The Journal of Veterinary Medical Science

NOTE

Virology

Isolation, genetic analysis of the first Akabane virus from goat in China

Hai-bo TANG[1–3)#], Pengfei REN[1)#], Shuying QIN[1)], Jun LIN[1)], Anbin BAI[1)], Shaomin QIN[1)], Fenglian CHEN[1)], Jinfeng LIU[1)] and Jianmin WU[1)*]

[1)]Guangxi Veterinary Research Institute, Nanning 530001, China
[2)]College of Basic Medicine, Guangxi University of Chinese Medicine, Nanning 530001, China
[3)]Guangxi Key Laboratory of Translational Medicine for Treating High-Incidence Infectious Diseases with Integrative Medicine, Nanning, 530200, China

J. Vet. Med. Sci.
81(10): 1445–1449, 2019
doi: 10.1292/jvms.18-0602

Received: 11 October 2018
Accepted: 23 July 2019
Advanced Epub:
5 August 2019

ABSTRACT. Akabane virus (AKAV) is an important insect-borne virus belonging to the genus *Orthobunyavirus*, the *Peribunyaviridae* family. An AKAV defined as GXDH 01 here, was isolated for the first time from blood from a sentinel goat in China in 2016, and its full-length open reading frames (ORFs) were sequenced in this study. Sequence analysis suggested that the isolate GXDH 01 probably had undergone a reassortment incident and acquired L segments from other strain originating from an attenuated vaccine, such as OBE-1. This study aims to provide more understanding as to the origin and epidemiology of AKAV in China.

KEY WORDS: Akabane virus, complete open reading frame, goat, phylogenetic analysis

Akabane disease is an important arthropod-borne disease of cattle and sheep caused by Akabane virus (AKAV). AKAV belongs to the species *Akabane orthobunyavirus*, the genus *Orthobunyavirus*, the *Peribunyaviridae* family [15]. AKAV is an enveloped virus with three segments of single-stranded negative-sense RNA genome, small (S), medium (M), and large (L), which differ in sizes with approximately 0.86, 4.3, and 7 kb, respectively [20].

Diseases caused by AKAV in cattle, goats and sheep are associated with abortions, premature delivery, stillbirths, and fetal malformation, mummified fetus, neonatal joint flexion, without brain and encephalitis [7]. The disease was prevalent in cattle and sheep in Australia in the 1930s and was subsequently reported in Japan. From 1972 to 1975, more than 42,000 calves were lost in the herds west of Kanto, Japan, associated with economic losses of more than five billion Japanese Yen [4, 13, 17]. Apart from Japan and Australia, Israel, Saudi Arabia, Kuwait, Yemen, Bahrain, Turkey, Indonesia, South Korea and the United Arab Emirates had reported this disease successively [1, 3, 6, 8, 19, 23]. It has caused serious economic losses to animal husbandry and posed a great threat to the development of cattle and sheep breeding [11]. It is classified as a classIIepidemic disease that must be in quarantine in the importing of cattle and sheep in China. Because of its wide distribution and damaging effects, AKAV is one of the key quarantine objects in international animal trade.

A previous serological survey of AKAVs in cattle, goats and sheep in Shaanxi, Inner Mongolia, Hunan, Hebei, Shandong, Anhui, Zhejiang, Fujian, and Shanghai displayed that the total neutralizing antibody positive rate of cattle reached 39.18%, and the neutralizing antibody positive rate of goats was 12.66% [14]. A large-scale serological survey of Akabane virus infection in cattle, yak, sheep and goats were performed in China. Results showed that the total neutralizing antibody positive rate of cattle reached 21.3%, and the neutralizing antibody positive rate of goats was 7.7% [24]. Tang *et al*. first reported natural infection of bamboo rats by AKAVs in Guangxi province, southern China [22]. The results indicated a widespread AKAV infection among cattle and sheep in China. Asymptomatic infection of AKAV has been demonstrated in wild animals such as impala, blue wildebeest, warthog and elephant [2]. Akabane disease rarely occurs in other animals. However, in the brain samples collected previously from domestic bamboo rats after death we confirmed that the bamboo rats were infected with AKAV [22]. So far, there has never been a report of direct isolation of AKAV from cattle, goat and sheep in China. The present study describes the first goat-derived AKAV isolate reported in China.

