CURRENT STATUS OF IMPORTANT
TRANSBOUNDARY ANIMAL DISEASES IN JAPAN

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ABSTRACT

In Japan, the re-emergence of foot-and-mouth disease and highly pathogenic avian influenza was observed in 2000 and 2004, respectively, after a long absence. Although these typical transboundary diseases have broken out twice or more since then, we have successfully eradicated by culling affected animals according to the basic policy on disease control measures. In a nationwide scale outbreak of porcine epidemic diarrhea in 2013, above 1.2 million pigs have shown typical symptoms and approximately 380,000 pigs have died within a year. Various arthropod-borne (arbo-) viruses have invaded repeatedly from overseas and caused the diseases mainly in cattle. Patterns of the prevalence of arboviruses in Japan have been changed recently. New pathogens, such as epizootic hemorrhagic disease virus serotypes 6 and 7, have been detected one after another since 1997. Unexpected emergence of variants and novel viruses will become serious threats to the livestock industry. It is urgently necessary for us to develop more accurate diagnostic and preventive methods for controlling transboundary animal diseases.

Keywords: Foot-and-Mouth Disease, Highly pathogenic avian influenza, Porcine epidemic diarrhea, Arthropod-borne viral diseases, Epidemiology, Diagnosis, Pathogenicity

INTRODUCTION

It is apparent that infectious diseases of animals threaten the livestock industry and food safety. In particular, highly contagious viral diseases which can spread over the borders cause socio-economic problems in the world. Many factors, like worldwide expansion of trade and movement of humans, rapid growth in the livestock industry mainly in Asia, climate change (global warming), artificial environmental changes, affect the occurrence and epidemiology of such transboundary animal diseases including emerging and re-emerging zoonotic diseases. Studies on the transboundary animal diseases have become increasingly essential for disease control in order to contribute to the progress of the livestock industry and the international trade of animals and animal products.

We have been continuously collecting and analyzing the field strains and sera obtained from the virus-infected animals in collaboration with the Ministry of Agriculture, Forestry and Fisheries, prefectural livestock hygiene service centers and veterinary diagnostic laboratories in Japan for monitoring important viral diseases. So far, our scientific data have contributed for the improvement of control measures of animal diseases so far. Indeed, we could successfully eradicate classical swine fever (April, 2007), foot-and-mouth disease (February, 2011), Newcastle disease (September, 2012) and highly pathogenic avian influenza (April, 2015). In this paper, the current status and research topics of representative transboundary animal
OUTBREAKS OF TRANSBOUNDARY ANIMAL DISEASES IN JAPAN

Foot-and-mouth disease

An outbreak of foot and mouth disease (FMD) was recorded in Japan in the spring of 2000, the first in 92 years (Sakamoto et al., 2002). Between 25 March and 11 May, four farms in two prefectures were infected. However, the disease was eradicated without resorting to vaccination, through a campaign of culling, movement control of cloven-hoofed animals in areas surrounding infected premises, and intensive clinical and serological surveillance. Japan regained FMD-free status by the end of September 2000. In this case, FMDV was isolated from probang (esophageal-pharyngeal fluid) materials of cattle and subsequent analyses indicated that the virus is classified into the PanAsia lineage in the Middle East-South Asia (ME-SA) topotype of serotype O. Coincidental outbreaks caused by the PanAsia strain occurred in the Republic of Korea, Russia and Mongolia from March to April 2000. Interestingly, native animals infected with the PanAsia strain did not always develop as an obvious clinical disease.

FMD occurred for the first time in a decade in Japan (Muroga et al., 2012). The index case was detected on a beef-breeding farm in Miyazaki Prefecture, Southern Japan, on April 20, 2010. FMD epidemics in this area have caused severe damage to the local livestock and its related industries. FMDV isolated from affected animal was identified as a serotype O strain in the Southeast Asia (SEA) topotype, Mya-98 lineage. After confirmation of this first case, control measures such as stamping out, movement restriction and disinfection were implemented. However, these strategies proved insufficient to prevent the spread of FMD and emergency vaccination was adopted. Up until the last outbreak on July 4, 2010, a total of 292 outbreaks had been confirmed, with about 290,000 animals having been culled. The epidemic occurred in an area with a high density of cattle and pigs, making disease control difficult. Invasion of the disease into a high-density area aided its rapid spread and led to difficulties in locating suitable burial sites. Epidemiological investigations indicated that the disease was introduced into Japan approximately one month before detection. This delay in initial detection is considered to have allowed an increased number of outbreaks in the early stage of the epidemic. Nevertheless, the epidemic was contained within a localized area in Miyazaki Prefecture and was eradicated within three months because of intensive control efforts including emergency vaccination.

