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The follow up study after massive outbreak of Akabane and bovine ephemeral fever viruses in Korea

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(Received 22 May 2013; revised 26 June 2013; accepted 29 June 2013)

Abstract

Since a large number of Akabane and bovine ephemeral fever (BEF) infection occurred in the southern part of Korea in 2010, recent information about seroprevalence of Akabane virus (AKAV) and bovine ephemeral fever virus (BEFV) has been required for preventing both diseases. In this study, serological assay against AKAV and BEFV using virus neutralization assay was conducted using 1,743 bovine sera collected from Namwon, Miryang, Yeongju and Uljin which located in Southern part of Korea from March to May in 2012. The overall seropositive rates for AKAV and BEFV were found to be 49.8% and 1.2%, respectively. The regional distribution of seroprevalence for AKAV ranged from 18.1% to 63.7%. Seroprevalences of AKAV were 63.7% in Miryang, 62.3% in Uljin, 50.7% in Namwon, and 18.1% in Yeongju. The seropositive rates for AKAV in southern part of Korea were higher than the annual average at the national level. On the other hand, seropositive rates of BEFV in four regions were from 0.3 to 3.1%. In detail, regional seroprevalences were 3.1% in Miryang, 2.0% in Uljin, and 1.7% in Yeongju, and 0.3% in Namwon. Even only one year after massive outbreaks, overall seropositive rates were very low, similar to the annual average at the nation level. This result indicates that many number of cattle infected with BEFV may be replaced by new born calf or cattle in farm may not be immunized with vaccines. To prevent another epidemic, a national wide warning should be issued and more aggressive control measure must be implied. Recent global warming phenomenon could lead to more vigorous activity of haematophagous vectors and it is possible that arboviral diseases such as AKAV and BEFV are increased. Therefore, continuous sero-monitoring and extensive vaccination combined with control of haematophagous vectors are important to effectively prevent and control diseases caused by AKAV and BEFV.

Key words : Akabane virus, Bovine ephemeral fever virus, VN test, Seroepidemiology

INTRODUCTION

Akabane and bovine ephemeral fever diseases are designated as a third-class infectious disease in the infectious prevention act and both diseases are an important arthropod-borne viral disease in ruminants. These arthropod-borne viruses (arboviruses) are widely distributed from the tropical to temperate zones of the

world and have been associated with hematophagous arthropod vectors such as *Culicoides* biting midges and mosquitoes. The particular vector species linked to the Akabane virus (AKAV) and bovine ephemeral fever virus (BEFV) are *C. brebitarsis*, *C. oxystoma*, *C. nebulchlsus* and *C. nipponensis* (Lee et al, 2002; Hsieh et al, 2005).

The AKAV causes epizootic and sporadic outbreaks of reproductive disorders such as abortions, stillbirths,

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premature births and congenital malformations in cattle, sheep and goats (Kono et al, 1975; Kono et al, 2008). AKAV is a member of the genus *Orthobunyavirus* in the family *Bunyaviridae*, and contains three segments of single-stranded negative RNA designated L (large), M (medium), and S (small). The L segment RNA encodes the polymerase gene, which plays an important role in transcriptase activity, whereas the M segment RNA encodes two envelop glycoproteins, Gn and Gc, which are responsible for viral neutralization. The S segment RNA contains nucleotides encoding two proteins, nucleoprotein (N) and nonstructural proteins (Elliott et al, 1990). Since the AKAV was first isolated in Japan in 1959, several Asian countries including Korea have reported outbreaks of AKAV infection in ruminant (Akashi et al, 1997; Bak et al, 1980; Lee et al, 2002; Liao et al, 1996; Nakajima et al, 1980). Therefore, there have been efforts to prevent Akabane disease with several kinds of vaccines in Korea (Kim et al, 2011).

