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Research Article

Seroprevalence of Brucellosis in Livestock in Khuzestan Province, Southwest of Iran, 2008-2012

Ehsan Gharib MOMBENI[1]*, *Manoochehr Gharib* MOMBEINI[2], *Mehdi Khalaj*[3], *Reza ASADI*[4], *Abdul Amir REZAEI*[5], *Karim AMIRI*[6], *Samed BROMAND*[7], *Mostafa KENARKOHI*[8], *Afshin Gharib MOMBENI*[9]

[1]*Expert of Animal Diseases Control and Monitoring, Ahvaz Veterinary Organization, Khuzestan province, Iran*
[2]*Provincial Head of Animal Disease Control and Monitoring, Veterinary Organization, Iran*
[3]*Head of Iran Veterinary Organization, Iran*
[4]*General Director of Ahvaz Veterinary Organization, Khuzestan province, Iran*
[5]*General Director of Veterinary Public Health and Animal Disease Management, Tehran, Iran*
[6]*Group Chief of Brucellosis Control Department, Iran Veterinary Organization*
[7]*General Director of Zoonotic Disease Control, Iran Veterinary Organization, Iran*
[8]*General Director of Khuzestan Province Veterinary Organization, Iran*
[9]*Engineering Department, Shaid Chamran University, Ahvaz, Iran*

**Corresponding Author: Ehsan Gharib MOMBENI Animal Diseases Control and Monitoring, Ahvaz Veterinary Organization, Khuzestan province, Iran*

e-mail: e.mombeni@hotmail.com

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ABSTRACT

Brucellosis is a zoonotic problem worldwide, especially in developing countries and specifically in Iran, where it is endemic. This study aimed to investigate the seroprevalence of brucellosis among livestock in the 18 districts of Khuzestan Province in Southwest Iran. Serum samples of 87798 cattle and 119020 sheep were tested using the Rose Bengal Plate Test, and positive results were confirmed with serum agglutination tests (SAT) and 2-mercaptoethanol SAT (2ME-SAT). The seroprevalence derived from total samples was 0.72% for cattle and 3.01% for sheep, revealing that though brucellosis is present among livestock populations in Khuzestan and that infection among sheep is significantly higher than among cattle ($P<0.05$), seroprevalence in general was much lower than indicated by results of other studies of livestock in the Middle East and neighboring regions. These results nevertheless recommend implementing a policy of transparency regarding brucellosis as well as measures to effectively eradicate the disease.

Key Words: Serology, ruminants, brucellosis, Iran

ÖZET

2008-2012 YILLARI ARASINDA GÜNEYBATI İRAN KHUZESTAN İLİNDE YETİŞTİRİLEN ÇİFTLİK HAYVANLARINDA BRUSELLOZUN SEROPREVALANSI

Bruselloz endemik seyrettiği İran ve gelişen ülkeler başta olmak üzere, dünya çapında yaygın görülen bir zoonotik hastalıktır. Çalışmada Güneybatı İran'da yer alan Khuzestan ilinin 18 farklı semtinde ki çiftlik hayvanları arasında brusellozun seroprevalansının araştırılması amaçlandı. 87798 sığır ve 119020 koyun serum örneği Rose Bengal Lam Aglütinasyon tekniği ile test edildi, pozitif örnekler serum aglütinasyon testi (SAT) ve 2-merkaptoetanol SAT (2ME-SAT) ile doğrulandı. Örneklerden elde edilen seroprevalans oranları, sığırlar için %0,72, koyunlarda ise %3,01 olup Khuzestan'da ki çiftlik hayvanlarında brusellozun varlığını ve sığırlara göre koyunlarda enfeksiyonun istatistiksel

olarak daha yüksek oranda (P<0,05) seyrettiğini ortaya çıkardı. Bununla birlikte belirlenen genel seroprevalansın komşu bölgelerdeki ve Orta Doğu'daki çiftlik hayvanları üzerinde yapılan diğer çalışmalardaki sonuçlara göre çok daha düşük olduğu saptandı. Bu sonuçlar yine de bruselloz ile ilgili olarak şeffaflık politikası ile birlikte etkili eradikasyon tedbirlerinin uygulanması gerektiğini göstermektedir.