Highly pathogenic avian influenza

In Japan, the outbreaks caused by H5N1 highly pathogenic avian influenza viruses (HPAIVs) occurred in chicken farms in 2004 and 2007. These isolates in 2004 and 2007 were classified genetically into clades 2.5 and 2.2, respectively. In addition, H5N1 HPAIVs were isolated from jungle crows, mountain hawk eagles and whooper swans in 2004, 2007 and 2008, respectively.

At lakes in the northernmost part of Hokkaido prefecture on 14 October 2010, H5N1 HPAIVs were isolated from fecal samples of ducks flying from their nesting lakes in Siberia. Since then, H5N1 HPAIVs have been isolated from 63 wild birds including migrating and resident birds in 17 prefectures, and caused large-scale outbreaks of HPAI in 24 chicken farms in nine prefectures by the end of March in 2011 (approximately 1,830,000 chickens were destroyed in the 2010-2011 winter season) (Sakoda et al., 2012, Soda et al., 2013). Each of these isolates was genetically closely related to the isolates at a lake in Hokkaido, and those in China, Mongolia, Russia and Korea, belonging to genetic clade 2.3.2.1. In addition, these isolates were genetically classified into three groups, suggesting that the viruses were transmitted by migratory water birds through at least three different routes from their northern territory to Japan. The viruses in each group were continuously isolated in respective limited areas, indicating that viruses were
maintained in local bird populations throughout the outbreak periods. Some viruses were genetically closely related to the Korean isolates around the same periods, suggesting that migratory birds were suspected of contributing to transportation of the viruses across the sea. Viruses were recovered from systemic tissues including digestive organs of the deceased raptors, indicating that they were infected with HPAIVs by their predatory behavior, eating infected birds or carrion in the environment.

Since 2013, H5N2, H5N6 and H5N8 HPAIVs have emerged in the world one after another by genetic reassortment among avian influenza viruses derived from poultry and wild birds. An H5N8 HPAI outbreak on a broiler chicken farm was confirmed in Kumamoto prefecture in Japan in April 2014 (Kanehira et al., 2015). H5N8 HPAIVs were also isolated from wild bird specimens in several prefectures, and this was followed by disease outbreaks in poultry in Miyazaki, Yamaguchi, Okayama and Saga prefectures in southern Japan from December 2014 to January 2015.

These HPAI outbreaks in 2004, 2007, 2010-2011 and 2014-15 in Japan were controlled by the culling of chickens in the relevant farms, intensive surveillance and improved biosecurity measures.

Porcine epidemic diarrhea

Since late 2010, severe porcine epidemic diarrhea (PED) outbreaks with considerable morbidity and high mortality among suckling pigs were reported in China. The first case of PED in the United States (US) occurred in April 2013, and PED spread rapidly to over 30 states in the first year. Two main types of PEDVs have been identified in the US based on the genetic analysis of the spike (S) gene: original highly virulent US PEDV strains (defined as the North American type) and S INDELs PEDV strains, which contain insertions and deletions in the 5′ terminus of the S gene.

In Japan, PED was first reported in 1982. In 1996, PED outbreaks occurred in 80,000 pigs in 102 farms in nine prefectures, and approximately half of the affected pigs died. Thereafter, PED was listed as a notifiable infectious disease in Japan, resulting in the establishment of immunohistochemical methods for detecting PEDV. In October 2013, an outbreak of PED re-emerged in Japan after a period of seven years without a reported case. Over 1000 outbreaks of PED in nearly all (39/47) prefectures of Japan have occurred, as reported by the Ministry of Agriculture, Forestry and Fisheries. Above 1.2 million pigs have shown typical symptoms such as anorexia, vomiting and watery diarrhea, and approximately 380,000 pigs have died from October 2013 through August 2014. Sequence analyses of the Japanese strains isolated from 2013 to 2014 indicated that these were genetically distinct from the strains reported previously in Japan, but were related to the strains recently circulating in the US, Korea, and China (Suzuki et al., 2015). It is suggested that PED virus strains detected almost simultaneously in the US, Korea, China and Japan are derived from the common origin. New strains have probably invaded from overseas and rapidly spread throughout Japan since 2013.

