Molecular epidemiology of Akabane virus in Taiwan

Hau-You Tzeng¹ | Cheng-Lung Tsai¹,² | Lu-Jen Ting³ | Kuei-Min Liao¹,⁴ | Wu-Chun Tu¹

¹Department of Entomology, National Chung Hsing University, Taichung City, Taiwan
²Department of Biomedical Science and Environmental Biology, Kaohsiung Medical University, Kaohsiung City, Taiwan
³Council of Agriculture, National Institute for Animal Health, New Taipei City, Taiwan
⁴National Mosquito-Borne Diseases Control Research Center, National Health Research Institutes, Kaohsiung City, Taiwan

Correspondence
Wu-Chun Tu, Department of Entomology, National Chung Hsing University, 145, Xingda Rd., South District, Taichung City, Taiwan.
Email: wctu@dragon.nchu.edu.tw

Funding information
Bureau of Animal and Plant Health Inspection and Quarantine, Council of Agriculture, Executive Yuan, Taiwan, Grant/Award Number: 104AS-10.10.3-BQ-B1

Abstract
Background: Akabane virus (AKAV) is a teratogenic and neuropathogenic arbovirus that infects livestock and wild animals. AKAVs are endemic arboviruses from dairy farms in Taiwan in 1989, and the first sequence was detected in cattle with nonsuppurative encephalitis in 1992.

Objectives: This study aims to understand the epidemiological relationships of the akabane viruses between Taiwan and nearby places.

Methods: In this study, 17 specimens were identified or isolated from vector insects, and ruminant fetuses collected from 1992 to 2015 were sequenced and analysed.

Results: Sequence analyses revealed all Taiwanese AKAVs belonged to genogroup Ia but diverged into two clusters in the phylogenetic trees, implying that at least two invasive events of AKAV may have occurred in Taiwan.

Conclusions: The two clusters of AKAVs could still be identified in Taiwan in 2015, and a reassortment event was observed, indicating that the two clusters of AKAVs are already endemic in Taiwan.

KEYWORDS
Akabane virus, cattle, Culicoides biting midge, mosquito, orthobunyavirus

1 INTRODUCTION

Akabane virus (AKAV; belonging to the genus Orthobunyavirus, family Peribunyaviridae) is an arthropod-borne virus that causes premature birth, abortion, stillbirth and congenital abnormalities with arthrogryposis hydranencephaly syndrome in ruminants (Yanase et al., 2020). This virus may also cause bovine encephalitis and encephalomyelitis in calves and cows (Kono et al., 2008; Liao, Lu, Goto & Inaba, 1996; Oem et al., 2012). There are two major endemic zones of AKAV distribution worldwide, where one extends from East Asia to Southeast Asia to Australia and the other extends from the Middle East to South Africa (Al-Busaidy et al., 1987; Ojuoţlu et al., 2015; Taylor & Mellor, 1994; Wang et al., 2019; Yanase et al., 2020).

The AKAV genome, similar to that of other orthobunyaviruses, consists of three negative-stranded RNA segments, that is S, M and L segments named according to size (small, medium and large, respectively) (Elliott, 2014). The encoded proteins in the genome corresponded with those of other orthobunyaviruses, and the genetic analysis exhibited higher conservation of nucleoprotein (N) on the S segment and RNA-dependent RNA polymerase (RdRp) on the L segment than those on the M segment, which encodes a polyprotein composed of virion glycoproteins (Gc and Gn) and a nonstructural protein (NSm) (Elliott, 2014). Four genogroups (I–IV) and two lineages in genogroup I (Ia and Ib) were identified through phylogenetic analysis of two full-length open reading frames of S and M segments and partial sequences of the L segment (Kobayashi et al., 2007).
A few AKAV isolates have been sequenced in Taiwan. PT-17 was first recognised in 1992 and was isolated from a calf with nonsuppurative encephalitis (Liao, Lu et al., 1996b); its sequence was found to be similar to that of the Iriki strain. NT-14 was identified in swine tonsils in 2000, indicating that AKAV could naturally infect not only ruminants but also swine (Huang et al., 2003). CY-77 was isolated from bovine erythrocytes in 1993, and the isolate was found to belong to genogroup Ia (Yamakawa et al., 2006).

