Korean Journal
of Veterinary Service

Korea J Vet Serv (2005) 28(4) : 387~392

Brucellosis outbreak of Korean indigenous cattle at Yeongwol and Pyeongchang county in Korea

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(Received 28 March 2005, accepted in revised from 5 December 2005)

Abstract

This study was attempted to investigate the properties of brucellosis in Korean indigenous cattle at the Yeongwol and Pyeongchang county. *Brucella* spp was differentiated and identified from cotyledons, amniotic fluids and supramammary lymph nodes which confirmed with clinical, serological, epidemiological evidences (69 cases) from January to June, 2004.

Isolation frequency of this causative agent from supramammary lymph nodes, cotyledons and amniotic fluids from 38 pregnant Korean indigenous cattle were 39.1%, 87.5%, and 63.2%, respectively, and finally confirmed with *Brucella abortus* biotype 1 through biochemical and serological test. A *Brucella* specific DNA with 711 bp band was detected by PCR assay using BCSP primer. The two cases were definite epidemiological evidences that infected Korean indigenous cattle acrossed the border to Yeongwol and Pyeongchang from near two provinces. Effective prevention programs are urgently needed for further spreading this epidemics.

Key words : *Brucella abortus*, PCR, Epidemiological evidences

Introduction

Brucellosis is one of great health significant and economic important infection in many countries. This organism infects domestic and wild animals, even to humans who have contact with infected animals or contaminated dairy products (zoonosis)[1, 2],

Brucellosis is categorized class II in

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"Code (Law) of Infectious Disease Prevention for Domestic Animals", class III in "Code of Infectious Disease" in Korea. *Brucella* subspecies consist of *B abortus, B suis, B melitensis, B neotomae, B ovis, B canis,* and *B maris* by their antigenicity and host-specificity. Among them, *B abortus, B suis* and *B melitensis* have reported strong pathogenicity for human[3, 4]. There were consistent reported for this infectious disease since 1955 with first occurrence of brucellosis in the following year in Korea But still stagnant stage for their eradication regardless of early detection and termination policy, as for now[5, 6].

Typical signs of bovine brucellosis are mainly genital organ related symptoms, Therefore brucellosis has been conceived as one of the most severe diseases in livestock industry[7, 8]. Before explosive occurrence of brucellosis in 2004 on Korean native cattle, anybody had no regard for korea native cattle as main host of *B abortus.*

Even though the mechanism of *Brucella* organism to Korean cattle be quite similar to dairy cattle, such as their migration and propagations in the reproductive system, we still have some doubts about possibly different mechanisms between both strains includes immunology against *B abortus*, nevertheless of different patterns of breeding style between them in Korea. Though microbiological isolation of *Brucella* spp be the definite evidence for diagnosis, there has been some difficulties such as, retardation for specific isolation, hard adaptation for early diagnosis, imperfect reliability for characterization and their bio-hazard itself too[9-14]. One of the reason for hard detection for Korean cattle could be explained as quite different breeding style when we compare to dairy cattle in Korea. Consequently, most screening methods has highly emphasized on early detection tools, such as milk ring test (MRT). Following explainable presumptions, their report for brucellosis on Korean cattle might be delayed or neglected until initial spreading stage as mentioned the above (as the above-mentioned).

We are surveyed and characterized the recent brucellosis cases in southern region of Gangwon province in Korea with serological, microbiological, PCR and epidemiological tools as initial intervention of Gangwon province.

Materials and Methods

Sampling of the specimen

A total of 69 serum and mammary lymph nodes, 48 cotyledons and 38 amniotic fluids were collected from 69 herds, which were confirmed as brucellosis in Yeongwol and Pyeongchang county under the 2004 Korea guideline of brucellosis prevention and control from January to June, 2004

Serological tests

Rose bengal test (RBT) : Agglutination reaction was determined as positive when *B abortus* 1119-3 media apply to 30 $\mu\ell$ of inactivated sera with 56℃, 30 minutes with 30 $\mu\ell$ supernatant (as antigen) which already stained with rose bengal.

Standard tube agglutination test (STAT) : Agglutination reaction in more than 100 fold-diluted sera was determined as positive when *B abortus* 1119-3 media apply to serially diluted sera (25, 100, 200 and 400 fold) from RBT-positive sera with 2 ㎖ creamy.

Microbiological test

All specimen were initially inoculated and incubated on Trypticase Soy Broth for 3 days, then selectively inoculated on antibiotics added Serum Dextrose agar and 3% sheep blood media, finally incubated for 3 to 5 days (cotyledon, amniotic fluid, mammary lymph node) and 30 days (blood sample)[7].

Each clusters were determined with Gram stain, CO_2 availability, H_2S productivity and hemolysis. Further biological typing proceeded by Woo's method. Verified colonies as *Brucella* spp were used by consecutive passages on agar added Tryptic Soy Broth[4, 18, 19]

Polymerase chain reaction (PCR) [3, 15-17]

Extractions of template DNA (processed by boiling and cooling stages) from the propagated *B abortus* colony were kept in -20℃ refrigerator until our experiments BCSP (*Brucella* 31 kd cell surface protein) primer used for specific detection of *Brucella* organism.

