Epidemiology of Bovine Brucellosis in Dairy Cattle in Northern Iran

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ABSTRACT

Brucellosis is a bacterial infectious disease in animals and humans and is caused by Brucella, spp. In this survey, the Brucella infection rate was studied in dairy cattle of industrial and non-industrial herds in Mazandaran province. One thousand one hundred and fifty-two milk specimens were randomly collected (384 samples per county and per month of study, According to Morgan table) from cows in the area. It must be indicated that 576 samples of milk samples were taken from dairy cows on industrial farms and 576 samples of them from dairy cows of traditional herds. The specimens were collected from 3 regions in this province. The milk ring test was done on milk samples. The results showed that Brucella infection in the province was 2.77% for industrial farms, 9.02% for non-industrial herds and 5.9% for total cows of the province (68 Reactor of 1152 samples). The results of months of the study did not show significant differences in the rate of disease, on traditional cattle and industrial cattle. Also significant difference in incidence was not observed in different geographic areas. But the increase in the prevalence of Brucellosis in traditional cattle than cows in the industrial farms was significant (P <0.05).

Keywords: Brucellosis, Cattle, Brucella abortus, Epidemiology, Mazandaran, Iran.

1. INTRODUCTION

Brucellosis is a zoonotic disease caused by Brucella bacteria, which can infect many species of animals, including humans [1-9]. The disease occurs throughout the world, except in countries where brucellosis has been eradicated [1]. Brucellae is a Gram-negative, facultative intracellular [2,10-12], small, non-motile, aerobic [11], immobile cocobacilli [10] and non-spore Bacterium [13], that major cause of economic losses around the world due to infection of livestock [2,4,14,15]. Ten species within the genus Brucella is known [11,12,16], that six of which are considered the classic species, *Brucella abortus* (cattle) (seven biovars), *B. melitensis* (goats) (three biovars), *B. suis* (pigs) (five biovars), *B. canis* (dogs), *B. ovis* (rams), and *B. neotomae* (desert rats). Four new species of marine mammals have been isolated, including *B. ceti* (marine mammals), *B. pinnipedialis* (marine mammals), *B. microti* (common vole), and *B. inopinata* [11,17,18], which *B. abortus*, *B. melitensis*, *B. suis*, and *B. canis* are known to occur in humans [16]. The disease affects in the reproductive system of animals, resulting in substantial productivity losses, such as decreased milk production, abortion, weak newborns, weight loss, cull and condemnation of infected animals because of infertility, lameness and an obstacle to trade and export [15,19-21]. Death can occur as a consequence of acute metritis, followed by preserving fetal membranes [21]. The sources of infection for animals such as aborted materials, vaginal discharges, milk and semen of infected animals [19]. The disease diffused via food and grass contaminated by bacteria, aerosol, broken skin, and mucus membrane contact with the contaminated environment, aborted tissue, fetal fluids, fetal placenta [1]. The occurrence of the disease in humans is thus closely tied to the prevalence of the infection in sheep, goats and cattle and methods that allow exposure of humans to potentially infected animals or their products [8]. Transmission to humans can occur through direct contact with infected animals, infectious animals' tissues, inhalation of aerosolized droplets and the use of infected unpasteurized milk and dairy products [3,6,9,11,14,22-24]. Brucellosis in humans is a primary reproductive disease clinically characterized by abortion in the last trimester and retained placenta in females, whiles orchitis and epididymitis with frequent sterility in males [4.5,8,21]. The most common clinical features of Brucellosis in humans are undulant fever, sweating, arthralgia, myalgias, lymphadenopathy and hepatosplenomegaly [22]. Bovine brucellosis, which is mainly caused by *Brucella abortus*, is predominantly detected in pregnant females, which abort may develop lifelong infection [3]. According to data from the World Health Organization, 500,000 new cases are recorded each year. This is a primary disease of animals, through direct contact with infected animals or consumption of contaminated animal products expands