Anahtar Kelimeler: Seroloji, ruminant, bruselloz, İran

Introduction

Brucellosis is a highly contagious, zoonotic, bacterial disease associated with significant morbidity that can lead to increased rates of spontaneous abortions in livestock and also in humans. It has important economic significance as it causes considerable financial losses due to abortion, decreased milk production, low fertility rates and stillbirth. It also affects industrial production (Bercovich, 1998; McDermott and Arimi, 2002; OIE, 2008; Poester et al., 2002; 2010).

It is the second most important zoonotic disease in the world after rabies. The importance of this widespread disease is due to its enormous hazard to human health, either through direct contact with infected animals or through consumption of unpasteurized milk and milk products. Small ruminants are considered to be the main hosts of this infection. It is also an occupational hazard. Brucellosis has an adverse effect on animal health and has a deep economic impact on the animal industry (Bale et al., 1982; Godfroid et al., 2004; Hugh-Jones 2000; Kumi-Diaka et al., 1980; McDermott and Arimi 2002; Nicoletti 1980; Ocholi et al., 2004; Shafee et al., 2011).

It is a worldwide problem of wild and domestic animals, especially cattle, sheep, and goats causing a decrease in reproductive efficacy and an increase in abortion rate. It has also been reported in most of the developing countries, such as Iran (Shafee et al., 2011).

In susceptible herds, abortion rates vary from 30 to 70% (Godfroid et al., 2004). The mortality of adult animals is insignificant (Rahman et al., 2011). Infection may be lifelong, and during ulterior pregnancies there is invasion of the pregnant uterus and allantochorion; abortion rarely recurs, but uterine and mammary infection recurs (Pappas et al., 2005).

Brucellosis as a widespread zoonosis disease is an important public health problem in many countries around the world, especially those in the Middle East (Araj et al., 2005).

In Iran, traditional food habits such as consumption of raw milk and used it for production of fine cheese, ice-cream and butter, is particularly common in the counties of Khuzestan province. Slaughterhouse workers and others involved in animal handling are at a higher risk of direct inoculation by skin abrasion, mucous membranes and inhalations (Al-Majali et al., 2009; Gwida, et al., 2010; Heydari et al., 2008; Nikokar et al., 2011) .

The calves born from infected non-vaccinated cows remain as carriers. Since the reproductive performance of these carrier animals is unaffected, they are retained in herds in Iran despite the presence of pathognomonic clinical signs in these cases, making the control of brucellosis very difficult.

In this study, we aimed at determining the seroprevalence of this infectious zoonotic disease in eighteen districts of Khuzestan province among the cattle and sheep/goats through Rose Bengal Plate Test [RBPT], Serum Agglutination Tests [SAT] and 2-mercaptoethanol SAT [2-ME SAT] kept at various government and private farms. RBPT is standardized, simple to perform, inexpensive and suitable to for screening individual animals, false negative reactions occur rarely, mostly due to prozoning with this test. Antibody resulting from *B. abortus* S19 and *B.melitensis* Rev.1 vaccination and some cross reacting antibodies are detected by these tests and it is necessary to use other test(s) to confirm reactor animals as infected (Alton et al., 1975; Garin-Bastuji et al., 1999; Moyer et al., 1987; Nielsen, 2002; Sareyyüpoğlu et al., 2010).

2-ME has sensitivity of 89.6% and specificity of 93.1-99.8%; RBPT has a

sensitivity of 89-93%; SAT has sensitivity of 93.9% and specificity of 100% (Baum et al., 1995; Blasco et al., 1994; Dohoo et al., 1986; Poester et al., 2010; Reviriego et al., 2000; Sareyyüpoğlu et al., 2010).

Materials and Methods

Study Population

Samples collected during each of four seasons from January 2008 until December 2012 using a random sampling approach within districts and regions to establish estimates of livestock brucellosis. A total of 206818 serum samples were collected from the Khuzestan province; 87798 from cattle and 119020 from sheep.

