

Maryam Salmani Seraji (MSc) ¹
 Jamshid Yazdani Charati (PhD) ^{2*}
 Farhang Baba Mahmoudi (MD) ³
 Reza Ali Mohammadpour
 Tahamtan (PhD) ⁴
 Habib Vahedi (PhD) ⁵
 Jalil Shojaei (PhD) ⁶

1. Department of Biostatistics,
 Faculty of Health, Mazandaran
 University of Medical Sciences,
 Sari, Iran

2. Department of Biostatistics,
 Faculty of Health, Mazandaran
 University of Medical Sciences,
 Sari, Iran

3. Department of Infectious
 Diseases, Faculty of Medicine,
 Mazandaran University of Medical
 Sciences, Sari, Iran

4. Department of Biostatistics,
 Faculty of Health, Mazandaran
 University of Medical Sciences,
 Sari, Iran

5. Department of Basic sciences,
 Faculty of Health, Mazandaran
 University of Medical Sciences,
 Sari, Iran

6. Health Sciences Research Center,
 Faculty of Health, Mazandaran
 University of Medical Sciences,
 Sari, Iran

*** Correspondence:**

Jamshid Yazdani Charati,
 Department of Biostatistics, Faculty
 of Health, Mazandaran University
 of Medical Sciences, Sari, Iran

E-mail:

jamshid.charati@gmail.com
 Tel: +98 1133542473

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Epidemiology of brucellosis in Mazandaran, North of Iran in a nine-year period (2009-2017)

Abstract

Background: Brucellosis is a common disease between humans and animals that still exists in most parts of Iran. This study investigated the epidemiology of brucellosis in Mazandaran province, Iran, during a nine-year period from the beginning of 2009 until the end of 2017.

Methods: This descriptive-analytical study was conducted based on the guidelines of Iran's Ministry of Health and Medical Education on the data of 3732 brucellosis patients. The data were retrieved from the private and public laboratories gathered in a Ministry's database. We used the generalized estimating equation (GEE) for the Poisson regression model (Poisson GEE) to examine the disease incidence based on the studied variables.

Results: The age-standardized incidence rate has been 13.2 per 100,000 people (15.6 in men and 21.3 in rural areas). The highest rate (17.2) was in 2012 and the lowest (10.6) was in 2014. The cities of Galugah (35.0) and Qaemshahr and Fereydunkenar (3.0) had the highest and lowest incidence rates. The patients' professions had been 30% housewives, 14.9% ranchers, and 14.4% livestock-related professions. The highest standard incidence was in people older than 55 years old. The results of the Poisson GEE regression model showed that the disease incidence has been higher in men compared to women and in rural compared to urban areas (risk ratio = 1.5, 3.4, P = 0.001).

Conclusion: Mazandaran faces the risk of increased brucellosis incidence rate. Taking preventive measures, including educating people, are recommended for the near future.

Keywords: Epidemiology, Brucellosis, Generalized estimating equation, Poisson GEE model, Mazandaran.

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Brucellosis is a common infectious disease between humans and livestock. It can be transmitted to humans by consuming unpasteurized dairy products, invisible cutaneous scratches, urine, discharges of infected livestock, inhaling contaminated air, accidental inoculation of animal vaccine, placenta, blood transfusion, bone marrow, and conjunctiva (1). The geographical situation of Iran, consumption of unpasteurized dairy products, and the job contacts between slaughterhouse workers, livestock farms, veterinarians, butchers, and laboratory workers make brucellosis an occupational disease (2). Those infected with brucella suffer from a chronic disease for weeks or months with symptoms like fever, frequent sweating, fatigue, anorexia, headaches, muscle aches, body pain, and depression (3). This disease is seasonal and its risk is higher in spring and summer, which is the time of pregnancy and childbirth of the livestock (4). *Brucella melitensis* is the most common disease-causing agent in humans (5). Because of the physical and mental complications, high diagnostic and medical costs, abortion, weight loss and production of milk and wool, infertility and loss of infected livestock, it imposes heavy economic costs and pressure on many countries (6).