In this study a survey on sera from 14 cities in Guangxi of China was conducted (Table 1). Serum samples were collected from cattle (983) and goats (1,428) in 2014 and 2015. All serum samples were collected randomly from animals over 6 months of age on large-scale farms or from free-range cattle or goats that had no record of AKAV vaccination. The serum antibody to Akabane

*Correspondence to: Wu, J.: wu-jm20@163.com
#These authors contributed equally to this work.

(Supplementary material: refer to PMC https://www.ncbi.nlm.nih.gov/pmc/journals/2350/)

Table 1. Distributions of the seroprevalence of Akabane virus in Guangxi province

Location	Cattle (%)		Goat (%)		Total (%)
	2014	2015	2014	2015	
Liuzhou	40.42 (19/47)	14.00 (7/50)	29.74 (69/232)	30.00 (6/20)	28.94 (101/349)
Guilin	65.21 (30/46)	56.67 (17/30)	12.85 (9/70)	13.33 (4/30)	34.09 (60/176)
Yulin		58.33 (35/60)		18.18 (4/22)	47.56 (39/82)
Hezhou		87.5 (35/40)	62.00 (62/100)	42.10 (8/19)	66.04 (105/159)
Beihai	100 (30/30)	68.33 (41/60)	62.50 (5/8)	48.89 (88/180)	58.99 (164/278)
Qinzhou		80.00 (48/60)	41.58 (42/101)		55.90 (90/161)
Laibin		50.00 (15/30)	25.00 (25/100)	23.33 (7/30)	29.38 (47/160)
Fangchenggang		55.00 (33/60)			55.00 (33/60)
Chongzuo		43.18 (19/44)	14.00 (7/50)	10.00 (2/20)	24.56 (28/114)
Wuzhou			60.00 (30/50)	66.67 (20/30)	62.50 (50/80)
Baise		67.24 (39/58)	44.19 (76/172)		50.00 (115/230)
Nanning	84.73 (111/131)	80.25 (126/157)	24.46 (23/94)	40.00 (4/10)	67.35 (264/392)
Guigang		26.67 (16/60)			26.67 (16/60)
Hechi		35.00 (7/20)	34.00 (17/50)	32.50 (13/40)	33.63 (37/110)
Total	74.80 (190/254)	60.08 (438/729)	35.54 (365/1,027)	38.90 (156/401)	47.66 (1,149/2,411)

virus was tested with ID Screen® Akabane Competition ELISA Kit (ID-VET, Montpellier, France) following the manufacturer's instructions. A total of 2411 serum samples obtained from cattle and goats in Guangxi were tested for the presence of antibodies to AKAV. There was evidence of AKAV antibodies in cattle and goats from the 14 cities. Overall AKAV seroprevalence in cattle was 63.89% (628/983 tested). And AKAV antibodies were 36.48% positive in goat serum samples from all 14 cities (521/1428 tested). AKAV seroprevalence was higher in cattle than in goats in the χ^2 test (P<0.01) (Table 1). The positive rate of our detection of AKAV antibodies is higher than previously reported. It is possible that because of the previously reported focus on neutralizing antibodies of AKAV, we detected the multiple antibodies of the virus. In addition, these differences may also have a certain relationship with the region. Previous reports were mainly distributed in the northern China, and our tests were in southern China. The climatic reasons determine the difference in the type and quantity of *Culicoides* biting midges.