The sequence of this primer was obtained from the Bioneer Corporation in Daejeon, Korea. The forward primer sequence is 5'-GTATCGTTCTTGAAGCC TAC-3', and the reverse primer sequence is 5'-GTGCATTTCAATAGGCTA GAG-3'.

PCR was performed in 94℃, 5 min denaturation of DNA as initial reaction, and 95℃, 15 sec denaturation, 55℃, 20 sec annealing, 72℃, 1 min DNA extension with consecutive 30 times and one final extension at 72℃ for 5 min.

Ten microliters of the amplification reaction mixture was taken and fractionated in a 0.7% agarose gel containing 50 X TAE buffer, stained with ethidium bromide solution (0.5㎍/㎖), and visualized under UV light.

Results

Positive sera by RBT and STAT

All of the sera were screened by the RBT and the STAT in our serological tests. Of 206 serum samples examined, 98 (47.5%) were identified positive for brucellosis by the RBT, 69 cases (70.4%) of 98 RBT-positive were also identified positive by the STAT (Table 1).

Table 1. Result of serological tests

	RBT	STAT
No. of test	206	98
No. of positive	98	69
%	47.5	70.4

Isolation rate from various specimens

On the results of isolation of *Brucella* organism from sera-positive cases, 42 cases (87.5%) were confirmed from cotyledons, 24 cases (63.2%) from amniotic fluids and 27 cases (39.1%) from supramammary lymph nodes (Table 2).

Table 2. Isolation rate of *Brucella* organisms from various specimens

	Specimens				Total
	Cotyledons	Amniotic fluids	Supramammary lymph node	Blood	
No. of test	48	38*	69	69	224
No. of positive	42	24	27	0	93
%	87.5	63.2	39.1	0.0	41.5

* : Pregnant cow

Biological properties and PCR results of isolates

Selected 93 isolates showed positive in CO_2, availability, H_2S productivity and urease/catalase/oxidase reaction. All 93 isolates also showed agglutination against anti-serum A as typical reaction of *B abortus* biotype 1[18].

Based on the results of PCR assays, randomly selected 8 isolates by farm were proceeded to PCR reaction. All *Brucella* specific DNA's with 711-bp band were detected by PCR assay (Fig 1).

High risk ages groups

Evaluation of epidemiological histories showed that *Brucella*-positive 50 cases (72.5%) among totally surveyed 69 cases purchased from at least near two county. In this survey, the high *Brucella*-risk group was noted at 2 to 3 year-old cattle as 76.8 % (53 cases) (Table 3).

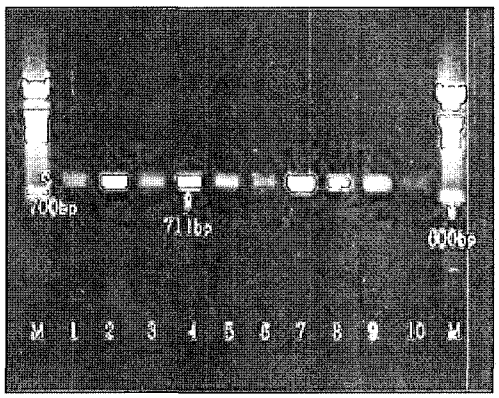

Fig 1. Amplification patterns of BCSP gene of *B abortus* isolates by PCR using BCSP primers.
M : DNA ladder marker, Lane 1 : Standard strain, *B abortus* (biotype1), Lane 2-9 : isolates, Lane 2 : A farm, Lane 3 : B farm, Lane 4 : C farm, Lane 5 : D farm, Lane 6 : E farm, Lane 7 : F farm, Lane 8 : G farm, Lane 9 : H farm, Lane 10 : Standard strain, *B abortus* (biotype 1), M : DNA ladder marker.

Table 3. Distribution of *Brucella* reactor Korean indigenous cattle by age and sex

Sex	Age						Total
	Below 1	1	2	3	4	Above 5	
Male	2	0	7	1	0	0	10
Female	2	1	26	19	7	4	59
Subtotal	4	1	33	20	7	4	69

Discussion

To control and eradicate bovine brucellosis in Korea, early detection and termination policy even to Korean indigenous cattle already adopted by the act (law) since 1985. However, we has been encountered complicated application for newly patterns of characterization ambiguous clinical signs except for abortion. So as to clarify this characterization in Yeongwol and Pyeongchang county in Gangwon province, we identified the causative microbes, as *B abortus* biotype 1, 21.7% (15 cases) had history about one or more than one case of abortion. On autopsy reports, all confirmed cases had severe haemorrhages of cotyledons with villi segregation.

On the basis of isolation rates, especially cotyledons and amniotic fluids were proper portion to Korean indigenous cattle as 87.5% and 63.2%, respectively[4].

When we resume to Korean indigenous oxen group, there might be no correlation between both sex. Because most oxen usually do not have bred to more than 2 years except as selected donors in Korea.

Since 2002, there has been increasing rate of oxen termination (slaughter) rather than cow. We might have to highly emphasize the proper preventive screening of *Brucella* contaminated oxen group especially for human public health.

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