Screening household members of shepherds in brucellosis endemic areas in China and reporting follow-up results of asymptomatic infections

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Research article

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Abstract

Background: Brucellosis is a zoonotic infectious disease caused by brucella, patients often show obvious clinical manifestation, however, many cases of asymptomatic brucella infection were reported. Previous scholars have described or screened the asymptomatic infection, but little attention has been paid to the results. This research focused on the short-term results in patients with asymptomatic brucella infection

Methods: 595 household members of shepherds in brucellosis endemic areas were included, all of them have questionnaires and laboratory tests. Based on inclusion and exclusion criteria for the cohort, 15 asymptomatic infections were included and followed-up for 18months.

Results: Among 595 subjects, 34(5.7%) were asymptomatic infections, 460(77.3%) were healthy, 58patients (9.7%) were diagnosed as brucellosis, 13(2.2%) suspected cases, 19(3.2%) cured cases and 11(1.8%) unclear diagnosis. Among 15 asymptomatic infections, the median age was 34 [12, 50] years, there were 40%cases <18 years old and the male-female ratio was 1.5:1, 60% cases were farmers and herdsmen, 11(73.3%) cases had a history of possible exposure to brucella. Average follow-up time was 10.47 ± 8.47 months. A total of 7 asymptomatic infections developed into brucellosis, of which five patients turned in the first month of follow-up, one patient in the second month, and one minor case turned in the seventh month. Remaining asymptomatic infections showed negative outcomes after 7 months of follow-up, among them, SAT titer decreased in two cases, no changes in SAT titer and clinical manifestations in six cases.

Conclusions: Continued exposure to brucella may be a major risk factor for asymptomatic infection turn to brucellosis.

Background

Brucellosis is a common disease caused by infection of *Brucella* spp, which can be transferred from animals to humans^[1, 2]. It ranks as one of the most important zoonotic diseases throughout the world^[3, 4]. Brucellosis patients are often accompanied by long-term fever, hyperhidrosis, joint pain, hepatosplenomegaly or other clinical manifestation^[5, 6]. However, there are still many cases of asymptomatic brucella infection in endemic areas^[7-9] because of individuals who were continuously in contact with high risk animals were infected^[10]. Asymptomatic infection is a condition of brucellosis. Although laboratory tests have confirmed brucella spp infection, subjects did not show any clinical manifestation. These subjects may present clinical manifestation in reinfection or immune deficiency conditions^[11]. December 2, 2019, the First Hospital of Lanzhou University reported that 4 researchers at the Veterinary Institute suspected of being infected with brucella. After standard agglutination test (SAT) titer test in 317 teachers and students of this institute, 96 people were found to be antibody positive, and none of them have clinical manifestation. They were all diagnosed as asymptomatic infection.

The appearance of asymptomatic brucellosis infection has been reported in dozens of literatures. A study indicated that the prevalence of asymptomatic brucellosis infections in Saudi Arabian was 32.4%^[12], which was higher than that (26.0%) from the study by Almuneef et al^[13]. A survey in Iran showed that 53.3% (8/15) seropositive subjects had asymptomatic infection of *Brucella*^[9]. Although these studies have carried out family screening or epidemiological characterization of asymptomatic infections, most have not conducted follow-up studies on results.

Our study searched brucella infections in family members of shepherds in endemic areas of China and described the results of follow-up of asymptomatic brucella infection for 18 months with the aim to provide more information on characteristics, results and positive result time of asymptomatic infection.

Methods

Study population

In November 2012, 595 subjects from shepherd families in Qianguoerluosi Mongolian Autonomous County, Songyuan District, Jilin Province, China were selected in this study by combination of multi-stage sampling for questionnaire survey. Meanwhile, blood samples were collected into vacuum tubes after the subjects signed the informed consent. Inclusion criteria for follow-up: laboratory serum agglutination test was positive (SAT > 1: 100 ++) and had no clinical manifestations. Exclusion criteria for follow-up: patients who had previously been treated or disagreed with follow-up. 15 subjects remained.

Follow-up

These subjects were seen for follow-up once every three months until May 2014, with a total of 6 follow-up visits. Telephone interviews were taken, its main contents included whether the subject had clinical manifestations such as fever and fatigue, and whether he had contacted the sheep in some way, and reviewed SAT at the same time. Participants who developed clinical manifestation or whose SAT titer increased during the investigation were defined as positive result. On the contrary, participants who continued to be asymptomatic infection or whose SAT titer decrease within the time of follow up were defined as negative result.

Laboratory test and data analysis

SAT examinations were performed at Plague and Brucellosis Prevention and Control Base, Chinese Centers for Disease Control and Prevention. Epidata3.1 software was used to enter the questionnaire, and SPSS24.0 was used for statistical analysis. All tests were 2-tailed with a 0.05 significance level.