Nowadays, despite the numerous control programs in different regions especially in developing countries, it is still a global issue (7). More than 500,000 new cases of brucellosis are reported to the World Health Organization (WHO) from one hundred countries annually, most of which are related to developing countries (8). Malt fever is still an important infectious disease with high prevalence in many provinces of Iran (9).

A prerequisite for implementing public health programs to prevent, control and eradicate a disease is precise epidemiological statistics and information, as well as an accurate and timely description of changes in disease behavior over time. This study was conducted to identify the high-risk areas of Mazandaran of Iran. We set out to evaluate the disease condition as well as to determine the factors affecting its incidence in a nine-year period.

Methods

In this descriptive-analytical study, the research population was 3732 patients with definite diagnosis who had been followed-up until being treated whose demographic and clinical chiropractic data were registered in an infectious disease management data base. The inclusion criteria according to the definitions of probable and definite cases of brucellosis were as follows:

Probable: Suspected cases whose Wright test titer was ≥ 1.80 . Definite: Suspected or probable cases with definitive laboratory diagnosis (a. Separation of the disease agent from clinical specimens in the culture medium, b. 2ME titer (equal to or greater than one-fourth, and c. Coombs Wright positive with a dilution interval more than Wright) (10). The gathered information in the epidemiological examination forms were age, gender, occupation, residence place, history of contact with livestock, history of livestock vaccination, history of using unpasteurized dairy products, etc. These data had been collected and recorded in an online

system by the city health centers, hospitals, laboratories, and private clinics. A team of medical professionals had treated the patients after doing their diagnosis.

Statistical analysis: The data were tested in the statistical package for social sciences (SPSS) software Version 25. We used the generalized estimating equation (GEE) with the Poisson log-linear link and autoregressive correlation structure or Poisson GEE model to examine the relationship between the studied variables and brucellosis incidence. The Poisson GEE model is a generalized linear model that is used to analyze the repeated measure count data that may be correlated (the response variable indicates the number of events in many periods) (11). A p-value was considered significant at 0.05. The incidence rate was calculated based on the distribution of new cases among the population at risk. Population census data and the population growth rate of the studied years were used to calculate the incidence. Then we used the direct standardization method to calculate the age-standardized incidence rate using the standard population (12).

Ethical consideration: This study was approved by the Ethics Committee affiliated with Mazandaran University of Medical Sciences (MUMS) (IR.MAZUMS.MED.REC.1398.3607).

Results

The age-standardized incidence rate has been 13.2 per 100,000 people. The highest incidence (17.2) was in 2012 and the lowest (10.6) in 2014. The cities of Galugah (35.0) and Qaemshahr and Fereydunkenar (3.0) had the highest and lowest incidence rates (table 1, figure 1). It was 15.6 in men and 10.4 in women (risk ratio = 1.5); 21.3 in rural areas and 6.4 in urban areas (rr = 3.4). The patients' professions had been 30% housewife, 14.9% ranchers, and 14.4% livestock-related works (housewives compared to children rr = 11.5).

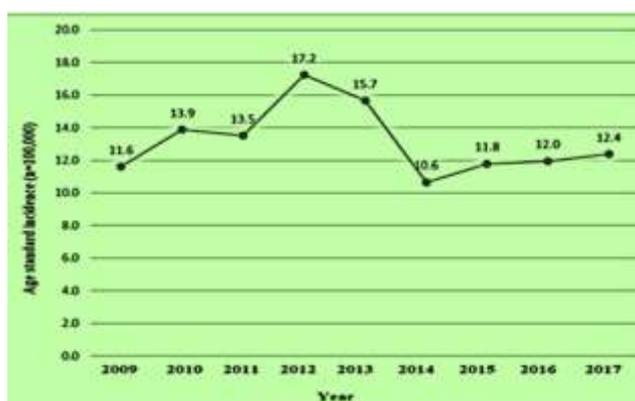


Figure 1. Trend of brucellosis incidence in Mazandaran (2009- 2017)

Table 1. Age-standardized incidence of brucellosis in Mazandaran (2009-2017)

