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Comparative serology of human brucellosis in Korea

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Abstract

The study was carried out to evaluate the diagnostic efficacy of the tube agglutination test (TAT), enzyme-linked immunosorbent assay (ELISA) and the 2-Mercaptoethanol agglutination test (2-MAT) to detect human brucellosis patients in Korea. We examined 87 serum samples of people in the rural farm areas where bovine brucellosis had been reported. People in this study were divided into seven groups- farmers and their families, veterinarians, veterinary quarantine workers, livestock health control officers, artificial inseminators, livestock traders and healthy control individuals. Among 87 people, 65 were males and 22 were females ranging in age from 13 to 72 years. Of 87 serum samples, ELISA detected 21.84%, TAT detected 11.50% and 2-MAT detected 8.05% *Brucella* positive sera. *Brucella* specific IgG ELISA antibody titer was recorder higher in the individuals between the ages of 50 and 65 years. The highest prevalence rate of brucellosis(29.4%) was recorded in the cattle farmers and their family members followed by quarantine veterinary office workers (25%) and practicing veterinarians (11.1%). The majority of the *Brucella* sero-positive individuals in this study had a history of direct contact with animals.

Key words : Human brucellosis, ELISA, Tube agglutination test, 2-Mercaptoethanol agglutination test, Korea.

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Introduction

Human brucellosis is a zoonotic disease caused by Gram-negative bacilli of the genus *Brucella*. Severe complications including meningitis, meningoencephalitis, brain abscess, epidural abscess, meningovascular syndromes have been reported in patients infected with brucellosis[1,2]. Brucellosis is a recently emerging zoonotic disease in the Republic of Korea (ROK). Human brucellosis in the ROK was first reported in 2002[3]. During 2005 to 2007 more than 500 human cases have been reported to the KCDC[4]. During the same period, more than 50,000 cattle have been slaughtered due to bovine brucellosis translating an estimated economic loss of US$ 500 million[5]. It had been previously predicted[6] that this disease has the potential to turn into a major epidemic since food animal farming in the ROK are expanding without any major regulatory change, mandating an integrated approach to control bovine brucellosis, and re-evaluating the current official policy of "test and slaughter"

The most specific diagnostic test for brucellosis is isolation of the etiologic agent by culture. Although serum-based antibody assays are less specific than isolation, serological methods are still in use for diagnosis of *Brucella*. Almost all available serologic assays have been used for diagnosis of brucellosis[7], illustrating that the ideal serological test has not yet been found.

The aim of this study was to evaluate the tube agglutination test (TAT), enzyme-linked immunosorbent assay (ELISA) and the 2-mercaptoethanol agglutination test (2-MAT) for diagnosis of brucellosis in people in a rural farm community in the ROK.

Materials and Methods

Study area and population

A total of 87 blood samples were collected from the residents in the Chungnam and Chonbuk provinces during May 2006. The residents were belonged to the seven groups-cattle farmers and their families (n=34), practicing veterinarians (n=18), veterinary quarantine workers (n=8), livestock health control officers (n=3), artificial inseminators (n=3), livestock traders (n=7) and adult healthy control individuals (n=14) with no history of exposure to livestock. Among 87 residents 65 were males and 22 were females ranging in age from 13 to 72 years. The mean age of the residents was 40.4 years. The average age of males was 41.8 and female was 37.77 years.

Serological tests

Sera samples (n=87) were individually placed into one ml aliquots and kept at -20℃ until tested. Aliquots of sera samples (n=87) were sent to the Baylor College of Medicine, Houston, Texas, USA via overnight express according to the manufacturer's protocol[8] using panbio diagnostics (USA) for measuring serum IgG antibody by the ELISA. TAT and 2-MAT were also performed for measuring serum antibody titers by the previously described routine protocols[9,10].

Determination of cut-off value

An initial cut-off value of IgG ELISA was determined by testing the negative control sera samples. The diagnostic specificity and sensitivity of serum IgG ELISA

were determined by plotting the ELISA OD values of the negative and positive sera samples in a frequency distribution histogram. The cut-off value of TAT and 2-MAT were also determined using *Brucella* positive and negative sera samples.

Measuring of *Brucella* specific antibodies in the sera samples by IgG ELISA, TAT and 2-MAT

A total of 87 sera samples were tested for measuring of *Brucella* specific antibodies by IgG ELISA, TAT and 2-MAT. Patients with the serum IgG ELISA OD value of ≥ 1.1 and serum TAT and 2-MAT antibody titers of ≥1:160 and ≥1:140, respectively, were considered to be positive for *Brucella*. Patients with serum IgG ELISA OD value of 0.9 - < 1.1 and serum TAT and 2-MAT antibody titers of ≥1 : 20 ≤1 : 80 and ≥1 : 20 - ≤1 : 80, respectively, were considered as equivocal. Patients with serum IgG ELISA OD value of ≤0.9 and TAT and 2-MAT antibody titers value of 0 were considered as negative to brucellosis.