BEFV is also an arthropod-borne rhabdovirus, which belongs to the genus *Ephemerovirus* in the family *Rhabdoviridae* showing a bullet or cone shaped virion and consists of a minus sense, single-stranded RNA genome (Wunner et al, 1998; Hsieh et al, 2005). Bovine ephemeral fever called "three day sickness" is a viral disease caused by BEFV. The cattle infected with BEFV clinically show acute fever, stiffness, lameness, nasal and ocular discharges, depression, cessation of rumination and constipation (Hsieh et al, 2005). This acute febrile disease occurred in cattle in tropical and subtropical regions of Africa, Asia, Australia and the Middle East (Walker et al, 1991; Walker et al, 1992; Walker et al, 1994; Dhillon et al, 2000). For the prevention of epidemic BEF infection, BEFV vaccine inactivated with binary ethylenimine and BEFV live vaccine were developed in Korea in the 1990's. The two kinds of BEFV vaccines have been applied for the control of BEFV infection in the field. (Shin et al, 2009)

Among the arboviruses, AKAV and BEFV are the most significant vector-borne viral agents in Korea and a large-scale epidemic of AKAV and BEFV occurred around Namwon of Korea in 2010 (<http://www.kahis.go.kr>). Follow up study after massive outbreak is critical for predicting potential outbreaks of vector-borne viral

diseases among cattle again. Therefore, recent information about seroprevalence against AKAV and BEFV has been required for establishing a system to prevent these diseases. In this study, we conducted a serological survey to determine the prevalence of antibodies against AKAV and BEFV in Korea.

MATERIALS AND METHODS

Cells and viruses

Vero cells (Africa green monkey kidney cell line: ATCC CCL81) were maintained in alpha-minimum essential medium (MEM; Gibco BRL, USA) containing 5% fetal bovine serum (Gibco BRL, USA), penicillin (100 unit/mL), streptomycin (100 unit/mL), and amphotericin B (0.25 µg/mL) at 37°C in a 5% CO₂ incubator. AKAV, KV0505 strain isolated from cattle blood in Jeju island in 2005 (Yang et al, 2008), and BEFV (DS11 strain) were used for the virus neutralization assay. Uninfected cell cultures were used as negative controls.

Collection of blood samples

For the seroprevalence study, a total of 1,743 bovine blood samples were collected from 101 farms located in southern part of Korea from March to May in 2012. There is no information about whether the beef cattle were vaccinated with both AKAV and BEFV vaccines. Clotted blood samples were separated by centrifugation and the sera were separated and stored at -20°C until use.

Virus neutralization assay (VNA)

The VNA was performed in 96-well microplates in duplicate using the sera inactivated at 56°C for 30 min. Fifty microliter of twofold serially diluted serum was mixed with an equal volume of AKAV and BEFV containing 200 TCID₅₀/0.1 mL, respectively. After incubation of mixture at 37°C for 1 h, 100 µL of Vero cells suspension containing 20,000 cells was added to

Table 1. Seroprevalence rates of Akabane virus and bovine ephemeral fever virus using virus neutralization assay in bovine blood samples collected from regions in Korea

| Region | No. of farms | No. positive / No. tested (%) | |
|---------|--------------|-------------------------------|------------------------|
| | | Akabane | Bovine ephemeral fever |
| Namwon | 97 | 513/1,012 (50.7) | 3/1,012 (0.3) |
| Miryang | 2 | 188/295 (63.7) | 9/295 (3.1) |
| Yeongju | 1 | 43/237 (18.1) | 4/237 (1.7) |
| Uljin | 1 | 124/199 (62.3) | 4/199 (2.0) |
| Total | 101 | 868/1,743 (49.8) | 20/1,743 (1.2) |

each well. The plates were incubated for 3 days in a humidified incubator with 5% CO₂. Each well was examined under microscopy for detection of virus specific cytopathic effect (CPE). The virus neutralizing antibody titers were expressed as the reciprocal of the highest serum dilution that inhibited CPE completely. A titer of 1:4 or greater was considered to be positive against AKAV and BEFV.