Ethical support and quality control

This study has been approved by the Ethics Committee of the School of Public Health of Jilin University. All participants were approved by the Institutional Review Board of the School of Public Health of Jilin University, signed an informed consent, and agreed to participate in the study. Professional training was

provided to researchers. The diagnosis and serological methods and results of brucellosis were performed in accordance with relevant guidelines and regulations.

Results

A total of 595 subjects were included in the study and all of them were Questionnaire, interviewed, and then tested by SAT. Among them, 332 (55.8%) cases were male and 263 (44.2%) were female. The mean age was 33.81 ± 18.12 , and 205(34.5%) subjects were under 18 years old. The distribution of brucella infection in 595 subjects was shown in Table 1.

Table 1
Brucella infection in 595 subjects

	Number	Percent (%)
Asymptomatic infection	34	5.7
Confirmed case	58	9.7
Healthy	460	77.3
Suspected case	13	2.2
Cured case	19	3.2
Unclear diagnosis	11	1.8

Detailed baseline demographics of follow-up subjects were listed in Table 2. The male to female ratio was 1.5:1, and nine (60%) were agro-pastoralists. The median age was 34 years (1st–3rd quartile: 12–50 years), and six (40%) subjects were under 18 years old. A majority (n = 11) of them has a history of potential exposure to brucella, such as contacting with abortion or brucella live attenuated vaccine, cleaning sheepfolds and delivering sheep.

Table 2
General characteristics of 15 asymptomatic brucellosis infections

Characteristic	Total	Male			Female		
		≤ 18 years	18– 30 years	> 30 years	≤ 18 years	18– 30 years	> 30 years
Occupation							
agro-pastoralist	9	0(0%)	1(11.1%)	5(55.6%)	0(0%)	0(0%)	3(33.3%)
student	6	3(50%)	0(0%)	0(0%)	3(50%)	0(0%)	0(0%)
History of possible exposure							
Raise sheep	11	1(9.1%)	1(9.1%)	5(45.4%)	1(9.1%)	0(0%)	3(27.3%)
Deliver	6	0(0%)	1(16.7%)	4(66.7%)	0(0%)	0(0%)	1(16.7%)
Contact with abortion	4	0(0%)	0(0%)	2(50%)	0(0%)	0(0%)	2(50%)
Contact with vaccine	5	0(0%)	0(0%)	3(60%)	1(20%)	0(0%)	1(20%)
Slaughter	3	0(0%)	0(0%)	2(66.7%)	0(0%)	0(0%)	1(33.3%)
Clean sheepfold	9	0(0%)	1(11.1%)	5(55.6%)	0(0%)	0(0%)	3(33.3%)
N	4	2(50%)	0(0%)	0(0%)	2(50%)	0(0%)	0(0%)

Follow up results of 15 asymptomatic subjects were shown in Table 3. Of the nine adult infections, five patients were diagnosed as brucellosis in the first month, one patient was diagnosed in the second month, one of them was still asymptomatic and two subjects' SAT titer descend until the 18th month. Among the six juveniles, only one patient had obvious clinical manifestations in the seventh month, and the other five subjects were still asymptomatic at the end of 18-month follow-up.

Table 3
Follow-up of 15 asymptomatic Brucella infections

No.	Age	Sex	Raising sheep	History of possible exposure during follow-up	Outcome	Follow-up (month)
X ₁	> 30	Male	Yes	Raise sheep, Graze, Deliver, Contact with abortion, vaccine, Clean sheepfold	symptom developed	1
X ₂	> 30	Female	No	No	symptom developed	2
X ₃	> 30	Male	Yes	Graze, vaccine, Clean sheepfold	symptom developed	1
X ₄	≤ 18	Female	No	No	symptom developed	7
X ₅	> 30	Male	Yes	Raise sheep, Graze, Deliver, Contact with abortion, vaccine, Slaughter, Clean sheepfold	symptom developed	1
X ₆	18–30	Male	Yes	Raise sheep, Graze, Deliver, Contact with abortion, vaccine, Clean sheepfold	symptom developed	1
X ₇	> 30	Male	Yes	Raise sheep, Graze, Deliver, Contact with abortion, vaccine, Slaughter, Clean sheepfold	symptom developed	1
X ₈	> 30	Male	Yes	Raise sheep, Graze, Deliver, vaccine, Clean sheepfold	SAT titer descend	18
X ₉	> 30	Female	Yes	Raise sheep, Graze, Deliver, Contact with abortion, vaccine, Clean sheepfold	SAT titer descend	18
X ₁₀	≤ 18	Male	NA	NA	Asymptomatic infection	18
X ₁₁	≤ 18	Male	NA	NA	Asymptomatic infection	18
X ₁₂	≤ 18	Female	NA	NA	Asymptomatic infection	18
X ₁₃	≤ 18	Female	NA	NA	Asymptomatic infection	18
X ₁₄	≤ 18	Male	NA	NA	Asymptomatic infection	18
X ₁₅	> 30	Female	Yes	Raise sheep, Graze, Deliver, Contact with abortion, vaccine	Asymptomatic infection	18
NA = Not Available						