Arthropod-borne viral diseases in cattle

Arthropod-borne virus (arbovirus) infections in cattle are frequently reported and have caused serious damage to the beef and dairy industries in Japan (Forman et al., 2008). Large outbreaks of abnormal deliveries in cattle, such as abortion, stillbirth, premature birth and congenital malformations, have been periodically caused by arboviruses, such as Akabane and Aino viruses of the genus Orthobunyavirus of the family Bunyaviridae, and Chuzan virus of the genus Orbivirus of the family Reoviridae. Akabane virus is considered to be the most important veterinary arbovirus in Japan because it has caused extensive damage at least five times, and a significant prevalence of the virus was detected almost every year over five decades from 1959 by virus isolation and serological surveillance. It is estimated that more than 42,000 abnormal calves were born during the largest outbreak, from 1972 to 1975, associated with economic losses of more than US$50 million. Akabane virus was also associated with bovine epizootic encephalomyelitis in 2006
and 2011 (Table 1). Seasonal epidemics of acute febrile illness in cattle caused by bovine ephemeral fever virus of the genus Ephemerovirus of the family Rhabdoviridae have been sporadically observed. Before the 1980s, Ibaraki virus, a strain of epizootic hemorrhagic disease virus (EHDV) serotype 2, was involved in large outbreaks of diseases in cattle characterized by fever and deglutitive disorder, so-called ‘Ibaraki disease’. A strain of EHDV serotype 7, which was initially regarded as an IBAV variant, was widely spread in the western part of Japan in 1997 and caused a large outbreak of bovine abortion in the spread area. In 2015, EHDV serotype 6 has also emerged in Japan and infected cattle showed Ibaraki-like disease. Recently, Peaton, Sathuperi and Shamonda viruses of the genus Orthobunyavirus and D’Aguilar virus of the genus Orbivirus were newly confirmed and were thought to be involved in congenital malformations of cattle. These viruses are thought to be transmitted by Culicoides biting midges (Diptera: Ceratopogonidae) and are probably introduced with the infected midges from overseas to Japan by seasonal winds every summer.

Subclinical infections are often caused by arboviruses in cattle. In the case of congenital abnormalities by Akabane, Aino and Chuzan viruses, the affected calves are generally noted several months after the virus has spread. Therefore, an early warning system is needed to detect the virus incursion and spread before clinical cases are apparent. Monitoring in vectors and sentinel animals is certainly an important component of systems for rapid and definite detection of arboviral activity. The southern end of the main islands of Japan is thought to be one of the gateways for arbovirus incursion from overseas. Indeed, the nationwide surveillance for bovine arboviral diseases in Japan indicated that the transmission was often started in the southwestern region.

Table 1. Bovine arboviruses identified in Japan since 1959

<table>
<thead>
<tr>
<th>Virus (Family, Genus, Species)</th>
<th>First isolation year</th>
<th>Symptoms of infected cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bunyaviridae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Orthobunyavirus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Akabane virus</td>
<td>1959</td>
<td>Abortion, Stillbirth, Premature Birth, AH syndrome, Encephalomyelitis</td>
</tr>
<tr>
<td>Aino virus</td>
<td>1964</td>
<td>Abortion, Stillbirth, Premature Birth, AHCH syndrome</td>
</tr>
<tr>
<td>Peaton virus</td>
<td>1987</td>
<td>AH syndrome ? (27 suspected cases)</td>
</tr>
<tr>
<td>Sathuperi virus</td>
<td>1999</td>
<td>AH syndrome ? (2 suspected cases)</td>
</tr>
<tr>
<td>Shamonda virus</td>
<td>2002</td>
<td>AH syndrome ? (3 suspected cases)</td>
</tr>
<tr>
<td>Batai virus</td>
<td>1994</td>
<td>Subclinical</td>
</tr>
<tr>
<td><strong>Reoviridae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Orbivirus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bluetongue virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serotype 2</td>
<td>2007</td>
<td>Subclinical</td>
</tr>
<tr>
<td>Serotype 3</td>
<td>1998</td>
<td>Subclinical</td>
</tr>
<tr>
<td>Serotype 9</td>
<td>2003</td>
<td>Subclinical</td>
</tr>
<tr>
<td>Serotype 12</td>
<td>1990</td>
<td>Subclinical</td>
</tr>
<tr>
<td>Serotype 16</td>
<td>1985</td>
<td>Subclinical</td>
</tr>
<tr>
<td>Serotype 21</td>
<td>1989</td>
<td>Fever, Facial edema and hemorrhages, Ulceration of the mucous membranes</td>
</tr>
<tr>
<td>Epizootic hemorrhagic disease virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serotype 1</td>
<td>1984</td>
<td>Subclinical</td>
</tr>
<tr>
<td>Serotype 2 (Ibaraki virus)</td>
<td>1959</td>
<td>Fever, Deglutitive disorder</td>
</tr>
<tr>
<td>Serotype 6</td>
<td>2015*</td>
<td>Fever, Deglutitive disorder</td>
</tr>
<tr>
<td>Serotype 7</td>
<td>1997</td>
<td>Fever, Deglutitive disorder, Abortion, Stillbirth</td>
</tr>
<tr>
<td>Palyam virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chuzan virus (Kasba virus)</td>
<td>1984</td>
<td>HCH# syndrome</td>
</tr>
<tr>
<td>D’Aguilar virus</td>
<td>1987</td>
<td>HCH syndrome</td>
</tr>
</tbody>
</table>
Bunyip Creek virus 2008 Subclinical