RESULTS

Annual incidence of vector-borne viruses including AKAV, Aino virus (AINV), Chuzan virus (CHUV), Ibaraki virus (IBAV) and BEFV in cattle was investigated using data from KAHIS of Animal and Plant Quarantine Agency (QIA) in Korea from 2007 to 2012. Among these viruses, only AKAV (132 cases) and BEFV (100 cases) were occurred during this period. In particular, the highest incidences of AKAV (124 cases) and BEFV (92 cases) were shown in 2010 and most of AKAV and BEFV cases occurred around Namwon. But there was no outbreak of AINV, CHUV and IBAV infection.

Seroprevalences of AKAV and BEFV using VNA were examined in 1,743 bovine sera collected from Namwon, Miryang, Yeongju and Uljin in Korea in 2012. The overall seropositive rates for AKAV and BEFV were found to be 49.8% (868/1,743) and 1.2% (20/1,743), respectively (Table 1). The regional distribution of seroprevalence for AKAV ranged from 18.1% to 63.7%. Seroprevalences of AKAV were 63.7% (188/295) in Miryang, 62.3% (124/199) in Uljin, 50.7%

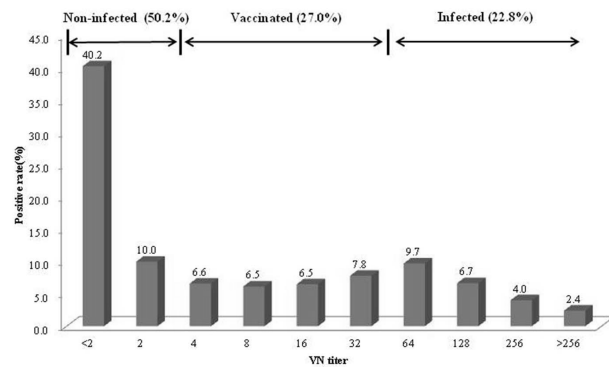


Fig 1. Distribution of virus neutralization (VN) titers against Akabane virus (AKAV) in 1,743 bovine sera. A VN titer of 1:4 or more was considered to be seropositive against AKAV. VN titers from 1:4 to 1:32 and above 5 indicate positive sera from vaccinated cattle and cattle infected by wild AKAV, respectively.

(513/1,012) in Namwon, and 18.1% (43/237) in Yeongju. On the other hand, seropositive rates of BEFV in four regions ranged from 0.3 to 3.1%. In detail, regional seroprevalences were 3.1% (9/295) in Miryang, 2.0% (4/199) in Uljin, and 1.7% (4/237) in Yeongju, and 0.3% (3/1,012) in Namwon (Table 1).

A virus neutralization (VN) titer of 1:4 or more (49.8%) was considered to be seropositive against AKAV. In distribution of VN titers against AKAV, VN titers of less than 1:4, from 1:4 to 1:32, and more than 1:32 were 50.2%, 27.0% and 22.8%, respectively. VN titers from 1:4 to 1:32 (27.0%) and above 1:32 (22.8%) presume respectively positive sera from vaccinated cattle and cattle infected by wild AKAV within positive range (Fig. 1).

DISCUSSION

AKAV and BEFV infections among vector-borne viral diseases have been a center of attention for the threat of economic loss. Recent records of AKAV and BEFV infections have been resulted in 132 and 100 cases in Korea from 2007 to 2012 and the highest incidences of AKAV (124 cases) and BEFV (92 cases) were particularly shown in 2010, whereas AINV, CHUV and IBAV infections did not occurred in the same period. For the prevention of arboviral diseases, both AKAV and BEFV vaccines were developed in the

1990's and have applied to cattle in Korea. Recently, a trivalent inactivated vaccine for AKAV, AINV and CHUV was developed in 2005 and are available commercially. Especially, large amount of AKAV and BEFV vaccines have been provided to cattle farm, because these kinds of vaccines are subsidized by the Korean government.