The AKAV positive serum samples collected from sentinel cattle and goat in Guangxi Province in 2015 and 2016 were for AKAV isolation and identification. A classical operation procedure for AKAV isolation was performed on these serum samples as described by Gard *et al.* [5]. Briefly, embryonated chicken eggs were inoculated with AKAV antibody-positive serum collected from sentinel cattle. Brains, lungs, spleens and livers were harvested from dead embryos at day 3–5 post inoculation and were homogenized, after which the supernatants of organization homogenates were passaged 3–5 times on BHK-21 cells. The culture was monitored daily for cytopathic effect (CPE). The cultured supernatants were harvested at 2–3 days post-inoculation and stored at −70°C as a viral stock. An isolate of AKAV was obtained from these serum samples by testing cell culture supernatants using a reverse transcription polymerase chain reaction (RT-PCR) method [22]. The AKAV isolate defined as GXDH 01, then 6-well tissue culture plates were used for plaque purification of the isolate in which each well was seeded with 1×10^6 BHK-21 cells in 10% growth medium and incubated at 5% CO_2 at 37°C. GXDH 01 was serially diluted 10^{-1} from to 10^{-6} in Minimum Essential Medium (MEM), in six wells, monolayer of five wells were infected with 0.5 m*l* of different virus dilution starting from 10^{-2} to 10^{-6} and 6th well was kept as cell control. This plate was kept in incubator with 5% CO_2 at 37°C for 1.5 hr involving swaying for every 15 min to ensure virus adsorption. After incubation, inoculum was drained off completely with the help of 1 m*l* micropipette to remove unadsorbed virus without disturbing the monolayer and immediately overlayed with 5 m*l* of 2× MEM with 2% Fetal Bovine Serum (FBS); 2% plaque agarose mixture in 1:1 ratio. Finally, overlayed plates were allowed to solidify and then transferred to incubator. Plaques which are more distinct and isolated from others were collected with the help of micropipette in 200 μl of 10% MEM. Collected individual plaques were infected to BHK-21 cells in 24-well plate for virus propagation. The purified the virus for three generations using the plaque purification method. The CPE in the infected BHK-21 cell culture was found 48 hr after inoculation (data not shown). The serum samples of the virus was collected from an 8 month old sentinel goat kept in Danzhang tun, Chengnei Village, Dahua County, Hechi City, Guangxi in April 2016.

For further analysis, twelve primer pairs were designed based on the published genome sequence of the KU375444, AB190458 and AB297832 strains and were used to amplify the whole AKAV genome (Table 2). For amplification of the complete genome segments of the isolate GXDH 01, total RNA was extracted from infected BHK-21 cells with RNA Kits (TransGen Biotech, Beijing, China) according to the manufacturer's instructions. The cDNA was synthesized using 1.0 μg total RNA, 1 μl (20 pmol/μl) forward primer, and 100 U Moloney murine leukaemia virus (MMLV) reverse transcriptase in a 25-μl reaction volume. PCR products were purified using a DNA purification kit (TianGEN, Beijing, China) according to the manufacturer's instructions and were submitted to Sangon Biotech Co., Ltd. (Shanghai, China) for sequencing, then the genome sequences were assembled using SeqMan software (DNASTAR Inc., Madison, WI, U.S.A.). The sequences of 3 genome segments of AKAV isolate GXDH 01 were submitted to GenBank under accession No. MH174977–MH174979. The S, M and L segments of AKAV isolate was 0.744, 4.190, and 6.816 kb in length excluding the primer binding sites respectively. Goat AKAV shared 95.6–98.3, 88.2–96.6 and 92.2–99.1%

Table 2. The primers amplification the S, M and L segments of Akabane virus isolated from goat, China used in this study