Culicoide-borne diseases are important transboundary diseases. Viral carriers can undergo long-distance dissemination through wind or monsoons, resulting in spread of the virus from hundreds to thousands of kilometres away from the original outbreak site (Aguilar-Vega et al., 2019; Braverman & Chechik, 1996; Walker & Klement, 2015). To date, nationwide serosurveillance and regional surveillance of vector specimens have shown that AKAV infection is prevalent in Taiwan (Tzeng et al., 2019). However, the origin of Taiwanese AKAVs and their epidemiology remains unclear.

Accordingly, in this study, we reconstructed the epidemiological history of Taiwanese AKAVs by analysing their phylogenetic relationships to improve our understanding of transboundary Culicoid-borne diseases in Taiwan and elsewhere.

2 MATERIALS AND METHODS

2.1 Sample source

In total, 15 samples of Taiwanese AKAVs were identified from Culicoides biting midges, mosquitoes, bovine tissues and goat tissues from 1992 to 2015, whereas the other two isolates Tainan/17H8 and 93H78 AKAV were provided by the Council of Agriculture National Institute for Animal Health, Taiwan (Table 1). The locations of all the AKAVs evaluated in this study are labelled on the map of Taiwan shown in Figure 1. The S, M and partial L segments were sequenced in all 17 samples, 14 samples and 12 samples, respectively. The GenBank accession numbers of the genome sequences are as follows: MF278855–MF278882, MT676825–MT676826 and MZ501031–MZ501043.

2.2 RNA preparation, polymerase chain reaction amplification and cloning

RNA extraction from vector insects was conducted using the standard protocol from the PureLink RNA extraction kit (Thermo Fisher Scientific, Waltham, MA USA), as described in our previous study (Tzeng et al., 2019). RNA from bovine tissues of infected cattle was obtained from the Animal Health Research Institute. cDNA synthesis was conducted using an iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA, USA), and viral genes were amplified using an AccurPrime pfX DNA polymerase kit (Thermo Fisher Scientific). The primers for the S segment were obtained from Yamakawa et al. (2006), and the primers for the M segment were obtained from An et al., and Park (2010). The primers for the L segment were designed from the consensus sequences of AKAV-7/SKR/2010 (JQ308779), OBE-1 (NC_009894.1) and DHL10M110 (KY284021) by an alignment result. All primers used are listed in Table S1. The amplicons were analysed on 1% agarose gels, and amplicons with the predicted size were extracted with a Quick Gel Extraction Kit (Thermo Fisher Scientific). To insert the amplicons into the desired plasmid vectors, a 3’ A was added to the amplicons extracted with rTaq DNA polymerase (Takara Bio, Shiga, Japan) at 72°C for 10 min, and the products were then directly ligated into the pGEM-T easy vector with T4 ligase (Promega, Madison, WI, USA). The transformation and colony selection procedures were described previously (Tzeng et al., 2019).

2.3 Sequencing and genome assembly

After the amplicon of each AKAV segment was cloned into a pGEM-T easy vector, at least three vectors were selected for sequencing, and both sites on the pGEM-T easy vector using T7 and SP6 primers were implemented for each sample for confirmation. Sequencing was
then conducted using a sequencing service (Genomic Ltd., Taipei, Taiwan). The sequencing data were trimmed using VectorNTI 10 Advanced (Thermo Fisher Scientific) to remove plasmids and unqualified sequences. Then, the partial genome sequences of the M segment were assembled into genome contigs using ContigExpress in VectorNTI 10 Advanced (Thermo Fisher Scientific).

2.4 Sequence analysis for elucidation of genetic characteristics and phylogeny

Phylogenetic analysis was carried out as described by Kobayashi et al. (2007) and Yanase et al. (2018). Each nucleotide sequence and its deduced amino acid sequence were aligned with downloaded sequences from GenBank (Table S2) using ClustalW in the AlignX program in VectorNTI 10 Advanced (Thermo Fisher Scientific). Construction of a neighbour-joining tree was performed using the alignment results of each nucleotide sequence with Molecular Evolutionary Genetics Analysis version X or MEGA X (Kumar et al., 2018), and the best nucleotide substitution model was determined using the best-fit model in MEGA X. The reliability of tree construction was examined using 1000 bootstrap test repeats. The sequences of the Sabo virus were employed in the analysis to determine the relative position of the Taiwanese AKAVs in the phylogenetic tree. All Taiwanese AKAV sequences were uploaded to GenBank, and the accession numbers for the obtained sequences are listed in Table 1.