Sample Collection

All blood samples were collected from the jugular vein, using individual needles and sterile plain vacuum tubes [Vacutainer®], which were immediately placed into an ice bath and transported to laboratory. The samples were centrifuged at 3.000 rpm for 15 minutes and the serum was removed and stored at -20°C until the laboratory analysis was performed. Additional information regarding the gender, disease history, age, pregnancy [determined by rectal palpation] and reproductive problems such as abnormal uterine discharge, abortion of the animals was also recorded for a subset of the sampled animals.

Sample Analysis

The RBPT (VLA, Weybridge, Uk) was done on all samples in accordance with the manufacturer`s instructions. All samples testing positive or which were inconclusive using the RBPT were further subjected to 2ME-SAT (Alton, et al., 1975; Brown, et al., 1981). In the RBPT any degree of agglutination was considered to be positive. For the SAT, visible agglutination at the dilution of 1/100 was considered to be positive and for the 2ME, visible agglutination at the dilution of 1/25 was considered to be positive.

Statistical analysis

The analysis was performed using SPSS version 21 for Windows. Chi-square and Fisher exact tests were used to compare categorical variables. P value less than 0.05 was considered as statistically significant. Categorical variables were shown by number and percentage.

Results

The prevalence of *Brucella* in cattle and sheep is summarised in Table 1, 2 and 3. The alteration of brucellosis prevalence in cattle and sheep over time is shown in Table 2. The overall seroprevalence of brucellosis in sheep located in the Shoosh, Shushtar, Ramhormoz and Dezful counties was significantly higher than that reported for other counties ($P<0.05$) (Table 3). There were no significant differences in overall prevalence by year in this study. We noticed that overall seropositivity was higher among females than males ($P<0.05$), which has been identified as a risk factor for the consumption of raw milk. Statistically, the difference in the results amongst RBPT and 2ME-SAT was found to be insignificant ($P>0.05$) (Table 1).

Discussion

Brucellosis is a worldwide disease, particularly prevalent in the Near East, the Middle East, Turkey, Iraq, and Iran. Several reports have previously indicated that brucellosis is on the increase in Iran and other developing countries (Mai et al., 2012; OIE, 2008). Underreporting brucellosis is a problem in the Near East and Middle East regions (Gargouri et al., 2009).

The high prevalence of *Brucella* seropositivity in sheep in the Shoosh, Shushtar, Ramhormoz, and Dezful regions of Iran needs to be controlled in order to curtail the spread of brucellosis in this geographical area. The increased prevalence of brucellosis in such regions may be attributable to poor husbandry methods. Previous research has shown that controlling this disease in sheep (mainly by Rev-1 vaccination) can be effective in reducing infection in cattle (Ahmed and Munir, 1995; Ahmed et al., 2010; Banai, 2002; Samaha et al., 2008).

Table 1. Prevalence of *Brucella* in domestic animal species based on RBPT, 2ME-SAT, Khuzestan province, January 2008-December 2012.

Tablo 1. Ocak 2008-Aralık 2012 tarihleri arasında, Khuzestan ilinde RBPT ve 2ME-SAT ile evcil hayvan türlerinde görülen bruselloz prevalansı.

Animal Species	Animals Tested	Proportion of Positive Animals [2ME-SAT]	Proportion of Positive Animals [RBPT]
Cattle	87798	(633) 0.72%	(790) 0.9%
Sheep	119020	(3639) 3.01%	(4285) 3.6%
Total	**206818**	**(4272) 2.07%**	**(9307) 4.5%**

Lack of control measures in most parts of this province may be contributed to this increase. Intermixing of animals, sharing of pasture lands and common trading at local stock yards may be a contributing risk factor to the disease status.

In present study overall the seroprevalence of brucellosis in cattle and sheep is 0.72% and 3.01% respectively. Similar finding were reported by Shimi (1998) and Zowghi, et al. (1990) giving 0.6 and 0.85% in cattle, respectively, and which carried out in Iran. This finding also was supported by Maadi (2011) giving 1.18% prevalence in cattle.

Table 2. Prevalence of brucellosis in cattle and sheep based on 2ME-SAT, Khuzestan province, during the January 2008-December 2012.

Tablo 2. Ocak 2008-Aralık 2012 tarihleri arasında, yıllara göre Khuzestan ilinde 2ME-SAT ile sığır ve koyunlarda görülen bruselloz prevalansı.