[7,10,14,16,23,25,26]. Brucellosis is primarily a disease of dairy cattle that causes economic damage to the livelihoods of many farmers around the world [4,5,13,27,28]. This is one of the main bacterial infectious diseases, effects on domestic animals in many developing countries [4]. Many countries have taken steps to control brucellosis disease, but infections in animals still exist in some areas as a result may be transmitted to humans [11]. The disease remains as an uncontrolled problem in high endemic regions such as Africa, the Mediterranean region, Middle East, parts of Asia and Latin America [4], as well as from India and Mexico have been reported [12,24]. In addition, Brucellosis is a cause of major economic losses worldwide; for instance, "it is estimated to lay a burden of 600 million dollars on Latin America's economy" [16]. Also in Iran, Brucellosis is a zoonotic disease and is endemic in many parts of Iran [17]. The results of epidemiologic studies in Iran have reported that B. abortus biovar 3 and B. melitensis biovar 1 are the dominant biovars [11]. For example, in Khoy city, nearby the Urmia, in West of eastern Azerbayjan province, northwestern Iran, prevalence of brucellosis has been reported 26.66% (positive MRT) in spring, 2008 [17]. Although several measures have been to prevent and control the disease in Iran, but the disease continues to cause enormous economic losses, especially in cattle and small ruminants and cause a serious public health problem in Iran. The objective of the present study was to estimate the epidemiology of brucellosis in cattle in Mazandaran province, Iran, to identify strategies for control and eradication of the disease in the country.

2. MATERIAL and METHODS

Three adjacent counties of Mazandaran province were included in the study; Sari, Babol and Noor. In this survey, the Brucella infection rate was studied in dairy cattle of industrial and non-industrial herds in Mazandaran province, north of Iran. One thousand one hundred and fifty-two milk specimens were randomly collected (384 samples per county and per month of study, According to Morgan table) from whole 400,000 heads of cow in the area. It must be indicated that 576 samples of milk samples were taken from dairy cows on industrial farms and 576 samples of them from dairy cows of traditional herds. The specimens were collected from 3 regions in this province in three months from April to June 2018 and each month 192 samples from industrial farms and 192 samples from non-industrial farms. The milk ring test was done on milk samples. The milk ring test is the most practical method for the detection of contaminated dairy animals and for surveillance of brucellosis-free herds. The test was performed by adding 30 µl (0.03 ml) of B. abortus Bang Ring Antigen (hematoxylin-stained antigen manufactured by the State Biological Laboratory, Institute of Veterinary Preventive Medicine, Ranipet, India). The height of the milk column in the tube was kept up to 25 mm. The milk (antigen) mixtures were incubated at 37°C for 1 h, together with positive and negative control samples. Agglutinated Brucella cells were picked up by fat globules as they rose, forming a dark cream layer on the top of the sample. A strongly positive reaction was indicated by formation of a dark blue ring above a white milk column. The test was considered negative if the color of the underlying milk exceeded that of the cream layer and when the cream layer was normal. Samples were read as negative, 1+, 2+, 3+ and 4+ depending on the intensity of color in the cream layer. The criteria of reactions are given below:

Negative reaction (-): cream ring white, skim milk fraction blue white;
Suspicious reaction (1+): cream ring pale pink, but less colored than the skim milk fraction;
Suspicious reaction (2+): the pink color of the cream ring equal to that of the skim milk fraction;
Positive reaction (3+): color of cream ring deeper pink than that of the skim milk fraction;
Positive reaction (4+): cream ring pink, skim milk fraction white.

Chi-squared test ($\chi 2$) was used to compare the prevalence among the three counties, and the prevalence in dairy cattle of industrial and non-industrial herds in each county. The differences were considered statistically significant when probability P-value was <0.05. The 95% confidence intervals (95% CI) of prevalence rates were calculated. Statistical analysis was performed using IBM® SPSS software version 17.