Gene	Primer	Sequence (5′-3′)	Position	Product size (bp)
N	AKVS1	TGCAATGGCAAATCAATTTATT	30–51[a)]	788
	AKVS2	CGAGCAGCTGAACAAAGGTGTG	796–817	
M	AKVM1	ATGATTATTACAATTCTCAACAT	2–24[b)]	816
	AKVM2	GCTAAAAGGATGAATTGGT	799–817	
	AKVM3	CTTTGTGGCCATTATCTTGAC	673–693	1,497
	AKVM4	ATTGGAGTTTGGCATTCTTTGAT	2,147–2,169	
	AKVM5	CGGTAATTTACTGCGACAAGTT	1,915–1,936	1,447
	AKVM6	CCCTTTGTACACCCTCCTATTT	3,340–3,361	
	AKVM7	GCCAAGATGTCATTAGAGAAGA	3,141–3,162	1,096
	AKVM8	ACCCTCCCATTATGTCTATTTA	4,215–4,236	
L	AKVL1	TAGTGTACCCCTAAATACAACA	3–24[c)]	1,050
	AKVL2	AATGCGTCCAAATATGTGC	1,034–1,052	
	AKVL3	TTGGCAACTAATGACAGAGAGA	864–885	1,258
	AKVL4	TCTTTGCTTATATGCGTTGTA	2,101–2,121	
	AKVL5	CGACGATTAAGATTGAGGAT	1,886–1,905	1,438
	AKVL6	AATATCGACGTCATCACATCAT	3,302–3,323	
	AKVL7	AGATGAGATGATTAGCGAACC	2,976–2,996	1,286
	AKVL8	ACAGAGACCGAGAACGCATATC	4,240–4,261	
	AKVL9	CAGGCAATCTGACTTATTTGGT	4,034–4,055	1,255
	AKVL10	CGCAATGACTCGTAGGCTAT	5,269–5,288	
	AKVL11	CAATTGACTTCTGTGGCATGAT	5,135–5,156	1,219
	AKVL12	TCGCATTCACGTACTAAAGC	6,334–6,353	
	AKVL13	AGCCAAACAAGGTCCAGAAGAA	5,848–5,869	1,013
	AKVL14	GCCCCTAAATGCAATAATATA	6,840–6,860	

The oligonucleotide position is based on the sequences of three strains (a) KU375444, b) AB297832, c) AB190458) of Akabane viruses.

of nucleotide identity with the known AKAV isolates in S, M and L genes, respectively. The putative proteins of goat AKAV was 233 amino acids (aa) for the nucleoprotein (N), 1401 aa for the polyprotein and 2251 aa for the RNA-dependent RNA polymerase protein respectively. Goat AKAV shared 97.9–99.1, 92.3–98.5 and 97.8–99.5% of amino acid identity with the known AKAV isolates in S, M and L genes, respectively.

To define the relationship of the newly isolated goat AKAVs within the AKAV family, phylogenetic trees were constructed based on the sequences of the S, M and L genes from the goat AKAV isolate and the known AKAV sequences from the GenBank (Supplementary Table 1). Intact open reading frames (ORFs) sequence alignments were performed by clustal W method using MEGA 5 software [21]. The neighbor-joining (NJ) phylogenetic trees based on the S gene, M gene and L gene were constructed using the MEGA 5.0 software and 1000 bootstrap replicates were used to statistically assess the branching pattern. The phylogenetic trees based on the sequences of the S gene revealed that the goat AKAV isolate from China fell into a single clade (defined as Group Ia) together with the bamboo rat AKAV isolates GXLCH 01, GXLCH 02 and GXLCH 04 from China (97.4–98.8%) (Fig. 1A). The phylogenetic trees based on the sequences of the M gene revealed that the goat AKAV isolate from China fell into the very same clade (defined as Group Ia) as the bovine AKAV isolate AKAV-7/SKR/2010 which caused encephalomyelitis in cattle in South Korea [18] and AKAV isolates GXLCH 01, GXLCH 02 and GXLCH 16–70 were recognized to cause encephalomyelitis in adult bamboo rats from China (95.1–96.6%) (Fig. 1B). The M genes were higher than the 88.3–88.7% homologies observed between the goat AKAV isolate and OBE-1, 93FMX, TS-C2 and K0505 from Japan and Korea. However, based on the sequences of the L genes, the goat AKAV isolate and GXLCH 01, GXLCH 02 and GXLCH 16–70 isolate were classified into two different clades (defined as Group IIa andIIb) (Fig. 1C). The L genes of goat isolate and GXLCH 01, GXLCH 02 and GXLCH 16–70 shared 92.2–92.4% homologies, lower than the 99.0–99.1% homologies observed between the goat AKAV isolate and OBE-1, 93FMX, TS-C2 and K0505. The phylogenetic trees based on the sequences of the L genes suggested that the goat AKAV isolate had close relationship with the OBE-1, 93FMX, TS-C2 and K0505 from Japan and Korea strains recognized to attenuate or were avirulent in mice [12, 16]. It was more distantly related to the GXLCH 01, GXLCH 02 and GXLCH 16–70 isolates (Fig. 1C).

