

Investigation of *Brucella* seroprevalence in human and livestock in Igdir, Turkey

Gülhan Bora^{1*}, Yasemin Akkoyunlu², Mehmet Berköz³, Güneş Açıkgöz⁴, Mustafa Berктаş⁵, Fikret Altındağ⁶, Aydoğan Bora¹

¹Yüzüncü Yıl University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Van, Turkey

²Bezmialem Vakıf University, Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, İstanbul, Turkey

³Yüzüncü Yıl University, Faculty of Pharmacy, Department of Pharmaceutical Biotechnology, Van, Turkey

⁴Mustafa Kemal University, Vocational School of Health Services, Hatay, Turkey

⁵Yüzüncü Yıl University, Faculty of Medicine, Department of Microbiology, Van, Turkey

⁶Igdir University, Medical Technology Vocational Training School, Department of Clinical Microbiology, Igdir, Turkey

ABSTRACT

Brucellosis is a zoonotic disease of worldwide and still remains endemic in some developing countries, especially in Turkey. Milk is the main food product serving as a vector for *Brucella*. In this study we aimed to investigate the seroprevalence of human and animal brucellosis. The study group consisted of 358 subjects who had high risk occupations of animal breeder and farmer. The test results were interpreted and titers equal or in excess of 1/40 were considered significant. 189 farm animals were incorporated to our research. Among these animals, 104 of them were sheep and goat and 85 of them were cattle. The Brucellosis Milk Ring Test was used to test raw milk samples collected from the selected herds in the study area. The seroprevalence of human brucellosis was 6.7%. Seroprevalence of animal brucellosis was found as 22.75% in livestock. This study is important for being the first study in recent years that demonstrates the *Brucella* seropositivity rates in Igdir Province. This high seropositivity in our region may depend on the high rates of animal brucellosis; those of which were inadequate under control by veterinary and by vaccination and people who consume the milk by without boiling.

Key Words: Brucellosis, seroprevalence, vaccination, livestock

Introduction

Brucellosis is a zoonotic disease with a global distribution caused by bacteria of the genus *Brucella*. Major efforts have been undertaken around the world to control brucellosis due to the significant economic losses associated with the occurrence of the disease in domestic livestock and the often debilitating disease associated with human infection (1). Clinical signs of brucellosis in domestic livestock include late-term abortions, decreased milk production and lowered fertility in both males and females. The disease in humans is associated with a number of nonspecific complaints including irregular or intermittent fevers, night sweats and headaches. Untreated brucellosis in humans can lead to chronic osteo-articular complications manifesting as joint pain, joint effusion and debilitating arthritis (2). Particular biovars of *Brucella melitensis* are associated with pathogenicity in different

domestic animals. *B. abortus* is the biovar most commonly associated with brucellosis disease in cattle (3).

Brucellosis in humans is considered as a food borne disease or a disease related to occupational exposures (4). The routes of infection for humans are similar to those for animals: ingestion, inhalation, or through direct contact of the organism with a break in the skin. The key feature of brucellosis as a zoonosis is that it is a pure zoonosis: a disease transferred only from animals to people. Human-to-human transmission has occurred, but is exceedingly rare (5). The *Brucella* spp. and their biovars have different zoonotic potential. *B. ovis* and *B. suis* biovars two, four and five have essentially no zoonotic potential. *B. canis*, *B. abortus* biovar five and *B. neotomae* have very low zoonotic risk, but all other biovars of *B. melitensis*, *B. abortus* and *B. suis* can cause illness in humans (5, 6).

*Corresponding Author: Dr. Gülhan Bora, Yüzüncü Yıl University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Van, TURKEY, Tel: +90 (432) 225 10 24, Fax: +90 (432) 216 75 19, E-mail: gulhanarvas@yahoo.com-gunesani@hotmail.com

Food borne illness is contracted through the consumption of raw milk or raw milk dairy products. Meat products are not considered high risk and the actual risk is likely negligible. The one exception is with pork meat products (7,8). Bacteremia in swine results in disseminated infection rather than an infection localized to the reticulo-endothelial system and reproductive tract; as a result there can be a substantial number of bacteria in the muscle tissue. In addition, the consumption of organ meats poses a risk. The control of animal brucellosis is very important for the prevention of human infection (9,10). Also important in the prevention of human infection is the use of pasteurized milk and milk products in developing countries (9, 11).

