flocks might be a risk factor for brucellosis at the herds /flocks level, in addition the contact between animal herds/flocks will increase the chance for disease transmission to susceptible animals (Crespo, 1994). On contrary, Izquier and Villanueva (1996), Al-Majali *et al*., (2009) stated that the contact between small ruminant flocks had no impact on seropositivity to *Brucella*. Veterinary services play a minor role in preventing the introduction of infection, while its role in preventing the spread of the infection inside the farms or herds has a major role. These results are coincide with Crespo (1994), Mainar–Jaine & Vazquez- Boland (1999) and AL-Majali (2005), who suggested that the absence of disinfection programs in raising farms considered a risk factor for *Brucella* seropositivity in small ruminants. Proper disposal of aborted materials and highly hygienic procedures are extremely important steps in any successful *Brucella* control program. It is well known that delivering adequate animal health services results in a low incidence of diseases, and especially those diseases that have an infectious nature. In addition, controlling brucellosis in small ruminants (mainly by Rev-1 vaccination) will indirectly reduce the prevalence of this disease in other animal species especially cattle. Poor veterinary service has been identified as a risk factor for brucellosis in Argentina (Samartino, 2002) and Mexico (Luna-Martínez and Mejía-Terán ,2002).

Attributable risk is a measure of how much of the disease risk is attributable to a certain exposure, and is useful in answering the question of how much can be prevented. The magnitude of the

risk factors (Farm visitors, addition of new animals, exogenous fertilizing system and multiple species raising) were 0.03, 0.32, 0.28 and 0.66, respectively, in other meaning these risk factors are responsible for the introduction of *Brucella* infection in farms in percentages of 3 %, 32%, 28% and 66%, respectively. In this study, multiple species raising, addition of new animals and the exogenous fertilizing system were identified as the risk factors associated with seropositivity to *Brucella* antigen. Prevention or at least the reduction of brucellosis can be achieved through the satisfactory or strict application of biosecurity measures in animal farms. The use of disinfectants and the presence of adequate veterinary services were identified as the factors that protect against bovine brucellosis. Similar observations were reported for sheep, goats and camels (AL-Majali, 2005).

Odds in favor of acquiring *Brucella* infection in animal farms and the risk of having risk factors (Fig.5)

Odds are commonly used as measures in epidemiology (the odds of *Brucella* infection is the ratio of the number of ways the infection can occurred to the number of ways the infection cannot occurred). The chance and the probability of *Brucella* occurrence in animal farms and the degree of association between risk factors and *Brucella* occurrence were calculated by omega measure. According to the association by this measure, the risk factors were arranged as following: visitors, exogenous fertilization & lack of sanitary measures (0.31), addition of new animals (0.26) and rearing of multi-species (0.25).

Odds ratio measures the relative frequency of the risk factors for brucellosis to be occurred in farms or the degree of association between the risk factors with brucellosis. According to this measure, the frequency of risk factors was arranged as following: rearing of multi-species (28.80), addition of new animals (7.77), visitors (1.20), lack of sanitary measures (0.95) and exogenous fertilization (0.40). The OR value showed that the farms that rear multi-species, added continuously new animals and didn't made biosecurity measures against visitors were about 28.80, 7.77 & 1.20 times more at risk to brucellosis than those rear only one species, didn't add new animals from external sources and made a strict biosecurity measures against visitors. Mixed farming which raising sheep and/or goats along with cattle, was reported by many researchers to be a risk factor for *Brucella* transmission between different animal species (Abbas and Agab 2002, Al-Majali *et al*., 2007). In addition, producers introduced new animals into the herd on a more frequent basis, and stock interchange for breeding is most frequent when the herd is large. This increase the risk of introducing an infected animal into the herd. Practices that involve movement of animals between herds are also likely to carry risks (Kabagambe *et al*., 2001). Such husbandry practices, with animals of different species being herded together, increase the likelihood of animals being exposed to the disease. This factor should be taken into consideration in the planning and execution of control programs. Movements of animals should be controlled by appropriate legislation and regulations.

This study indicated high prevalence of *Brucella* seropositive reactors which pose a human health risk. Lack or mal-practices in the application of sanitary program in farms (such as rearing of multi-species, addition of new animals, and lack of sanitary measures) are highly risk factors which result in the maintenance and spread of brucellosis among animal farms and humans.

5 .Conclusions

Our results could make a useful contribution towards preventing brucellosis in small ruminants and decreasing losses in the livestock industry. More attention should be paid towards implementing a proper control program for brucellosis and more efforts should be directed towards improving the animal health biosecurity program in those Governorates that are large in size. In addition, controlling brucellosis in small ruminants (mainly by Rev-1 vaccination) will indirectly reduce the prevalence of this disease in other animal species especially cattle. Control progress should be monitored serologically and evaluated epidemiologically; Veterinary extension should be played a major role to guarantee the application of the sanitary procedures and measures in rearing, raising and breeding places and education of personnel and dissemination of awareness as well as veterinary public health culture through various multimedia.

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