3. RESULTS

The results showed that Brucella infection in the province was 2.77% for industrial farms, 9.02% for non-industrial herds and 5.9% for total cows of the province (68 Reactor of 1152 samples) ($P<0.05$). Total Prevalence of Bovine Brucellosis in dairy cattle in Mazandaran province in April, May and June was 6.77%, 5.20% and 5.73%, respectively (Table 2). Prevalence in non-industrial herd in April, May and June was 9.89%, 8.33% and 8.85%, respectively. Also the rate of Prevalence in industrial cattle in April, May and June was 3.64%, 2.08% and 2.60%, respectively (Table 2). The total prevalence in Sari, Babol and Noor was 4.94%, 5.73% and 7.03%, respectively (Table 1). The highest prevalence was observed in April. Geographically, the highest prevalence in the Noor city with a humid subtropical climate was observed ($P <0.05$).

***Table 1**: Prevalence of Bovine Brucellosis in dairy cattle of industrial and non-industrial herds in three adjacent counties of Mazandaran province, north of Iran*

County	No. of dairy cattle tested	No. of dairy cattle non-industrial	No. of dairy cattle industrial	No. of Positive non-industrial (Prevalence %)	No. of Positive industrial (Prevalence %)	Total (Prevalence %)
Sari	384	192	192	15 (7.81)	4 (2.08)	19 (4.94)
Babol	384	192	192	17 (8.85)	5 (2.60)	22 (5.73)
Noor	384	192	192	20 (10.41)	7 (3.64)	27 (7.03)
Total	1152	576	576	52 (9.02)	16 (2.77)	68 (5.9)

***Table 2:** Prevalence of Bovine Brucellosis in dairy cattle of industrial and non-industrial herds by Month of Study in Mazandaran province, north of Iran*

Month	No. of dairy cattle tested	No. of dairy cattle non-industrial	No. of dairy cattle industrial	Total Prevalence (%)	No. of Positive non-industrial	No. of Positive industrial	non-industrial Prevalence %	Industrial Prevalence %
April	384	192	192	26 (6.77)	19	7	9.89%	3.64%
May	384	192	192	20 (5.20)	16	4	8.33%	2.08%
June	384	192	192	22 (5.73)	17	5	8.85%	2.60%

4. DISCUSSION

The results of months of the study did not show significant differences in the rate of disease, on traditional cattle and industrial cattle. Also significant difference in incidence was not observed in different geographic areas. But the increase in the prevalence of Brucellosis in traditional cattle than cows in the industrial farms was significant ($P < 0.05$).

Many studies have been conducted about the incidence of Brucellosis in different parts of Iran. In a Study by *Gharib Mombeni et al. 2012* among livestock in the 18 districts of Khuzestan Province in Southwest Iran, the seroprevalence derived from total samples was 0.72% for cattle and 3.01% for sheep, and that infection among sheep was significantly higher than cattle [20]. *Maadi et al, 2011* in a same Survey was done for Prevalence of Brucellosis in Cattle in Urmia, Iran by MRT, 1.22% showed positive in spring and 1.17% showed positive in autumn [17]. In a survey by *Adamu et al, 2016* in Nigeria was done, from a total of 336 cattle screened by RBPT and SAT, 18 (5.4%) and 13 (3.9%) were seropositive to Brucella infection in cattle respectively [1]. *Assenga et al. 2015* in a Study of the prevalence of Brucellosis in Tanzania indicated in humans 0.6%, cattle 6.8%, goats 1.6% and buffaloes 7.9% [19]. *Mohammed et al, 2015* in Ethiopia reported that the prevalence of brucellosis in goats was 1.56% [8]. *Aznar et al, 2015* in a Study of Brucellosis in Argentina indicated, prevalence in Cow and herd prevalence were 1.8% (95% CI: 1.3–2.2; n = 157) and 19.7% (95% CI: 17.0–22.4; n = 89), respectively [3]. In a survey by *Bayemi et al, 2015* in Cameron was indicated, Overall prevalence of brucellosis was found to be 5.2%. There was strong evidence that cows in the extensive system (6.5%) had a higher infection rate than those in the semi intensive system (2%). Bovine overall brucellosis infection rates were higher in the dry season (67%) than the rainy season (33%). Healthier cattle (78%), older cattle (64%) and cows (75%) were more infected [4]. In an another Study by *Gwida et al, 2015* in Egypt reported, that the overall seroprevelance among the tested cows in animals showed reproductive disorders group was 52.2%. ELISA showed the highest number of positive reactors 67.9%, followed by FPA 59.11% and RBT 53.7%; while in cows were apparently healthy, the number of positive animals was 4.2%, 3.3% and 1.8% by using RBT, ELISA and FPA, respectively [25]. *Bertu et al, 2015* in Nigeria showed, four (33.3%) of the 12 milk samples from cattle tested positive by the Milk ring test [5]. In Bangladesh in 2011 by *Rahman et al,* the overall serological prevalence derived from the samples was 2.87% in buffaloes, 2.66% in cattle, 3.15% in goats, and 2.31% in sheep [15]. In Ghana, the MRT detected