County	Average population	Age standard incidence	County	Average population	Age standard incidence	County	Average population	Age standard incidence
Amol	403452	12.8	Noor	110108	25.8	Jouybar	75949	11.1
Behshahr	165567	15.8	Noshahr	122446	14.0	Galougah	39248	35.0
Tonekabon	181259	14.9	Babolsar	127803	6.2	Fereydunkenar	59722	3.0
Ramsar	70493	28.6	Babol	508345	7.7	Abbas Abad	54476	8.6
Sari	496084	14.1	Mahmudabad	91184	6.9	Miandoroud	58208	20.8
Savadkouh	56429	29.6	Neka	113996	27.9	Simorgh	17626	7.2
Qaemshahr	318784	3.0	Chalous	126932	12.9			

The incidence rate is per 100,000 people

The highest standard incidences were in spring (3.9) and summer (4.4) (summer compared to winter $rr=2$) and people older than 55 years old (22.6) (rr compared to children = 4.2). About 40%, 60% and 80% had a history of livestock vaccination and contact with them and consuming non-pasteurized dairy products, respectively ($rr=1.4, 1.6, 7.8$). About 70% of the patients had no other family history ($rr=5.4$) (tables 2 and 4) ($P=0.001$). The highest frequency of clinical symptoms included fever (71%) and muscle-

bone pain and back pain (75.5%). Also, 4.2% had arthritis. The most prescribed drugs were doxycycline (73.9%) and rifampin (68.8%) (table 3). The Poisson GEE model was applied for data analysis. Its result showed that: gender, age groups (with increasing effect, occupation, season, other family members suffering, history of livestock vaccination, type of contact with livestock, and history of using dairy products had a significant effect on brucellosis infection ($p<0.05$) (table 4).

Table 2. Frequency distribution (%) of demographic and epidemiological information of brucellosis in Mazandaran (2009-2017)

Variable	Category	Frequency (%)	Variable	Category	Frequency (%)	Variable	Category	Frequency (%)	Variable	Category	Frequency (%)
Gender	Female	1460 (39.1)	Age category	0-14	302 (8.1)	History of livestock vaccination	Has	1263 (33.8)	History of using dairy products	Has	2984 (80)
	Male	2272 (60.9)		15-24	530 (14.2)		Does not have	924 (24.8)		Does not have	384 (10.3)
Address	Urban	1023 (27.4)		25-44	1390 (37.2)		Not responded	1545 (41.4)		Not responded	364 (9.8)
	Rural	2709 (72.6)		45-54	630 (16.9)	History of contact with livestock	Has	2221 (59.5)	Type of dairy product	Milk	1664 (44.6)
Occupation	Child	97 (2.6)	>= 55	870 (23.3)	Does not have		1384 (37.1)	Cheese		1770 (47.4)	
	Student	333 (8.9)	Not answered	(0.3)10							

Variable	Category	Frequency (%)	Variable	Category	Frequency (%)	Variable	Category	Frequency (%)	Variable	Category	Frequency (%)
Occupation	Housewife	1118 (30)	Season	Spring	1108 (29.7)	Type of contact with livestock	Not responded	127 (3.4)	Diet	Cream	155 (4.2)
	Farmer	109 (2.9)					Contact with alive livestock	2056 (55.1)		Butter	307 (8.2)
	Rancher	556 (14.9)		Summer	1253 (33.6)		Slaughter of animals	676 (18.1)		Colostrum	96 (2.6)
	Related to livestock	537 (14.4)		Fall	747 (20)		Presence at livestock storage place	365 (9.8)		Kaymak	296 (7.9)
	Others	924 (24.8)		winter	624 (16.7)		Other family members suffering	Has		490 (13.1)	Ice cream
Not answered	58 (1.6)	Nationality	Iranian	3710 (99.4)	Does not have	2657 (71.2)					
			Non-Iranian	22 (0.6)	Not responded	585 (15.7)					

Student: University student and student- Rancher: Rancher, ranch worker- Related to livestock: butcher, butchery worker, employee-rancher, and farmer-rancher- Slaughter of animals, contact with blood and secretions: contact with secretions of aborted or aborted animals, contact with carcasses and secretions after slaughter, contact with meat and organs.