### TABLE 1 Taiwanese AKAVs isolated from 1992 to 2015

| Isolate/sample | Source | Year | County | Accession no. of genome segment |
|---------------|--------|------|--------|---------------------------------|
| PT-17         | Bovine blood | 1992 | Pingtung | AF034940 |
| CY-77         | Bovine erythrocytes | 1993 | Chiayi | AB232319 AB297851 AB297886 |
| Tainan/17H8   | Bovine | 1993 | Tainan | MF278879 MF278865 MZ501032 |
| 93T78         | Bovine | 1993 | Tainan | MF278877 MF278864 MZ501031 |
| NT-14         | Swine tonsil | 2000 | Nantou | AF529883 |
| 08H65-1       | Bovine | 2008 | Hualien | MF278881 MF278862 MZ501033 |
| 08H65-2       | Bovine | 2008 | Hualien | MF278880 MF278863 MZ501034 |
| YL-1/AKA/C/12/TW | Culicoides spp. | 2012 | Yunlin | MF278866 MF278855 MZ501035 |
| YL-2/AKA/C/12/TW | Culicoides spp. | 2012 | Yunlin | MF278867 MF278857 MZ501036 |
| PT-1/AKA/C/12/TW | Culicoides spp. | 2012 | Pingtung | MF278870 MF278857 MZ501038 |
| HL-1/AKA/C/12/TW | Culicoides spp. | 2012 | Hualien | MF278868 MF278858 MZ501039 |
| YL-3/AKA/C/12/TW | Culicoides spp. | 2012 | Yunlin | MF278871 MF278859 MZ501037 |
| HL-3/AKA/M/12/TW | Anopheles spp. | 2012 | Hualien | MF278869 |
| 12H37         | Bovine | 2012 | Tainan | MF278873 MF278861 MZ501040 |
| 13H33         | Bovine | 2013 | Yunlin | MF278872 |
| 13H35         | Bovine | 2013 | Taoyuan | MF278875 MF278860 MZ501042 |
| 13H22         | Goat | 2013 | Changhua | MF278876 |
| KH-1/AKA/C/13/TW | Culicoides spp. | 2013 | Kaohsiung | MF278882 MT676825 MZ501043 |
| YL-1/AKA/C/15/TW | Culicoides oxystoma | 2015 | Yunlin | MF278874 MT676826 |
| YL-2/AKA/M/15/TW | Anopheles sinensis | 2015 | Yunlin | MF278878 MT676827 |

3 | RESULTS

All Taiwanese AKAVs belonging to genogroup Ia were examined by phylogenetic analyses of the S, M and L segments (Figure S1). Determination of clusters in genogroup Ia relied on two criteria: (1) clusters should be monophyletic groups and (2) partial members in clusters should have relationships identified in prior phylogenetic or spatial-temporal analyses. In total, nine clusters were identified (clusters A–J) in the phylogenetic tree of the S segment (Figure 2a), and the same clusters were also identified in the phylogenetic trees of the M and L segments (Figure 2b and c). The Taiwanese AKAVs were distributed in clusters A and J on the phylogenetic trees. The intracluster nucleotide identities were 98.7–100% in cluster A and 98.3–100% in cluster J; intercluster identities for the S, M and partial L (543 bp) segments were 95.6–96.1%, 95.3–97.7% and 93.5–96.4%, respectively. In addition, the intracluster amino acid identities were 99.1–100% in each cluster; intercluster identities for the S, M and partial L segments were 98.3%–100%, 96.1–100% and 97.7–100%.

The AKAVs within cluster A were isolated since 2012 in Taiwan and in 2012 from Okinawa, Japan. Cluster J consisted of genogroup Ia of AKAVs isolated from mainland Japan in the 1980s and Okinawa, Japan in the 1990s. The Taiwanese AKAVs were distributed in clusters A and J on the phylogenetic trees. The intracluster nucleotide identities were 98.7–100% in cluster A and 98.3–100% in cluster J; intercluster identities for the S, M and partial L (543 bp) segments were 95.6–96.1%, 95.3–97.7% and 93.5–96.4%, respectively. In addition, the intracluster amino acid identities were 99.1–100% in each cluster; intercluster identities for the S, M and partial L segments were 98.3%–100%, 96.1–100% and 97.7–100%.