*Rhabdoviridae*

Ephemerovirus

Bovine ephemeral fever virus 1966 Fever, Anorexia, Muscle stiffness, Sternal recumbency, Ruminal stasis, Lameness

Kern Canyon group

Fukuoka virus 1982 Subclinical

*a* Epizootic hemorrhagic disease virus serotype 6 was genetically identified. Virus isolation is still in progress.

*b* AH: arthrogryposis and hydranencephaly

*c* AHCH: arthrogryposis, hydranencephaly and cerebellar hypoplasia

*d* HCH: hydranencephaly and cerebellar hypoplasia

**RECENT RESEARCH TOPICS ON TRANSBOUNDARY ANIMAL DISEASES**

Representative results of recent studies on transboundary animal diseases performed in National Institute of Animal Health, Japan, are as follows.

**Development of diagnostic methods using monoclonal antibodies for foot-and-mouth disease**

We developed monoclonal antibody-based sandwich direct enzyme-linked immunosorbent assay (MSD-ELISA) for antigen detection of foot-and-mouth disease virus (FMDV) (Morioka *et al*, 2014). We evaluated and compared the sensitivity and specificity of MSD-ELISA with conventional indirect sandwich (IS)-ELISA by using both experimental samples and field samples. We developed two types of MSD-ELISA, using different detection monoclonal antibodies: (1) for multiple serotypes and (2) for single serotypes for each serotype (O, A, Asia1). The MSD-ELISAs could detect the antigen in saliva samples of experimentally infected pigs of other topotypes of serotypes O, A and Asia1 for a longer term than IS-ELISA. We also used 178 FMDV positive field samples from cattle and pigs affected by the 2010 type-O FMD outbreak in Japan, and found that the sensitivity of both MSD-ELISAs was about 7 times higher than that of the IS-ELISA for each sample. Further, the sensitivity of the MSD-ELISAs was about 6 times higher than that of the IS-ELISA for each farm, with respect to the FMD-positive farm detection rate. Our MSD-ELISAs could be a better method than IS-ELISA for FMD antigen detection.

We also developed a lateral flow strip using monoclonal antibodies which allows for rapid antigen detection and serotyping of FMDV (Morioka *et al*, 2015). This serotyping strip was able to detect all 7 serotypes and distinguish serotypes O, A, C and Asia1. Its sensitivity is equal to those of the commercial product Svanodip (Boehringer Ingelheim Svanova, Uppsala, Sweden), which can detect all seven serotypes of FMDV, but does not distinguish them. Our evaluation of the FMDV serotyping strip using a total of 118 clinical samples showed highly sensitive antigen detection and accuracy in serotyping in accordance with ELISA or reverse transcription-polymerase chain reaction (RT-PCR). This FMDV serotyping strip provides both rapid antigen detection and serotyping of FMDV at the same time on one strip without extra devices. This method will be useful in both FMD-free countries and FMD-infected countries, especially where laboratory diagnosis cannot be carried out.