B. abortus antibodies in 21.9% of the milk samples while the ELISA detected specific antibodies in 58.9% of the samples [14]. In Turkey in 2008, of the cattle sera analyzed, (32.92%) and (34.64%) were determined as positive by RBPT and SAT, respectively [29]. The occurrence of reactors in newly established cattle farms may be more than 30%, though, the highest rate (72.9%) of infection till now has been reported in the Palestinian Authority. The interesting thing is that the second highest prevalence (71.42%) of Brucellosis has been reported in mules from Egypt. Brucellosis in buffaloes has been reported from Egypt (10.0%) and Pakistan (5.05%). As respects cattle are found around the world, the prevalence of brucellosis (0.85 to 23.3%) in cattle has been reported from a wide range of countries [21]. In another Study by *Cadmus et al.* in southwestern Nigeria in 2008 Overall, 18.61%of the milk samples were positive, according to the MRT [30].

In the current study, we tried to provide more data on the prevalence of brucellosis in Iranian cattle and to identify the potential risk factors of Bovine brucellosis in Iran. We found that 5.9% of all the tested cows were Brucella positive. Lack of adequate control measures in most parts of the province may have contributed to this increase. Intermixing of animals, sharing of pasture lands and common trading at the local stockyards can be a contributing risk factor to the disease condition. In present study overall the prevalence of brucellosis in dairy cattle is 5.9%. More than finding were recorded 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 more than by *Maadi (2011)* giving 1.18% prevalence in cattle [20].

Compared with the findings of previous studies in Iran, we can say that the prevalence of brucellosis has decreased in recent years. This may be causes, vaccination, implementation of a test and slaughter program, and the movement toward industrial livestock production. The milk ring test is a simple method for the day to day monitoring of *B. abortus* in single cow herds. Conclusion by using this test method may not have certainty. Accordingly, it is proposed that other confirmatory tests, like the milk ELISA, are to be used in conjunction for organizing disease status. Nevertheless, it is obvious that the MRT is the first line of screening test for brucellosis exclusively in single cow herds. This Survey indicates that the prevalence of Brucellosis in this province of the country in industrial farms is relatively low. It was assumed that vaccination significantly extended the effective methods to control Brucellosis in Mazandaran province. However, more research in order to implement a policy of transparency and effective strategy for eliminating brucellosis in cattle, especially in rural areas and non-industrial herds requirements. Generally, matching to the results of this study, the prevalence and gradual increasing of the infection in Mazandaran province is not due to consumption of raw cow's milk and its products, and the prevalence of human Brucellosis infection in this region should be searched in other animals such as sheep and goats.

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