Brucellosis is still endemic in many regions of the world, although it has been successfully eradicated in some countries (12,13). The infection threatens both domesticated animal and human health and causes considerable economic losses. It is especially widespread in South Europe, North Africa, Middle East and Near East (12-14).

In the Near East region countries, social and economic factors play a major role in the spread of brucellosis (15,16). Turkey is considered to be endemic for brucellosis, although little information is available; previous studies are limited to food-producing animals such as cattle and ewes and reports of human brucellosis in Turkey are limited to a few cases (17).

Turkey occupies a unique geographical, cultural and economic position at the crossroads between Europe and Asia. It is surrounded by the Black Sea in the north, the Mediterranean Sea in the south, and the Aegean Sea in the west. It shares land boundaries with Greece and Bulgaria in the northwest, Georgia, Armenia and Nakhchivan in the northeast, Iran in the east, and Iraq and Syria in the southeast (18).

The geographic situation of Turkey is always a risk factor for the dissemination of contagious diseases, mainly from the eastern and southeastern neighbors (17). Iğdır province is one of the most important city in this region and located in a strategic area that has border to three countries the border of three country (Nakhichevan, Armenia and Iran) (Figure 1).

In this study we aimed to investigate the seroprevalence of human and animal brucellosis in Iğdır province, North-east Anatolia. Since there is scarce information on the epidemiology of brucellosis in Iğdır province, particularly in those whose job put them at risk of contracting the disease, this study was conducted to determine the risk of exposure to Brucella in people who have direct contact with livestock as a consequence of their job (19). We also tried to elucidate the correlation between consumption of dairy products and seropositivity for Brucella.

Material and methods

This study was conducted in Iğdır province, districts and neighboring districts of Iğdır between June 2012 and October 2012. Iğdır and the surrounding regions were divided into the certain regions, as Iğdir Center, Aralık, Tuzluca, Doğubeyazıt, Karakoyunlu and Kağızman. The study group consisted of 358 subjects who had high risk occupations of animal breeder and farmer, living in the randomly selected villages from these regions. The ethical approval was taken from the local committee. The study group consisted of 78 male (21.79%) and 280 female (78.21%), with a median age 48.67 ± 19.09 years, ranging between 33-71 years.

Researchers in this study interviewed the subjects using a standard form. Questions included breeder name and surname, date, education, age, gender



Fig. 1. Map of Turkey and geographical location of Iğdır Province.

and number of cattle, sheep and/or goat and their races.

Prevalence of brucella-specific antibodies was investigated in sera of the subject. Firstly, blood samples collected from all subjects were centrifuged at 1200 *g* for 5 minutes at +4°C. Serum samples were harvested and kept at -20°C until used. Serum tube agglutination test including Coombs reactive was performed with commercial Brucella antigen according to the manufacturer's instructions (20,21). The test results were interpreted and titers equal or in excess of 1/40 were considered significant.

In the second part of this study, we collected milk samples from the farm animals that were bred by the farmers for reveal of brucellosis. For this purpose, 189 farm animals were incorporated to our research. Animals were separated as dairy cattle, sheep and goat and milk analysis was performed from the herds. Among these animals, 104 of them were sheep and goat and 85 of them were cattle. Milk analysis of sheep and goats were performed with the aid of selecting in 1/10 ratio from the single race animal herds and in 1/5 ratio from the different race animal herds by the method of randomize sampling. In the same way, milk analysis of cattles were performed with the aid of selecting in 1/5 ratio from the domestic and culture race animal herds.

Milk samples were collected soon after the morning or evening milking had been completed. Individual cattle were routinely milked into buckets that were then emptied into aluminum milk cans or an equivalent small bulk storage container. The milk samples were tested for the presence of Brucella antibodies using standard *B. abortus* strain 1119-3 Brucellosis Milk Ring Test antigen provided by the Pendik Veterinary Control Institute, Istanbul, Turkey.

One ml of whole milk from each sample was transferred to a narrow, sterile plastic test tube.

BRT antigen (0.03 ml) was added to each of the samples and mixed well. The milk samples were then incubated for 1 hour at 37°C in a portable incubator powered by a 12-volt car battery. Concentration of dyed antigen in the cream layer, forming a dark blue ring, indicated a positive reaction. Results were read at the conclusion of incubation and recorded.

Data were coded and analyzed by SPSS for Windows. Categorical variables were analyzed by χ^2 or Fisher exact test when appropriate. *p* value <0.05 was considered statistically significant.