**Characterization of highly pathogenic avian influenza virus and attempts to develop new vaccines**

A highly pathogenic avian influenza virus (HPAIV) of subtype H5N8, A/chicken/Kumamoto/1-7/2014 (HA clade 2.3.4.4.), was isolated from a Japanese chicken farm during an outbreak in April 2014. All eight genomic segments of this strain showed high sequence similarity to those of the H5N8 subtype HPAIVs which were isolated in Korea in January 2014 (Kanehira *et al*, 2015). Intranasal experimental infection of chickens and ducks with A/chicken/Kumamoto/1-7/2014 was performed to assess the pathogenicity of the
virus in chickens and the potential for waterfowl to act as a virus reservoir and carrier. A high-titer virus challenge was lethal in chickens, but they were unaffected by lower virus doses. Virus challenge at all doses examined was found to result in asymptomatic infection of ducks. A/chicken/Kumamoto/1-7/2014 possessed relatively low cross-reactivity with H5 viruses belonging to clades other than clade 2.3.4.4. These results suggest that waterfowl may be able to spread the virus even if they possess antibodies resulting from a previous infection with H5 HPAIV that was antigenically distinguishable from viruses belonging to clade 2.3.4.4.

The series of basic amino acids at the cleavage site of the hemagglutinin protein (HA) of the HPAIV is responsible for pathogenicity; however, the role of the internal gene products of HPAIVs in their pathogenicity has not been well established. Reverse genetics was utilized to generate artificial viruses with amino acid substitutions in the PB1 protein, one of the components of viral RNA polymerase, along with the HA from an HPAIV (Suzuki et al., 2014). A substitution at amino acid position 38 of the PB1 protein from cysteine to tyrosine (C38Y) enhanced viral polymerase activity by 5-fold. A valine-to-alanine substitution at position 14 (V14A) of the PB1 protein reduced the polymerase activity by 5-fold. An experimental infection study with the artificial viruses demonstrated that the C38Y substitution recovered the lethality of the virus and that the V14A substitution reduced the transmissibility of the virus in chickens. These results demonstrated that amino acid substitutions in the PB1 protein are involved in the pathogenicity of HPAIVs.

A vaccine for HPAIVs was produced using attenuated H5 subtype vaccine strains generated by reverse genetics (Uchida et al., 2014). The strain contained the HA gene from the H5N1 subtype HPAIV attenuated by genetic modification at the cleavage site and the neuraminidase (NA) gene derived from the H5N1 subtype HPAI or the H5N3 subtype of avian influenza virus. The vaccinated chickens could be distinguished from unvaccinated, infected chickens by detection of N3 antibody in chickens vaccinated with H5N3 subtype strain, after challenge with H5N1 subtype HPAIV. There were no differences in hemagglutinin inhibition titer, the survival rate of chickens, and the titer of shed virus, upon vaccination with either H5N1 or H5N3 strains followed by viral challenge. Vaccination with five times higher dose of antigen than the normal dose was effective in increasing survival and efficiently reduced viral shedding even on challenge with a virus of a different HA clade. The use of reverse genetics would be an option for prompt production of an inactivated vaccine with better matching of antigenicity to a circulating strain.

Molecular and pathogenic characterization of porcine epidemic diarrhea virus

We determined the whole-genome sequences of 38 PED virus (PEDV) strains from diarrheal samples collected at swine farms in 18 prefectures between 2013 and 2014 using next-generation sequencing technology (Suzuki et al., 2015). Eleven out of 38 PEDV strains were isolated successfully and subjected to genome sequence analysis. In a comparative genome analysis, we detected two novel PEDV variants, TTR-2/JPN/2014 and MYG-1/JPN/2014, with large deletions in the spike (S) and ORF3 genes, respectively. A phylogenetic analysis based on the S gene showed that the 38 Japanese PEDV strains were classified into two PEDV types: the North American type with high virulence and the INDEL type. In addition, the recent Japanese PEDV isolates had a close relationship to global PEDV strains isolated in recent years than to the classical PEDV strains detected in Japan the past decades ago. Moreover, the phylogenetic tree of the complete genomes also indicated that the 38 Japanese PEDV strains, including the two novel PEDV variants discovered in this study, are closely related to the PEDV strains that were widespread in the United States and Korea in 2013-2014. These findings suggest that the re-emergence of PED outbreaks since the last reported case in 2006 was caused by the introduction of recent PEDV strains to Japan from overseas.