The AKAVs within cluster A were isolated since 2012 in Taiwan and in 2012 from Okinawa, Japan. Cluster J consisted of genogroup Ia of AKAVs isolated from mainland Japan in the 1980s and Okinawa, Japan in the 1990s. The AKAVs within-cluster J were highly related to the Taiwanese isolates, including PT-17, NT-14 and CY-77. The same clustering on the S segment tree was also mapped on the phylogenetic tree of the M and L segments, indicating that there was some consistency in the Taiwanese AKAVs in comparison to the phylogenetic
FIGURE 2  Three phylogenetic subtrees of Akabane orthobunyavirus (AKAV) in Taiwan were constructed with the sequences of S, M and L segments. The three comprehensive trees are shown in Figure S1 in the Supplementary Information. (a) The phylogenetic tree was constructed with the 699-bp full-length nucleoprotein (N) gene on the S segment using the T92+G nucleotide substitution model. (b) The phylogenetic tree was constructed with the 4206-bp full-length Gc-NSm-Gn polyprotein gene on the M segment using the GTR+G+I nucleotide substitution model. (c) The phylogenetic tree was constructed with the 543-bp partial RNA-dependent RNA polymerase gene on the L segment using the T92+G nucleotide substitution model. Clusters A–J were determined using the S segment-based tree, and the same clustering is labelled on the M segment- and L segment-based subtrees behind the names of the sequences.
FIGURE 2 Continued
A reassortment AKAV, KH-1/AKA/C/13/TW, was observed in our analytical results indicated that at least two invasive events have become endemic viruses, and the new more invasive virus has established the current AKAV populations in Taiwan. The serological prevalence of AKAV in dairy farms indicated an 89% infection rate in 1989 (Liao et al., 1996a); however, the first S segment of Taiwanese AKAV, that is, PT-17, from cattle with encephalitis was isolated and sequenced in 1992 (Liao et al., 1996b). From these findings, it was still unclear whether the Taiwanese AKAVs found in 1992 were caused by the resurgence of the endemic AKAV prevalent before 1989 or represented a new invasive strain. Our data combined with records supported that a single invasion occurred before 1989 to establish Taiwanese AKAVs (cluster J). Based on Liao et al. (1996a) that observed a sustained epidemic of AKAVs in Taiwan from 1989 to 1994 that corresponded to PT-17, 93H78 and CY-77 were isolated in 1992 and 1993, which were grouped in cluster J. This indicated the isolates from 1989 to 1994 might have come from the same epidemic event, although AKAVs did not be isolated or sequenced in 1989 and 1994. In addition, Taiwanese AKAVs (cluster J) formed a monophyletic group on the phylogenetic trees of the S and L segments, indicating that they may have originated from a single strain and then spread to the whole island of Taiwan, although the members of cluster J did not form a monophyletic group in the phylogenetic tree of the M segment. A possible reason for this may be that members of cluster J included Japanese AKAVs isolated from 1984 to 1985. Our phylogenetic analysis showed that these strains had multiple origins; however, the M and partial L segments were sequenced for only two of these isolates, suggesting that insufficient information was available for analysing the M segment of cluster J. Despite this, the phylogenetic results obtained using sequences of the S and L segments still supported that there was a single invasion of Taiwanese AKAVs (cluster J). Thus, the population of Taiwanese AKAVs (cluster J) may have been established via a single invasion event before 1989. In the case of Taiwanese AKAVs isolated in 2012 (cluster A), the Okinawa isolate ON-1/P/12 was grouped with Taiwanese AKAVs from 2012 to 2015 in cluster A on the trees of the S, M and L segments, indicating that those of Okinawa and Taiwan had the same origin, although that specific origin has not yet been determined. Overall, our findings supported that the population of Taiwanese AKAVs was established through a single invasion event that may have occurred in 2012 and before 1989; however, the data were still insufficient to conclusively determine the origins of Taiwanese AKAVs.