An isolate Tinaroo virus (TINV) from Australia was reported to have genomic reassortment events AKAV S and L RNA segments with an M RNA segment of an unknown orthobunyavirus [10]. The goat AKAV reported here suggests that isolate GXDH 01 is a reassortant virus which has undergone a reassortment event and has probably obtained L gene segments from other strain originating from an attenuated vaccine, such as OBE-1. To determine the pathogenicity of GXDH 01, 1day-old suckling mice and adult mice free of AKAV neutralizing antibody were average divided into intracerebral (IC), and negative control groups (n=6 mice/group). The mice were anesthetized by halothane inhalant in a container and then each was inoculated intracerebrally

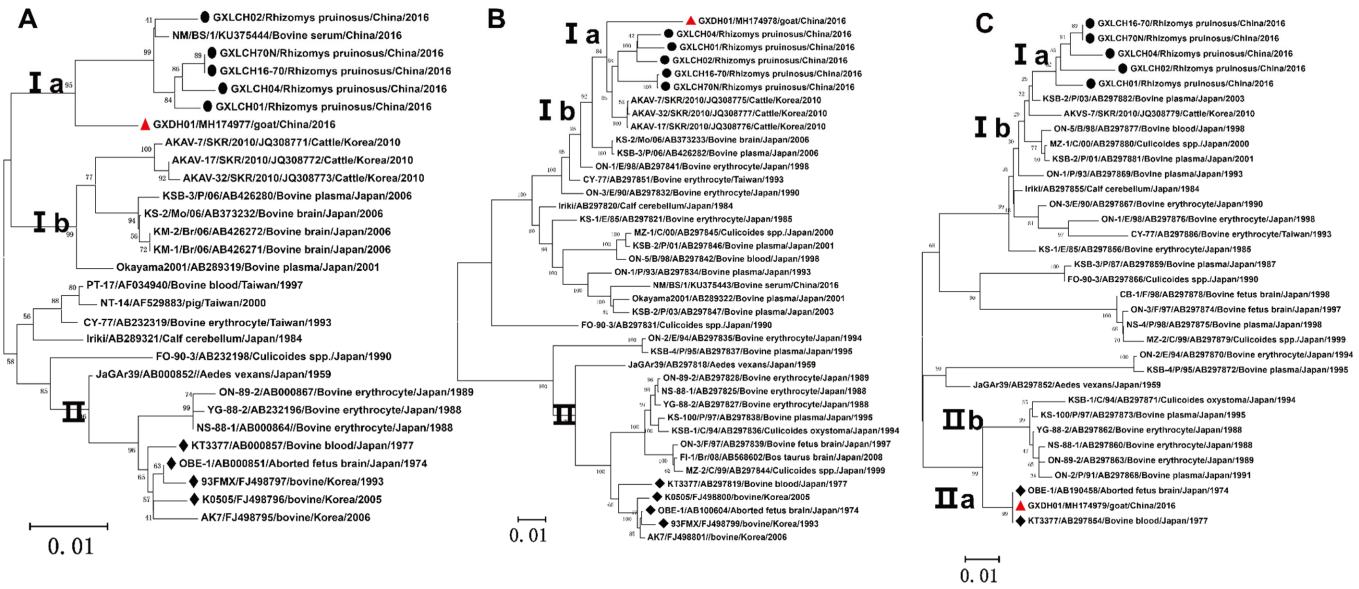

Fig. 1. Phylogenetic tree of AKAV based on the sequence of the S, M and L genes. Sequences of the goat AKAV isolate from southern China was indicated by red triangles (▲). The nucleotide sequences of other AKAV strains used in this study were obtained from GenBank. Details for the origin of each strain were given in Supplementary Table 1.

with 0.01–0.03 m*l* of GXDH 01 strain at a titer of $10^{5.2}$ $TCID_{50}$/0.1 m*l*. After inoculation, the mice were observed daily for clinical signs. The GXDH 01 strain was avirulent or, at most, caused slightly transient weight loss in mice. Jeong et al performed experimental inoculation of AKAV-7 on goats via different routes. During the experimental period, infected goats showed no clinical signs, but some animals showed encephalomyelitis on histopathological examination after IC and IV inoculation and lesions in the brain and spinal cord after IS inoculation [9]. The isolate GXDH 01 of AKAV from southern China maybe have genomic reassortment events AKAV can cause encephalomyelitis in goats S and M RNA segments with an L RNA segment of an avirulent AKAV. The pathogenicity of reassortment AKAV cause attenuate in infection mice or goats. Since GXDG 01 was isolated from asymptomatic sentinel goats of 8 months old, its pathogenicity in sheep and cattle remains unknown. Thus, further study is necessary to evaluate the pathogenicity.