Analysis of serum samples by ELISA, TAT and 2-MAT

Sera obtained from 87 individuals were divided into five different groups (Group A, B, C, D and E) based on their clinical histories. Individuals in the Group A (n=8) were considered as the highest risk group in term of exposure to livestock. Individuals in Group B (n=11) were reported to be serologically positive to brucellosis by Korean Center for Disease Control (KCDC). Individuals in Group C (n=6) were reported to be serologically positive to brucellosis by the KCDC and they were recovered from brucellosis following antibiotic treatment. Individuals in Group D (n=48) were known to moderately expose to livestock. Individuals in Group E (n=14) were adult healthy controls who had never been exposed to livestock and did not show any clinical sign characteristic of brucellosis.

Statistical analysis

Results of the serological tests were analyzed by the Student's t-test. Additionally, ELISA IgG OD values were correlated to different cut-off values in TAT and 2-MAT by Bayesian statistical approach[11].

Results

Cut-off value of ELISA, TAT and 2-MAT

The cut-off points of ELISA was established at OD value of 1.1. On the other hand, cut-off value of TAT and 2-MAT were established at antibody titer below 1:160 and 1:140, respectively. The TAT and 2-MAT reacted only with sera that had an ELISA OD value of at least 0.9. The relative sensitivity and specificity of ELISA is shown in the Table 1.

Table 1. Relative sensitivity and specificity of ELISA compared with TAT and 2-MAT

Diagnosis Cut off Analysis	Mean ± 2SD	
	1:160	1:140
Sensitivity	100	100
Specificity	76.6	80.3
Accuracy	79.5	83.6

Comparison of ELISA, TAT and 2-MAT for detection of brucellosis in human

A total of 87 sera samples were screened for human brucellosis by ELISA, TAT and 2-MAT. Among 87 patients 19 were diagnosed positive for *Brucella* by the serum IgG ELISA. On the other hand 10 and 7 patients were found positive for *Brucella* by the TAT and 2-MAT, respectively. The comparison of three serological tests results is shown in Table 2.

Table 2. Comparison of ELISA, TAT and 2-MAT for diagnosis of human brucellosis

Methods	ELISA (Mean ±2 SD)		TAT		2-MAT	
Results	O. D.	No (%)	Titer	No (%)	Titer	No (%
Positive	≥ 1.1	19 (21.84)	≥ 1 : 160	10 (11.50)	≥ 1 : 140	7 (8.05)
Equivocal	0.9 - < 1.1	5 (5.75)	≥1 : 20 - ≤1 : 80	12 (13.79)	≥1 : 20 - ≤1 : 80	15 (17.24)
Negative	≤ 0.9	63 (72.41)	0	65 (74.71)	0	65 (74.71)

Mean ± 2 SD: 0.219 ± 0.154, Student's t-test: 9.837 with $P<0.0001$.

Prevalence of brucellosis among peoples of different occupations and ages

Brucellosis was prevalent mostly in the cattle farmers and their families (n=10), practicing veterinarians (n=7) and the workers in the quarantine veterinary office (n=2) (Table 3). Farmers were the owners of the cattle farm where bovine brucellosis had been reported. Practicing veterinarians were involved in calf delivery and in disposal and/or slaughter of infected cattle under the governmental surveillance program.

Overall seroprevalence of brucellosis was recorded as 21.83% in the individuals involved in cattle-based industries. Brucellosis was recorded as 29.4% in the farmers and their family members, 11.1 % in the practicing veterinarian, and 25% in the workers in quarantine veterinary office. Equivocal cases (n=5) were only found among practicing veterinarians (27.7%) (Table 3).

The distribution of serum IgG ELISA OD values in different age groups were spread over a wide range (OD = 0.02-5.107). The peak OD values were found between the ages of 50 and 63 years (Fig 1).

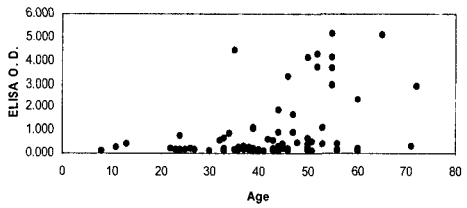

Fig 1. Age-specific distribution of ELISA OD values.

Analysis of serum samples belonged to Group A, B ,C, D and E by ELISA, TAT and 2- MAT

A total of 87 serum samples belonged to Group A (n=8), Group B (n=11), Group C (n=6), Group D (n=48) and Group E (n=14) were tested by the IgG ELISA, TAT and 2-MAT. Out of 87 individuals, 19 patients belonged to Group A and B were found to be serologically positive to brucellosis by IgG ELISA. Patients belonged to Group C, D and E was negative to brucellosis by

IgG ELISA. The average serum IgG ELISA OD value of the patients group A and B were recorded as 2.574 and 3.113, respec tively.

Out of 87 individuals, 10 patients belonged to Group A (n=4) and Group B (n=6) were serologically positive to brucellosis by TAT. Patients in the Group C, D and E were nega tive to brucellosis by TAT. The average TAT antibody titers in seropositive patients were ranged from 162 to 640. In case of 2-MAT seven patients from Group A (n=3) and Group B (n=4) were positive to brucel losis. Patients in the Group C, D and E were found serologically negative to brucellosis by 2-MAT. The average 2-MAT antibody titers in the *Brucella* seropositive patients ranged from 160 to 320.