The genetic divergence of AKAV clusters (clusters A and J) observed in our analytical results indicated that at least two invasive events have become endemic viruses, and the new more invasive virus has established the current AKAV populations in Taiwan. The serological prevalence of AKAV in dairy farms indicated an 89% infection rate in 1989 (Liao et al., 1996a); however, the first S segment of Taiwanese AKAV, that is, PT-17, from cattle with encephalitis was isolated and sequenced in 1992 (Liao et al., 1996b). From these findings, it was still unclear whether the Taiwanese AKAVs found in 1992 were caused by the resurgence of the endemic AKAV prevalent before 1989 or represented a new invasive strain. Our data combined with records supported that a single invasion occurred before 1989 to establish Taiwanese AKAVs (cluster J). Based on Liao et al. (1996a) that observed a sustained epidemic of AKAVs in Taiwan from 1989 to 1994 that corresponded to PT-17, 93H78 and CY-77 were isolated in 1992 and 1993, which were grouped in cluster J. This indicated the isolates from 1989 to 1994 might have come from the same epidemic event, although AKAVs did not be isolated or sequenced in 1989 and 1994. In addition, Taiwanese AKAVs (cluster J) formed a monophyletic group on the phylogenetic trees of the S and L segments, indicating that they may have originated from a single strain and then spread to the whole island of Taiwan, although the members of cluster J did not form a monophyletic group in the phylogenetic tree of the M segment. A possible reason for this may be that members of cluster J included Japanese AKAVs isolated from 1984 to 1985. Our phylogenetic analysis showed that these strains had multiple origins; however, the M and partial L segments were sequenced for only two of these isolates, suggesting that insufficient information was available for analysing the M segment of cluster J. Despite this, the phylogenetic results obtained using sequences of the S and L segments still supported that there was a single invasion of Taiwanese AKAVs (cluster J). Thus, the population of Taiwanese AKAVs (cluster J) may have been established via a single invasion event before 1989. In the case of Taiwanese AKAVs isolated in 2012 (cluster A), the Okinawa isolate ON-1/P/12 was grouped with Taiwanese AKAVs from 2012 to 2015 in cluster A on the trees of the S, M and L segments, indicating that those of Okinawa and Taiwan had the same origin, although that specific origin has not yet been determined. Overall, our findings supported that the population of Taiwanese AKAVs was established through a single invasion event that may have occurred in 2012 and before 1989; however, the data were still insufficient to conclusively determine the origins of Taiwanese AKAVs.

Our results revealed that Taiwan and the islands in Okinawa Prefecture shared AKAV epidemics. Indeed, the geographic distance between Okinawa and Taiwan is closer than that between Okinawa and mainland Japan (Figure 3). Notably, some AKAVs from Ishigaki Island and Iriomote Island in the Yaeyama Islands were found to have closer genetic features to Taiwanese AKAVs, indicating that these isolates may have originated from Taiwan (Figures 2a and 3). However, most AKAVs of Okinawa are genetically similar to those from mainland Japan (Yamakawa et al., 2006), implying that the islands of Okinawa may be an intersection for transboundary transmission of AKAVs from both Taiwan and mainland Japan. The long-distance dissemination of Culicoides biting midges by wind is determined to involve in the transmission of bluetongue virus, bovine ephemeral fever virus and AKAV (Aguilar-Vega et al., 2019; Braverman & Chechik, 1996; Walker & Klement, 2015); however, further studies are needed to elucidate the
migrant patterns of Culicoides biting midges among Taiwan and the islands of Okinawa Prefecture.

The geographic distributions of AKAV genogroups exhibited indistinct boundaries. Genogroup Ia is prevalent in southern China and Taiwan but sporadically appears in Japan and South Korea (An et al., 2010; Cao et al., 2019; Kono et al., 2008; Liang et al., 2018; Tang et al., 2017; Tang et al., 2019; Tzeng et al., 2019; Yamakawa et al., 2006; Yanase et al., 2018). Genogroup Ib has been detected sporadically in Israel, Turkey and Indonesia (Oğuzoğlu et al., 2015; Purnomo Edi et al., 2017; Şevik, 2017; Stram et al., 2004; Yamakawa et al., 2006). Furthermore, genogroup II is endemic to Japan and South Korea (An et al., 2010; Kobayashi et al., 2007; Yamakawa et al., 2006), whereas genogroup III only exists in Australia, and genogroup IV is found only in Africa (Wang et al., 2019; Yamakawa et al., 2006). In East Asia, all provinces of China have experienced AKAV epidemics (Wang et al., 2017); however, AKAV sequences have only been obtained from the southern provinces of Yunnan, Guangdong, Guangxi and Hainan. Our results showed that the isolate GD18134, which was from Guangdong, China, was phylogenetically related to isolates from Japan in 2013 (Wu et al., 2020; Yanase et al., 2018) (Figures 2 and 3a). Further analyses of AKAV sequences from China and Southeast Asia are essential to improve our understanding of the temporal and spatial patterns of transboundary transmission of viruses. These studies will also provide insights into the origins of Taiwanese AKAVs. Herein, we provided the first comprehensively phylogenetic data to describe the interaction between AKAVs in Taiwan and nearby islands, also filling up a blank for understanding about molecular epidemiology of AKAV in East Asia.