In conclusion, we isolated and sequenced the complete ORFs segments of strain GXDH 01 for the first time from goat in China. The strain was further determined to be a reassortant virus that contained L segments from an isolate of OBE-1 originating from Japan. Further experimental studies should be performed to confirm the biological characteristics of GXDH 01, especially its pathogenicity in sheep and cattle. This study will enhance our understanding of the epidemiology of AKAV in China.

FUNDING. This study was funded by the grant from the Natural Scientific Fund from Guangxi province (2017GXNSFBA198016) and the Open Project Foundation of State Key Laboratory of Veterinary Biotechnology (Project No. SKLVBF201613).

CONFLICT OF INTEREST. The authors declare no conflict of interests.

ETHICAL APPROVAL. The animal experiments were approved and performed in accordance with the animal ethics guidelines and approved procedures of Guangxi Veterinary Research Institute.

ACKNOWLEDGMENT. We are grateful to Associate Prof. Shaoqiang Ruan at the College of Foreign Language, Guangxi University, for her help in editing the manuscript.

REFERENCES

1. Al-Busaidy, S. M., Mellor, P. S. and Taylor, W. P. 1988. Prevalence of neutralising antibodies to Akabane virus in the Arabian peninsula. *Vet. Microbiol.* **17**: 141–149. [Medline] [CrossRef]
2. Al-Busaidy, S., Hamblin, C. and Taylor, W. P. 1987. Neutralising antibodies to Akabane virus in free-living wild animals in Africa. *Trop. Anim. Health Prod.* **19**: 197–202. [Medline] [CrossRef]
3. EIhassan, A. M., Mansour, M. E., Shamon, A. A. and EI Hussein, A. M. 2014. A serological survey of akabane virus infection in cattle in Sudan. *ISRN Vet. Sci.* 123904. [CrossRef]
4. Forman, S., Hungerford, N., Yamakawa, M., Yanase, T., Tsai, H. J., Joo, Y. S., Yang, D. K. and Nha, J. J. 2008. Climate change impacts and risks for animal health in Asia. *Rev. - Off. Int. Epizoot.* **27**: 581–597. [Medline] [CrossRef]
5. Gard, G. P. and Kirkland, P. D. 1993. Bluetongue virology and serology. *In*: Australian Standard Diagnostic Techniques for Animal Diseases