Year	No. Cattle	No. Seropositive cattle	Proportion of positive cattle	No. Sheep	No. Seropositive Sheep	Proportion of Positive Sheep
2008	22215	116	0.52%	37091	838	2.3%
2009	23325	138	0.6%	33084	1051	3.2%
2010	19275	180	1%	18365	641	3.5%
2011	12442	114	1%	15770	715	4.5%
2012	10541	85	1%	14710	394	3%

Brucellosis has been reported in numerous countries throughout the world. This study revealed that the prevalence of bovine and sheep brucellosis in Khuzestan province was (in your study individual prevelances were detected, whereas most of the work you cited in here were based on herd prevelance rates and that is why you are having lower rates. For this reason you must not say that your prevelance rate much lower than those of others). In Syria (Darwesh and Benkirane, 2001), Bangladesh [cattle, 2.66%] (Amin et al., 2005; Rahman et al., 2011), Israel (Refai 2002), India [cattle, sheep and goats, 6.37%, 3.42% and 5.53% respectively] (Sharma et al., 1979), Jordan (Al-Majali et al., 2009), Sri Lanka [cattle, 4.7%] (Silva et al., 2000), Pakistan [cattle 3%] (Ahmed and Munir 1995; Shafee et al., 2011), Libya (Ahmed et al., 2010), Afghanistan (Ajmal et al., 1989), Egypt (Refai, 2002), Saudi Arabia (Memish, 2001), Iraq (Shareef, 2006), Plateau state in Nigeria [sheep and goats, 14.5% and 16.1% respectively] (Berto, et al., 2010), Kars district of Turkey [bovine brucellosis, 34.64%] (Otlu et al., 2007), Ethiopia [bovine brucellosis, 4.9%] (Mekonnen et al., 2010) and Zambia (Muma et al., 2006).

Table 3. Prevalence of brucellosis in cattle and sheep based on 2ME-SAT, Khuzestan province, during the January 2008-December 2012.

Tablo 3. Ocak 2008-Kasım 2012 tarihleri arasında, köylere göre Khuzestan ilinde 2ME-SAT ile sığır ve koyunlarda görülen bruselloz prevalansı.

County	No. Cattle	No. Seropositive Cattle	Proportion of Positive Cattle	No. Sheep	No. Seropositive Sheep	Proportion of Positive Sheep
Abadan	3257	15	0.5%	5300	20	0.4%
Ahvaz	10537	96	1%	14453	503	3.5%
Andimeshk	3277	40	1.2%	9097	297	3.3%
Baghmalek	5112	19	0.4%	7791	109	1.4%
Behbahan	5470	41	1%	6038	99	1.6%
Dashte Azadegan	7783	23	0.3%	8711	149	1.7%
Dezful	4657	77	1.7%	8199	409	5%
Haftgel	2527	14	0.55%	2043	29	1.4%
Hendijan	595	9	1.5%	1640	25	1.5%
Izeh	6959	22	0.32%	10623	215	2%
Khorramshahr	956	13	1.36%	1835	22	1.2%
Mahshahr	2602	14	0.54%	4577	118	2.6%
Masjed Soleyman	5003	60	1.2%	8827	269	3%
Omidieh	3424	26	0.8%	4348	49	1.1%
Ramhormoz	7310	53	0.7%	7090	350	5%
Shadegan	5951	15	0.25%	3364	45	1.3%
Shoosh	3700	36	1%	5550	382	6.9%
Shushtar	8678	50	0.6%	9534	549	5.8%

The results of the three serodiagnostic tests used in the present study indicated that RBPT detected a higher percentage of seropositive animals compared to 2ME-SAT; however, this difference was not significant ($P>0.05$). According to Flad (1983), Blasco (1994), Dohoo et al. (1986), Poester et al. (2010), and Khan and Khan (2009), RBPT is a rapid, simple and sensitive method.

This study demonstrates that the prevalence of brucellosis in this region of the country is relatively low. It was assumed that effective vaccination strategies have significantly controlled the widespread of brucellosis in Khuzestan province. However, additional research is required in order to implement a transparency policy and effective strategy to eradicate brucellosis.

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