AUTHOR CONTRIBUTIONS
H. Y. Tzeng and W. C. Tu devised the project, the main conceptual ideas and proof outline. C. L Tsai provided the details of phylogenetic techniques. H. Y Tzeng and K. M. Liao collected all haematophagous insects from sampling farms. L. J. Ting collected the fetus samples and extracted RNAs from the samples. H. Y. Tzeng work for cloning and sequencing of target viral sequences. H. Y. Tzeng and W. C. Tu wrote the manuscript.

ACKNOWLEDGEMENTS
We thank Dr. Fan Lee of the Council of Agriculture National Institute for Animal Health for assistance in the preparation of this manuscript and Prof. Roger F. Hou, Department of Entomology, National Chung Hsing University for critical reading of this manuscript.

CONFLICT OF INTEREST
The authors declare no conflict of interest.
DATA AVAILABILITY STATEMENT
The data that support the findings of this study are openly available in GenBank at https://www.ncbi.nlm.nih.gov/nucleotide/.

ETHICS STATEMENT
The conduction of all experiments followed by the guideline of experimental animal management in Taiwan.

PEER REVIEW
The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.887.

ORCID
Hau-You Tzeng https://orcid.org/0000-0001-6245-8154

REFERENCES
Aguilar-Vega, C., Fernandez-Carrion, E., & Sanchez-Vizcaino, J. M. (2019). The possible route of introduction of bluetongue virus serotype 3 into Sicily by windborne transportation of infected Culicoides spp. Transboundary and Emerging Diseases, 66, 1665–1673.

Al-Busaidy, S., Hamblin, C., & Taylor, W. P. (1987). Neutralising antibodies to Akabane virus in free-living wild animals in Africa. Tropical Animal Health and Production, 19, 197–202.

An, D.-J., Yoon, S. H., Jeong, W., Kim, H.-J., & Park, B.-K. (2010). Genetic analysis of Akabane virus isolates from cattle in Korea. Veterinary Microbiology, 140, 49–55.

Braverman, Y., & Chechik, F. (1996). Air streams and the introduction of animal diseases borne on Culicoides (Diptera, Ceratopogonidae) into Israel. Revue Scientifique Et Technique (International Office of Epizootics), 15, 1037–1052.

Cao, Y., Fu, S., Song, S., Cai, L., Zhang, H., Gao, L., Cao, L., Li, M., Gao, X., He, Y., Wang, H., & Liang, G. (2019). Isolation and genome phylogenetic analysis of arthropod-borne viruses, including Akabane virus, from mosquitoes collected in Hunan Province, China. Vector-Borne and Zoonotic Diseases, 19, 62–72.

Elliot, R. M. (2014). Orthobunyaviruses: Recent genetic and structural insights. Nature Reviews Microbiology, 12, 673–685.

Huang, C. (2003). Natural infections of pigs with Akabane virus. Veterinary Microbiology, 94, 1–11.

Kobayashi, T., Yanase, T., Yamakawa, M., Kato, T., Yoshida, K., & Tsuda, T. (2007). Genetic diversity and reassortments among Akabane virus field isolates. Virus Research, 130, 162–171.

Kono, R., Hirata, M., Kaji, M., Goto, Y., Ikeda, S., Yanase, T., Kato, T., Tanaka, S., Tsutsui, T., Imada, T., & Yamakawa, M. (2008). Bovine epizootic encephalomyelitis caused by Akabane virus in southern Japan. BMC Veterinary Research, 4, 20.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution, 35, 1547–1549.

Liang, G., Li, X., Gao, X., Fu, S., Wang, H., Li, M., Lu, Z., Zhu, W., Lu, X., Wang, L., Cao, Y., He, Y., & Lei, W. (2018). Arboviruses and their related infections in China: A comprehensive field and laboratory investigation over the last 3 decades. Reviews in Medical Virolology, 28, e1959.