Table 3. Clinical and medical information of brucellosis patients in Mazandaran (2009-2017)

Variable	Category	Frequency (%)	Variable	Category	Frequency (%)	Variable	Category	Frequency (%)
Clinical symptoms	Fever	2651 (71.0)	Disease side-affects	arthritis	155 (4.2)	Prescribed medicine	Tetracycline	232 (6.2)
	Anorexia	1375 (36.8)		Spondylitis	21 (0.6)		Doxycycline	2757 (73.9)
	Weight Loss	1119 (30.0)		Encephalitis	1		Streptomycin	956 (25.6)
	Musculoskeletal pain, low back pain	2817 (75.5)		Orchitis	11 (0.3)		Gentamicin	708 (19)
	Adenopathy (enlargement of spleen or liver)	51 (1.4)		Endocarditis	1		Cotrimoxazole	587 (15.7)
	Weakness and lethargy	399 (10.7)		Mental disorder	6 (0.2)			
	Depression	37 (1)		Others	37 (1)		Rifampicin	2566 (68.8)
	Chills and nausea	2 (0.1)		Total	232 (6.2)			

Table 4: GEE for the Poisson regression model for brucellosis in Mazandaran (2009-2017)

Variable	Estimate(β)	Standard error	EXP(β) (Risk ratio)	Sig.	Confidence interval 95% for risk ratio
Gender (reference group = Woman)	0.413	0.034	1.5	0.001	(1.415, 1.613)
Location (reference group = Urban)	1.220	0.037	3.4	0.001	(3.155, 3.640)
Age Group (reference group = 0-14)					
15-24	0.626	0.072	1.9	0.001	(1.623, 2.153)
25-44	0.943	0.063	2.6	0.001	(2.268, 2.910)
45-54	1.281	0.070	3.6	0.001	(3.139, 4.129)
≥ 55	1.434	0.067	4.2	0.003	(3.684, 4.783)
Occupation (reference group = child)					
Student	1.230	0.115	3.4	0.001	(2.729, 4.284)
Housewife	2.440	0.106	11.5	0.001	(10.309, 14.112)
Farmer	0.116	0.139	1.1	0.404	(0.855, 1.476)
Rancher	1.742	0.110	5.7	0.001	(4.604, 7.078)
Related to livestock	1.707	0.110	5.5	0.001	(4.442, 6.841)
Others	2.249	0.106	9.5	0.001	(7.698, 11.681)
Year of Study (reference group = 2009)					
2010	0.178	0.075	1.2	0.018	(1.031, 1.384)
2011	0.181	0.075	1.2	0.016	(1.035, 1.387)
2012	0.420	0.071	1.5	0.001	(1.324, 1.749)
2013	0.352	0.072	1.4	0.001	(1.235, 1.637)
2014	0.048	0.078	1.0	0.541	(0.818, 1.112)
2015	0.080	0.076	1.1	0.291	(0.933, 1.257)
2016	0.100	0.075	1.1	0.184	(0.953, 1.280)
2017	0.144	0.074	1.2	0.052	(0.999, 1.336)
Study Season (reference group = Winter)					
Spring	0.574	0.050	1.8	0.001	(1.610, 1.958)
Summer	0.697	0.049	2	0.001	(1.824, 2.210)
Fall	0.180	0.054	1.2	0.001	(1.077, 1.331)
Other family members suffering (reference group =no)	1.690	0.049	5.4	0.001	(4.918, 5.966)
History of livestock vaccination (reference group = Does not have)	0.312	0.043	1.4	0.001	(1.256, 1.487)
Type of contact with livestock (reference group = Does not have)	0.473	0.034	1.6	0.001	(1.501, 1.716)
History of using dairy products (reference group = Does not have)	2.049	0.054	7.8	0.001	(6.980, 8.628)