Table 3. Seroprevalence of human brucellosis in the Chungnam and Chonbuk provinces determined by the IgG ELISA

Person categories	No of Persons (%)	Positive Cases (%)	Equivocal Cases (%)	Negative (%)	Prevalence Rate (%)
Farmers & family	34 (39.08)	10 (29.4)	0 (0)	24 (70.58)	11.4
Practicing Veterinarians	18 (20.68)	2 (11.1)	5 (27.7)	11 (61.11)	8.04
Workers in quarantine Veterinary Office	8 (9.19)	2 (25)	0 (0)	6 (75)	2.29
Livestock Health Control Officers	3 (3.44)	0 (0)	0 (0)	3 (100)	0
Artificial inseminators	3 (3.44)	0 (0)	0 (0)	3 (100)	0
Livestock Traders	7 (8.04)	0 (0)	0 (0)	7 (100)	0
Healthy - Control	14 (16.09)	0 (0)	0 (0)	14	0
Subtotal	87 (100)	14 (16.09)	5 (5.74%)	68 (78.16)	21.83[a]

[a]Indicates prevalence rate of brucellosis among 87 persons

Discussion

Prevention of brucellosis in the human populations largely depends on the control or eradication of the brucellosis from the animals. Accurate diagnosis is essential for controlling human brucellosis in Korea. In this study we found that the IgG ELISA along with TAT and 2-MAT can be used to detect human brucellosis. These tests had a high degree of specificity and sensitivity (Table 1). Furthermore, these tests could be used for monitoring the *Brucella* specific serum antibody level in human patients treated with antibiotics. This is the first independent comprehensive serological study which provides important baseline data for the accurate diagnosis of human brucellosis in Korea. Our serological data for *Brucella* diagnosis are in agreement with the find ings of Memish et al[12]. In our study high est prevalence of brucellosis was found among the farmers and their families (29.4 %) along with veterinarians (11.1%), similar observation was also made by park et al[3].

In Korea, brucellosis is fast growing newly emerging zoonoses. First diagnosed in cattle in 1959[13] and in humans in 2002[3]. Brucellosis is threatening to turn into a major

epidemic[3,6,14] in Korea since food animal farming are expanding without implementing effective control measure of bovine brucellosis. Current strategy for controlling bovine brucellosis is based on test and slaughter of the serologically reactor animals. In spite of the intense "test and slaughter" of the reactor animals, human brucellosis has become a serious public health problem in Korea[14-17]. In 2002 there was only one case of human brucellosis found in Korea who believed to acquire *Brucella* infection through drinking of unpasteurized milk[3].

By 2003, 16 cases of human brucellosis were reported in Korea[15]. There are more than 300 human brucellosis patients have been detected between January 2005 and December 2006, during that time a total of 47,752 *Brucella* infected cattle have been slaughtered[5]. The majority of the *Brucella* infected human patients in Korea were farmers and their family and practicing veterinarians, who were exposed to cattle infected with *Brucella*.

ELISA is a rapid, sensitive and specific assay, provides a profile of IgG immunoglobulin classes for the diagnosis of acute and chronic brucellosis. It is useful for mass serological screening and could be considered as the best serological method for diagnosis of brucellosis[8]. In our study the highest number of human brucellosis (21.84%) was identified by ELISA followed by TAT (11.50%) and 2-MAT (8.05%). We found higher IgG ELISA antibody titer among individuals between the ages of 50 and 65 years which might be resulted from either repeated exposure to *Brucella* infected cattle or with continued residence in a high-risk farm environment.

TAT is the most widely used laboratory test for the diagnosis of human brucellosis with a high degree of reproducibility and accuracy[2]. In this study the TAT antibody titers in *Brucella* positive human patients were recorded as 1 : 160 which are in agreement of the findings of Lopez-Merimo and Lopez-Santiago[18]. Yeom et al[19] also investigated human brucellosis in Korea and measured *Brucella* specific serum antibody by TAT. Our findings are in agreement with the observations of Yeom et al[19].

Our investigation was focused on the rural residents in a farming community. All principal participants in our study, excluding the healthy controls, were constantly exposed to *Brucella* infected cattle and might get infected due to direct contact. In an other study, one human brucellosis case was reported through direct contact with infected cattle[20]. Bovine brucellosis is endemic in Korea. The rate of human brucellosis in Korea is increased with the increased of bovine brucellosis. Human brucellosis in Korea could be eradicated with the eradication of bovine brucellosis. Along with the test and slaughter policy, vaccination of cattle with *B abortus* strain RB51[21,22] could be incorporated into the current bovine brucellosis eradication policy in Korea. Our current data under scores the public health significance of brucellosis in Korea. It is therefore suggested that development of effective serological screening tests as well as integration of cattle vaccination is very important to control human brucellosis in Korea.

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