Liao, Y. K., Cheng, C. Y., & Lu, Y. S. (1996a). The serological investigation of Akabane disease in Taiwan. Experimental Report of Taiwan Animal Health Research Institute, 30, 47–53.

Liao, Y. K., Lu, Y. S., Goto, Y., & Inaba, Y. (1996b). The isolation of Akabane virus (iriki strain) from calves in Taiwan. Journal of Basic Microbiology, 36, 33–39.

Oem, J. K., Lee, K. H., Kim, H. R., Bae, Y. C., Chung, J. Y., Lee, O. S., & Roh, I. S. (2012). Bovine epizootic encephalomyelitis caused by Akabane virus infection in Korea. Journal of Comparative Pathology, 147, 101–105.

Oğuzoğlu, T. Ç., Toplu, N., Koç, B. T., Doğan, F., Epikmen, E. T., İpek, E., & Akkoç, A. N. (2015). First molecular detection and characterization of Akabane virus in small ruminants in Turkey. Archives of Virology, 160, 2623–2627.

Purnomo Edi, S., Ibrahim, A., Sukoco, R., Bunali, L., Taguchi, M., Kato, T., Yanase, T., & Shirafuji, H. (2017). Molecular characterization of an Akabane virus isolate from West Java, Indonesia. Journal of Veterinary Medical Science, 79, 774–779.

Şevik, M. (2017). Molecular detection and genetic analysis of Akabane virus genogroup Ib in small ruminants in Turkey. Archives of Virology, 162, 2769–2774.

Stram, Y., Brenner, J., Braverman, Y., Banet-Noach, C., Kuznetzova, L., & Ginni, M. (2004). Akabane virus in Israel: A new virus lineage. Virus Research, 104, 93–97.

Tanaka, S., Tsutsui, T., Imada, T., & Yamakawa, M. (2008). Bovine epi- zootic encephalomyelitis caused by Akabane virus in southern Japan. Virus Research, 137, 162–171.

Taylor, W. P., & Mellor, P. S. (1994). The distribution of Akabane virus in the Middle East. Epidemiology and Infection, 113, 175–185.

Tzeng, H.-Y., Wu, H.-H., Ting, L.-J., Chang, N.-T., Chou, Y.-C., & Tu, W.-C. (2019). Monitoring Taiwanese bovine arboviruses and non-arboviruses using a vector-based approach. Medical and Veterinary Entomology, 33, 195–202.

Walker, P. J., & Klement, E. (2015). Epidemiology and control of bovine ephemeral fever. Veterinary Research, 46, 124.

Wang, J., Blasdell, K. R., Yin, H., & Walker, P. J. (2017). A large-scale serological survey of Akabane virus infection in cattle, yak, sheep and goats in China. Veterinary Microbiology, 207, 7–12.

Wang, J., Firth, C., Amos-Ritchie, R., Davis, S. S., Yin, H., Holmes, E. C., Blasdell, K. R., & Walker, P. J. (2019). Evolutionary history of Simbu serogroup orthobunyaviruses in the Australian eusystem. Virology, 535, 32–44.

Wu, D., Zhang, X., Zhang, H., Tan, Q. Q., Zhou, H. Q., Wang, H. Y., Liang, G. D., & Song, T. (2020). Detection and molecular characterization of the Akabane Virus and Oya virus in Guangdong Province, China. Chinese Journal of Virology, 36, 84–91.

Yamakawa, M., Yanase, T., Kato, T., & Tsuda, T. (2006). Chronological and geographical variations in the small RNA segment of the teratogenic Akabane virus. Virus Research, 121, 84–92.

Yanase, T., Kato, T., Hayama, Y., Akiyama, M., Itoh, N., Horiuchi, S., Hirashima, Y., Shirafuji, H., Yamakawa, M., Tanaka, S., & Tsutsui, T. (2018). Transl action of Akabane virus genogroups and its association with changes in the nature of disease in Japan, Transboundary and Emerging Diseases, 65, 41-41.

Yanase, T., Murota, K., & Hayama, Y. (2020). Endemic and emerging arboviruses in domestic ruminants in East Asia. Frontiers in Veterinary Science, 7, 168.

SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Tzeng, H. Y., Tsai, C. L., Ting, L. J., Liao, K. M., & Tu, W. C. (2022). Molecular epidemiology of Akabane virus in Taiwan. Veterinary Medicine and Science, 8, 2215–2222. https://doi.org/10.1002/vms3.887