Discussion

Asymptomatic brucellosis patients may develop into brucellosis patients, causing serious damage to their health^[14]. Nine farmers and herdsman in this study may have been exposed to brucella through sheep raising, deliver, contact with aborted lambs and brucella live attenuated vaccine. These work methods give shepherds the opportunity to directly contact the liquid of diseased animals or the partially inactivated brucella, and to be exposed to the polluted environment for a long time, thus leading to infection. During this follow-up, it was found that a total of 7 (46.67%) asymptomatic infections were positive and all showed obvious clinical manifestations of brucellosis, of which 5 patients turned positive in the first month. All 5 patients had an epidemiological history of exposure to diseased animals during the follow-up period, which may make them more likely to be exposed to brucella, it leads to repeated infection and progression from asymptomatic infection to brucellosis. There was one asymptomatic patient showed clinical manifestation after two months of follow-up, but she did not contact brucella directly during the follow-up period, the possible reason was the decline of the body's immune function. In addition. It was also found that two asymptomatic infection adults showed decreased SAT titers and no clinical manifestation. Possible causes were (1) Brucella antibodies had poor persistence in asymptomatic infections (2) Short follow-up time has not been observed the final outcomes of these two people.

Brucellosis reportedly occurs mainly in adult populations^[10]. However, there were 6 (40%) minors had asymptomatic infections in this study, most of them (83.3%) had no brucella direct contact history. Possible causes of asymptomatic brucella infection in minors who were not in contact with diseased animals are (1) Minors have less direct contact with brucella, mainly due to ingestion of contaminated food or contaminated environment and aerosol infections^[15-17] such as eating unpasteurized dairy products and local traditional foods containing dairy products^[18]. (2) Minors, especially children, usually show fewer or milder symptoms than adult patients. However, we also found that only one of the minors had a positive outcome at the seventh month at the same time. This may be due to the lower frequency of contact or contamination with brucella-bearing animals, and the possibility of repeated infection was lower.

We found that it is because of their continuous exposure to pathogenic animals or contaminated environments that asymptomatic infections had progressed from asymptomatic infection to brucellosis. For this asymptomatic brucella outbreak in the laboratory, we supposed that timely inspection and treatment of sources of infection should be performed to prevent asymptomatic infections from re-contacting brucella and reduce the possibility of becoming infected. Similar happened in Thailand^[19]. They also supposed that the most likely mechanism was air-borne inhalation. At the same time, researchers should strengthen personal protection in the search for the source of infection and terminal disinfection in order to avoid biosecurity issues from happening again. Follow-up observations should be performed on those diagnosed with asymptomatic infection. Fortunately, previous studies had

shown that polymerase chain reaction (PCR) testing has an important role in predicting the subsequent outcome of asymptomatic brucella infection^[20–23].

There are several advantages in our study. First, the annual incidence rate of brucellosis in qianguoerluosi mongolian autonomous county was high, about 0.36 per thousand. It is a representative area with high incidence of brucellosis in China. Second, we surveyed all members of shepherds. However, only 15 asymptomatic infections were observed in our study, asymptomatic infection rates(5.7%)are lower than previous studies^[9, 24]. We supposed that the infection may show obvious clinical manifestation because of the strong virulence of sheep brucella. Previous research has shown that *Brucella abortus* is less pathogenic and virulent than sheep brucella, leading to asymptomatic and suspected infections in humans after exposure to infected cattle. Our current study also had limitations. First, we only observed 15 asymptomatic infections. Second, the average follow-up time was 10.47 ± 8.47 months, which only reflects the short-term outcomes of asymptomatic infections. Long-term outcomes and influencing factors cannot be obtained. Obviously, more cases and long-term follow-up can get more accurate results.

Conclusion

Continued exposure to brucella may be a major risk factor for asymptomatic infection turn to brucellosis.

Declarations

Ethics approval and consent to participate

This study has been approved by the Ethics Committee of the School of Public Health of Jilin University. All participants signed an informed consent and agreed to participate in the study.

Consent to publication

Written informed consent for publication was obtained from the patient.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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Authors' Contributions

ZQ, SN, CZ and JS: conception, Questionnaire design, acquisition analysis of data, interpretation of results, and manuscript writing. JH, HY, XZ, SZ, TW: acquisition of the data, interpretation of results, and revision of the manuscript. DW, FH, JL: acquisition of the Laboratory examination results, interpretation of results, and revision of the manuscript. All authors read and approved the final manuscript.

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