Results

Totally 358 individual who had high risk occupations of animal breeder and farmer in Iğdir province and its surrounding regions were included to our study. This study was arranged in Iğdir Center, Aralık, Tuzluca, Doğubeyazıt, Karakoyunlu and Kağızman and from these regions, 36, 55, 45, 118, 18 and 86 subjects were admitted to our study, respectively.

The seroprevalence of Brucella specific antibodies were based on titers of 1/40 by Serum tube agglutination test including Coombs reactive. The seroprevalence of human brucellosis was 6.7% in our study (positive in 24 subjects). Samples of 334 subjects (93.3%) resulted negative in the agglutination test. Seroprevalence of Brucella antibodies were listed in Table 1 according to the subjects' location and antibody titers.

Seroprevalence of Brucellosis was found as 11.5% in male subjects (9 of 78 individuals) and 5.4% in female subjects (15 of 280 individuals). According to this data, there were no significant differences between the genders (*p*>0.05). Seroprevalence of Brucella antibodies were listed in Table 2 according to the subjects' gender.

Table 1. Seroprevalence of Brucella antibodies according to the subjects' location and antibody titers

	1/40 (n)	1/80 (n)	1/160 (n)	1/320 (n)	1/640 (n)	Total (n)
Aralık	-	1	1	-	-	2
Tuzluca	-	-	-	-	-	-
Doğubeyazıt	5	4	4	4	1	18
Karakoyunlu	-	-	-	-	-	-
Kağızman	-	1	-	-	-	1
Iğdir Center	1	2	-	-	-	3
Total	6	8	5	4	1	24

(n= number of individuals)

Table 2. Seroprevalence of Brucella antibodies according to the subjects' gender

	Male (n) (%)	Female (n) (%)	Total (n) (%)	p value
Positive	9 (11.5 %)	15 (5.4 %)	24 (6.7%)	p= 0.054
Negative	69 (88.5%)	265 (94.6%)	334 (93.3%)	
Total	78 (100%)	280 (100%)	358 (100%)	

(n= number of individuals)

Totally 189 livestock in Iğdır province and its surrounding regions were included to our study. Seroprevalence of Brucellosis was found as 22.75% in livestock (43 of 189 animals). Seroprevalence of Brucella antibodies according to the livestock's location and antibody titers was shown in Table 3.

Seroprevalence of Brucellosis was found as 26.92% in sheep and goat and 17.65% in cattle. Seroprevalence of Brucella antibodies according to the livestock's species and location was shown in Table 4. Seroprevalence of Brucellosis in sheep and goats was 1.53 fold higher than the cattle. A significantly higher seroprevalence of brucellosis among sheep and goats compared to the cattle was found ($p < 0.05$).

Discussion

The geographic position of Turkey is always a risk factor for the dissemination of infectious diseases, especially from the eastern and southeastern neighbors. Brucellosis is endemic in all of the countries around Turkey, especially in Iran, Iraq and Syria (22). In Iran, the prevalence of brucellosis among sheep and goats was 10.2% and among cattle were 17.5% in the 1990s. In Iran, also, human brucellosis is a serious public health problem, with almost 240 cases per million (23). In Iraq, the seroprevalence among sheep and goats was 15% and among investigated cattle was 3%, with approximately 280 human cases per million. Syria has the world's highest reported incidence of human brucellosis, with over than 1600 cases per million (24).

In Turkey, the reported incidence was over 18 000 cases in 2004, although, incidence of brucellosis was only 37 cases in 1970s (25). Probably, this significant increase reflects improvements in diagnosis, healthcare, and reporting, not an increase in the real incidence of the disease. When the Turkish literature was reviewed between 1915 and 1963 years, seropositivity of brucellosis was found as 9%, approximately (26). In 1937, Celik found brucellosis in 2.6% (27). In 1943, Golem found brucellosis in 5.9% (28). In 1957, Akyay

and Gursel found brucellosis in 4.3% in Eskisehir Province in Central Turkey (29). In 1990, Cetin et al. (30) found active brucellosis in 1.8% in different major cities throughout Turkey. The highest level of seroprevalence observed among healthy individuals was 3.6% in Diyarbakır, which is in eastern Turkey (30).