AKAV infection has been reported in many cattle-raising countries, and their seroprevalences have been estimated to range from 7.7% to 83.3% depending on locations and seasons (Mohamed et al, 1996; Kang et al, 2000; Shin et al, 2009). Antibodies against AKAV have also been detected in several other species such as goats, sheep, horses and rhinoceros with the prevalence ranging from 3.8% to 59.8%. (Kono et al, 2008; Elliott et al, 1990; Kim et al, 2011; Kurogi et al, 1987; Farag et al, 1998). Neutralizing antibodies against BEFV has also been reported in many countries in buffalo, sheep and cattle and is highly variable depending on the age and immune status of ruminants (Farag et al, 1998; Zheng et al, 2010; Jun et al, 2012). Our sero-monitoring after massive Akabane and BEF outbreaks reveals that the overall seropositive rates against AKAV and BEFV are moderate (49.8%) and very low (1.2%), respectively. In addition, the regional sero-prevalence against AKAV ranged from 63.7% to 18.1%. The seropositive rates for AKAV in southern part of Korea were higher than the annual average at the national level, reported as 10 to 20%. This result deduces that antibodies, derived from AKA infection at the time of massive outbreak still remain in those cattle population. On the other hand, seropositive rates of BEFV in four regions were from 0.3 to 3.1%. In detail, regional seroprevalences were 3.1% in Miryang, 2.0% in Uljin, and 1.7% in Yeongju, and 0.3% in Namwon. Surprisingly, even only two years after massive outbreaks, overall seropositive rates for BEFV were not affected by that outbreak and similar to the annual average at the nation level, reported as around 5%. This result indicates that many number of cattle infected with BEFV may be replaced by new born calf or cattle in farm may not be immunized with vaccines. The sero-surveillance of five arboviral diseases has been annually conducted since 1993 as preventive measures against epidemics. If the seropositive rate is

under the 30% for any of the diseases, a warning against the disease is issued. A warning against AKAV and BEFV was issued in May 2009 (Shin et al, 2009). With these seropositive rates, if the AKAV and the BEFV are introduced again and propagated in cattle, the economic loss would be high. So, a warning against both diseases may be issued again to prevent epidemic.

It was reported that live attenuated Akabane vaccine (JH-20 strain) induced low neutralizing antibodies level (1:4 to 1: 8) in cattle and low maternal antibodies level (1:8) in calves (Kim et al, 2011). Maternally derived antibodies to Akabane vaccine estimated to be decayed around 4.8 months with 90% probability (Tsutsui et al, 2009). Therefore, we categorized as that VN titers from 1:4 to 1:32 (27.0%) and above 1:32 (22.8%) presume respectively positive sera from vaccinated cattle and cattle infected by wild AKAV within positive range. Based on our results, approximately 13% of the tested population, which shown above 1:64 VN titers, were suspected to be infected with AKAV before summer season of 2011.

The glycoprotein of BEFV is considered as the major neutralizing antigen. When the variation of neutralizing epitope occurred in the recent epidemic isolate, the cattle inoculated with BEFV vaccine containing attenuated strain isolated in the 1980's may not show neutralizing antibody titer in VNA. In general, natural BEFV infection induces a strong neutralizing antibody response and infection usually keeps durable immunity. It was reported that BEFV isolates from mainland China and Taiwan are closely related to Australian isolates, but some variations have been detected (Hsieh et al, 2005).

Epizootic of Arboviruses such as AKAV and BEFV is associated with host factors such as health condition and population immunity, and seasonal conditions such as temperature, humidity, and activity of haematophagous vectors (Hsieh et al, 2005; Lim et al, 2007; Jun et al, 2012). Summer season (June to August) is suitable for proliferation and activity of haematophagous vectors in Korea. Before appearance of abundant haematophagous vectors, it is important to keep healthy condition and population immunity in the host ruminants. Recent global warming or climate change could lead to more vigorous activity of haematophagous vectors and it is

possible that arboviral diseases such as AKAV and BEFV are increased. Therefore, continuous monitoring, and control of haematophagous vectors are important to effectively prevent and control diseases caused by AKAV and BEFV. It is required to develop new vaccines using recent isolates circulating in Korea.

ACKNOWLEDGMENTS

This work was financially supported by a grant from Korean Institute of Planning & Evaluation for Technology in Food, Agriculture, Forestry & Fisheries (IPET).

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