We identified a third PEDV S variant with a large deletion of 582 nucleotides in the S gene, in addition to the North American type and the S INDELs type. To investigate the pathogenicity of this variant, TTR-2/JPN/2014, we performed experimental infection using colostrum-deprived piglets and compared the
results with those from the North American type PEDV, OKN-1/JPN/2013 (Suzuki et al., 2016). Fifteen newborn piglets were divided into two groups of 7–8 piglets each and inoculated orally with the one of PEDV isolates maintained at the eighth passage in Vero cell culture. Although all PEDV-inoculated piglets showed acute watery diarrhea, lethality clearly differed between both PEDV-inoculated groups. Moreover, there were differences in virus distribution and lesions on the intestines between the two PEDV-inoculated groups. Therefore, our data suggest that the OKN-1/JPN/2013 PEDV isolate is virulent, whereas the TTR-2/JPN/2014 PEDV isolate is avirulent.

**Ecological analyses and development of molecular diagnostic methods for arthropod-borne viruses**

Epizootic congenital abnormalities, encephalomyelitis and febrile illnesses in cattle caused by arthropod-borne viruses (arboviruses) are prevalent in Japan. Causative viruses including orthobunyaviruses, orbiviruses and rhabdovirus are thought to be transmitted by *Culicoides* biting midges (Table 1). Recently, the incursions of several arboviruses, potentially *Culicoides*-borne, were newly confirmed in Japan. However, their spread pattern and exact vector species are currently uncertain. Attempts to isolate arboviruses from *Culicoides* biting midges and sentinel cattle were conducted at the southernmost end of the main islands of Japan, a potentially high-risk area for incursion of arboviral diseases and outbreak of endemic ones. Seventy-eight isolates comprising Akabane, Peaton and Sathuperi viruses of the genus *Orthobunyavirus* of the family *Bunyaviridae*, bluetongue virus serotype 16, D’Aguilar virus, Bunyip Creek virus and epizootic hemorrhagic disease virus serotype 1 of the genus *Orbivirus* of the family *Reoviridae*, a potentially novel rhabdovirus of the genus *Ephemerovirus* and unidentified orbivirus-like viruses were obtained from *Culicoides* biting midges and sentinel cattle between 2003 and 2013 (Kato et al., 2015). Akabane, Sathuperi, D’Aguilar and Bunyip Creek viruses were selectively isolated from *Culicoides oxyстoma*, suggesting this vector’s responsibility for these arbovirus outbreaks. The results of virus isolation also implied that *C. tainanus*, *C. jacobsoni* and *C. punctatus* are competent for the transmission of bluetongue virus serotype 16, Peaton virus and epizootic hemorrhagic disease virus serotype 1, respectively. Our monitoring in *Culicoides* biting midges and sentinel cattle detected the circulation of Akabane virus just prior to the accumulations of bovine congenital abnormalities and encephalomyelitis by it around study sites in 2003, 2006, 2008 and 2013. Silent circulations of the other arboviruses, including potentially new viruses, were also detected during the study period.

TaqMan assays were developed for the broad-range detection of arboviruses belonging to Simbu serogroup lineage 1 in the genus *Orthobunyavirus* and also for the specific detection of three viruses in the lineage, Akabane, Aino and Peaton viruses (Shirafuji et al., 2015). All of the four primer and probe sets successfully detected targeted viruses, and thus broad-range and specific detection of all the targeted viruses can be achieved by using two multiplex assays and a single assay in a dual (two-color) assay format when another primer and probe set for a bovine-actin control is also used. Diagnostic sensitivity of the assays was tested with field-collected bovine samples, and the results suggested that the sensitivity was higher than that of a conventional reverse transcription-polymerase chain reaction (RT-PCR). These data indicate that the newly developed TaqMan assays will be useful tools for the diagnosis and screening of field-collected samples for infections of Akabane virus and several other arboviruses belonging to the Simbu serogroup lineage 1.

**CONCLUSION**

Emergence of variants and novel viruses will lead to unprecedented outbreaks of transboundary infectious diseases that cause serious losses of livestock and problems of human health. From now on, it will be necessary to strengthen international relationship among veterinary organizations through comparing and sharing of scientific data on transboundary animal diseases.
REFERENCES