Direct contact with livestock is a well-documented source of infection. Infection may occur through cuts and abrasions on the skin, via the conjunctiva and by inhalation. These routes of infection are important for farmers, veterinarians, and butchers, who are all at increased risk of infection through

Table 3. Seroprevalence of Brucella antibodies according to the livestock's location and antibody titers

	+	++	+++	Total
	(n)	(n)	(n)	(n)
Aralık	6	2	4	12
Tuzluca	3	2	1	6
Doğubeyazıt	8	3	0	11
Karakoyunlu	0	0	0	0
Kağızman	5	3	4	12
Iğdır Center	2	0	0	2
Total	24	10	9	43

(n= number of livestock)

Table 4. Seroprevalence of Brucella antibodies according to the livestock's species and location

	Sheep and goat (n)	Cattle (n)	Total (n)	p value
Aralık	10	2	12	p=0.032
Tuzluca	4	2	6	
Doğubeyazıt	11	0	11	
Karakoyunlu	0	0	0	
Kağızman	3	9	12	
Iğdır Center	0	2	2	
Total	28	15	43	

(n= number of livestock)

their contact with animals and animal products. A different seroprevalence study was carried out on occupational risk groups consisting of veterinarians and veterinary assistants, slaughterhouse workers, and controls in Kocaeli, a city in northwest Turkey and the researchers found brucellosis seroprevalence of 4.8% in the risk groups (17). In our study, we found seroprevalence of brucellosis as 6.7% in whole study group. The seroprevalence in communities in the provinces of northeast Turkey was lower than in the provinces in the other regions, and we attributed this to the vaccination of livestock in the year preceding the survey.

As livestock herders, all of the household members had regular and repeated contact with livestock. Potentially high-risk interactions with livestock included home slaughter of multiple livestock species and hand milking of cattle, sheep and goats (8,9). Generally adults were involved in livestock slaughter with men involved in the killing, skinning and deboning of the animals and women in the cleaning and preparing the gastrointestinal tract and internal organs. Both adults and children were observed milking cattle, sheep and goats (11,13). In our study, seroprevalence of Brucellosis was found as 11.5% in male subjects and 5.4% in female subjects. There were no significant differences between the genders. The questionnaire did not include specific information on human exposure to livestock placentas, uterine fluid or aborted material.

Animal brucellosis was a serious problem in both state and private farms. A serological study mainly on sheep and goats in state farms was conducted and showed 2.6% reactors. The clear relationship between infected animals and humans was shown in a report in 1957; a herd in Eskisehir suffering acute brucellosis showed 11% reactors, with 23% of workers testing positive (29). In our current study, seroprevalence of Brucellosis was found as 26.92% in sheep and goat and 17.65% in cattle.

Seroprevalence of Brucellosis in sheep and goats was 1.53 fold higher than the cattles. There was a significantly higher seroprevalence of brucellosis among sheep and goats compared to the cattles ($p < 0.05$). Of greater concern in this population was the high percent of interviewees who reported consuming raw cow's milk and preparing dairy products with raw milk. It is probable that the habits of the entire household would reflect the actions of the head of household in terms of raw

milk consumption and that all members of the household would consume dairy products produced from the raw milk (8,10). Although milk is routinely boiled immediately after collection these responses indicate that there is the potential for a significant level of human brucellosis exposure through the consumption of raw milk and dairy products in this population (11,12).

Although brucellosis has been eradicated from several developed countries, it still continues to be a major public health problem in many regions of the world. There are many reasons why brucellosis remains endemic in Turkey (17,19). Uncontrolled movements of livestock herds and flocks, geopolitical situation with endemic brucellosis in surrounding countries and political instability are main reasons of endemic brucellosis in Turkey. Inadequate veterinary support services and husbandry practices trigger the spread of infection in Turkey (18, 19, 22). Traditional use of raw milk products and following close contact with infected animals occur permanence of human cases (8, 17, 18). A distinct reduction in the levels of animal and human brucellosis had observed after the initiation of the national brucellosis control and eradication plan in 1984 and the new plan that was initiated in 2009 will continue to make progress (17).

The prevalence of brucellosis-seropositive cattle herds in Iğdır, Turkey, is undoubtedly associated with livestock production losses and probably associated with the occurrence of clinical signs and symptoms suggestive of brucellosis reported by the humans in the region (19). Although this study represents data from only a small portion of Turkey's livestock population there is no reason to believe that the occurrence of brucellosis in cattle is restricted to this study region. In any disease eradication and control program, strategies have to be made on the basis of the information available (17). We thought that, in national brucellosis control and eradication program, decisions have to be made on the basis of the information available. Therefore, this study may lead the new strategies for the national brucellosis control and eradication program in Iğdır Province and surrounding regions.

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