(Corner, L. A. and Bagust, T. J. eds.), CSIRO for the Standing Committee on Agriculture and Resource Management, East Melbourne.
6. Haughey, K. G., Hartley, W. J., Della-Porta, A. J. and Murray, M. D. 1988. Akabane disease in sheep. *Aust. Vet. J.* **65**: 136–140. [Medline] [CrossRef]
7. Inaba, Y., Kurogi, H. and Omori, T. 1975. Letter: Akabane disease: epizootic abortion, premature birth, stillbirth and congenital arthrogryposis-hydranencephaly in cattle, sheep and goats caused by Akabane virus. *Aust. Vet. J.* **51**: 584–585. [Medline] [CrossRef]
8. Jagoe, S., Kirkland, P. D. and Harper, P. A. 1993. An outbreak of Akabane virus-induced abnormalities in calves after agistment in an endemic region. *Aust. Vet. J.* **70**: 56–58. [Medline] [CrossRef]
9. Jeong, H., Oem, J. K., Yang, M. S., Yang, D., Kim, M. S., Lee, K. H., Lee, M. H., Lim, C. W. and Kim, B. 2017. Experimental infection of goats with a newly isolated strain of Akabane virus that causes encephalomyelitis. *J. Comp. Pathol.* **157**: 220–229. [Medline] [CrossRef]
10. Kobayashi, T., Yanase, T., Yamakawa, M., Kato, T., Yoshida, K. and Tsuda, T. 2007. Genetic diversity and reassortments among Akabane virus field isolates. *Virus Res.* **130**: 162–171. [Medline] [CrossRef]
11. Kurogi, H., Inaba, Y., Goto, Y., Miura, Y. and Takahashi, H. 1975. Serologic evidence for etiologic role of Akabane virus in epizootic abortion-arthrogryposis-hydranencephaly in cattle in Japan, 1972–1974. *Arch. Virol.* **47**: 71–83. [Medline] [CrossRef]
12. Kurogi, H., Inaba, Y., Takahashi, E., Sato, K., Akashi, H., Satoda, K. and Omori, T. 1979. An attenuated strain of Akabane virus: a candidate for live virus vaccine. *Natl. Inst. Anim. Health Q. (Tokyo)* **19**: 12–22. [Medline]
13. Lee, J. K., Park, J. S., Choi, J. H., Park, B. K., Lee, B. C., Hwang, W. S., Kim, J. H., Jean, Y. H., Haritani, M., Yoo, H. S. and Kim, D. Y. 2002. Encephalomyelitis associated with akabane virus infection in adult cows. *Vet. Pathol.* **39**: 269–273. [Medline] [CrossRef]
14. Li, C., Feng, Q., Zhang, R., Zheng, Z. and Zhou, L. 1994. Serological investigation Akabane disease in China. *China. Animal. Health. Inspection.* **11**: 35–36 [in Chinese].
15. Maes, P., Alkhovsky, S. V., Bào, Y., Beer, M., Birkhead, M., Briese, T., Buchmeier, M. J., Calisher, C. H., Charrel, R. N., Choi, I. R., Clegg, C. S., de la Torre, J. C., Delwart, E., DeRisi, J. L., Di Bello, P. L., Di Serio, F., Digiaro, M., Dolja, V. V., Drosten, C., Druciarek, T. Z., Du, J., Ebihara, H., Elbeaino, T., Gergerich, R. C., Gillis, A. N., Gonzalez, J. J., Haenni, A. L., Hepojoki, J., Hetzel, U., Hồ, T., Hóng, N., Jain, R. K., Jansen van