Discussion

This was an epidemiological study of brucellosis in Mazandaran. The results of the Poisson GEE regression model showed that the studied variables have a statistically significant effect on this disease's incidence. The disease prevalence has been different over the years. Some of the

reasons for trend changes could have been improved statistical system, increased livestock health and vaccination and people's increased willingness to use traditional dairy products and excessive import of livestock from neighboring countries such as Iraq, Pakistan, and Afghanistan (13). Having an age-standardized incidence

rate of 13.2 in a 9-year period, which is in line with the Ministry's report, shows that Mazandaran still faces a moderate pollution (14). The difference in the incidence rate of the counties is expected, despite more livestock in some of them and that the situation of jobs requiring to be in contact with livestock (15). Risk of brucellosis in rural area is higher than urban area. It can be due to the more exposure with animals and good screening system for better disease registry. The higher incidence rate in men compared to women and also in rural compared to urban areas in most studies is the frequency of contact with livestock and livestock products (16) This is in line with Zeinali et al.'s study results (14).

Most of the cases in our study occurred in housewives, followed by ranchers, and people with livestock-related occupations. In most related studies in Iran, housewives (because of their activity in agriculture and animal husbandry alongside men) have been the most affected group (16). Increasing awareness, and changing the attitude and behavior of rural livestock farmers can help to reduce many zoonotic infections (17). The standard disease incidence was more common in people older than 55 years old, which could be due to age-related immune deficiency. As it can be seen, this disease occurs in all ages, so all people are at risk economically and socially, and the importance of fighting this disease becomes clearer. In Hamedan, the incidence of this disease was much higher in adults (18).

Brucellosis can occur in all seasons, but it is more common in spring and summer. It can transmit while calving and livestock lactation and from being in contact with pregnancy residues after abortion and consuming contaminated dairy products. This is in line with most studies (19). Higher risk of brucellosis infection in spring and summer seasons maybe due to more consumption of unpasteurized food diet in this time. Therefore, the best intervention for prevention of brucellosis is vaccination of livestock.

The frequency of patients with a history of livestock vaccination was higher than those who had never been vaccinated. Various strategies have been presented for controlling this disease, but most of them are based on veterinary activities (including the regular program of testing and killing livestock and their vaccination). This was similar to the study conducted in Khorasan Razavi (10).

The frequency of people with a history of contact with livestock (especially live animals) and consuming non-pasteurized dairy products (especially cheese and milk) was more than those who did not. Insufficient rules regarding inspection control systems has led to the mixing of infected

animals in herds in villages during summer migrations, leading to prevalence of infection in livestock and disease incidences in humans (13). In Zeinali et al.'s study on 173,526 cases with Malt fever, most patients had a history of contact with livestock and a history of consuming non-pasteurized dairy products (20).

The frequency of patients that had no other family history was higher. In Nabavi et al.'s study, 18.5% of their patients had an infected family member too (21). The most common clinical manifestations were musculoskeletal pain, low back pain, fever, anorexia and weight loss. Hashartkhani et al.'s study (22) achieved similar results in their study. Brucellosis is a systemic infection that can affect any organ and system of the body. The most commonly prescribed drugs have been doxycycline and rifampicin which is similar to other reports (18, 22). The National Technical Committee of Iran recommends treating brucellosis with a combination of antibiotics. The most prevalent complication was arthritis. The results of Ebrahimpour et al.'s study showed that about one-third of human brucellosis is associated with skeletal disorders (23). The complications of the disease vary depending on the type of bacteria and disease severity.

A challenge of this kind of research is differences in the types of laboratory kits. This can interfere with our interpretations. It would be better to use the findings from a reference laboratory. Still, many patients who refer to a reference laboratory are from other cities or provinces, and this should be considered in future researches. Despite the implementation of preventive measures, education about this disease, including ways of transmission, its symptoms and ways to prevent it, is still the first priority and basic education should be emphasized in rural areas and among people at risk, e.g. the livestock farmers, village housewives and others with livestock-related occupations.

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M.S.S, J.Y.C and R.A.M.T. M.S.S, J.Y.C analyzed data. Interoperation of result and writing manuscript were done by all authors (M.S.S, J.Y.C, F.B.M, R.A.M.T, H.B and J.SH).

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