Vuren, P., Jin, Q., Jonson, M. G., Junglen, S., Keller, K. E., Kemp, A., Kipar, A., Kondov, N. O., Koonin, E. V., Kormelink, R., Korzyukov, Y., Krupovic, M., Lambert, A. J., Laney, A. G., LeBreton, M., Lukashevich, I. S., Marklewitz, M., Markotter, W., Martelli, G. P., Martin, R. R., Mielke-Ehret, N., Mühlbach, H. P., Navarro, B., Ng, T. F. F., Nunes, M. R. T., Palacios, G., Pawęska, J. T., Peters, C. J., Plyusnin, A., Radoshitzky, S. R., Romanowski, V., Salmenperä, P., Salvato, M. S., Sanfaçon, H., Sasaya, T., Schmaljohn, C., Schneider, B. S., Shirako, Y., Siddell, S., Sironen, T. A., Stenglein, M. D., Storm, N., Sudini, H., Tesh, R. B., Tzanetakis, I. E., Uppala, M., Vapalahti, O., Vasilakis, N., Walker, P. J., Wáng, G., Wáng, L., Wáng, Y., Wèi, T., Wiley, M. R., Wolf, Y. I., Wolfe, N. D., Wú, Z., Xú, W., Yang, L., Yāng, Z., Yeh, S. D., Zhāng, Y. Z., Zhèng, Y., Zhou, X., Zhū, C., Zirkel, F. and Kuhn, J. H. 2018. Taxonomy of the family Arenaviridae and the order Bunyavirales: update 2018. *Arch. Virol.* **163**: 2295–2310. [Medline] [CrossRef]
16. Murata, Y., Uchida, K., Shioda, C., Uema, A., Bangphoomi, N., Chambers, J. K., Akashi, H. and Nakayama, H. 2015. Histopathological studies on the neuropathogenicity of the Iriki and OBE-1 strains of Akabane virus in BALB/cAJcl mice. *J. Comp. Pathol.* **153**: 140–149. [Medline] [CrossRef]
17. Noda, Y., Yokoyama, H., Katsuki, T., Kurashige, S., Uchinuno, Y. and Narita, M. 2001. Demonstration of Akabane virus antigen using immunohistochemistry in naturally infected newborn calves. *Vet. Pathol.* **38**: 216–218. [Medline] [CrossRef]
18. Oem, J. K., Yoon, H. J., Kim, H. R., Roh, I. S., Lee, K. H., Lee, O. S. and Bae, Y. C. 2012. Genetic and pathogenic characterization of Akabane viruses isolated from cattle with encephalomyelitis in Korea. *Vet. Microbiol.* **158**: 259–266. [Medline] [CrossRef]
19. Oğuzoğlu, T. Ç., Toplu, N., Koç, B. T., Doğan, F., Epikmen, E. T., İpek, E. and Akkoç, A. N. 2015. First molecular detection and characterization of Akabane virus in small ruminants in Turkey. *Arch. Virol.* **160**: 2623–2627. [Medline] [CrossRef]
20. Pattnaik, A. K. and Abraham, G. 1983. Identification of four complementary RNA species in Akabane virus-infected cells. *J. Virol.* **47**: 452–462. [Medline]
21. Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**: 2731–2739. [Medline] [CrossRef]
22. Tang, H. B., Chen, F., Rao, G., Bai, A., Jiang, J., Du, Y., Ren, P., Liu, J., Qin, S., Yang, L. and Wu, J. 2017. Characterization of Akabane virus from domestic bamboo rat, Southern China. *Vet. Microbiol.* **207**: 280–285. [Medline] [CrossRef]
23. Taylor, W. P. and Mellor, P. S. 1994. The distribution of Akabane virus in the Middle East. *Epidemiol. Infect.* **113**: 175–185. [Medline] [CrossRef]
24. Wang, J., Blasdell, K. R., Yin, H. and Walker, P. J. 2017. A large-scale serological survey of Akabane virus infection in cattle, yak, sheep and goats in China. *Vet. Microbiol.* **207**: 7–12. [Medline] [CrossRef]