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Phylogenetic analysis of Gansu sheeppox virus isolates based on *P32*, *GPCR*, and *RPO30* genes

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ABSTRACT. Two outbreaks of sheeppox in sheep have occurred in Gansu Province, China. The *P32*, *GPCR*, and *RPO30* genes were used as markers for differential diagnosis. We confirmed that the outbreaks were caused by sheeppox virus. Sequence and phylogenetic analysis of the *P32*, *GPCR*, and *RPO30* genes revealed a close relationship between the 2 isolates and Chinese sheeppox viruses. Because ill sheep were imported from Jingyuan, another county of Gansu Province, our results strongly suggest the importance of veterinary surveillance prior to transportation.

Key words: *GPCR*; *P32*; *RPO30*; Sheeppox virus

INTRODUCTION

Sheeppox virus (SPPV) is a member of the genus *Capripoxvirus* of the family Poxviridae, which also contains 2 other members, goatpox virus (GTPV) and lumpy skin disease virus (LSDV) (Fields et al., 2007). SPPV can infect both sheep and goats and has been reported in many areas in the world, including Asia, Europe, Africa, America, and Oceania (Asagba and Nawathe, 1981; Afshar et al., 1986; Oğuzoğlu et al., 2006; Roy et al., 2008; Beard et al., 2010; Bhanuprakash et al., 2006, 2010). Infections by SPPV can cause high morbidity and mortality, particularly in lambs (Rao and Bandyopadhyay, 2000), subjecting sheepherders to considerable economic losses. Because of its great impacts on animal health, sheeppox is categorized as a notifiable disease by the World Organization for Animal Health.

Clinically, animals infected by SPPV and GTPV exhibit a fever with the development of macules in the skin, making primary diagnosis easy; however, differential diagnosis is difficult (Bhanuprakash et al., 2006; Gulbahar et al., 2006; Oğuzoğlu et al., 2006; Embury-Hyatt et al., 2012). Therefore, several molecular markers, including *P32*, G-protein-coupled receptors (*GPCR*), and RNA polymerase subunit (*RPO30*), have been proposed for use in differential diagnosis. *P32* is an envelope protein of the capripoxvirus and is homologous to the *P35* protein encoded by the vaccinia virus *H3L* gene (Johnson et al., 1993; Heine et al., 1999). Because of the size difference of *P32* between SPPV and GTPV, they can be distinguished by sequence comparison (Tian et al., 2010). *GPCR*, which is involved in defense against the aggressive assault executed by host inflammatory responses (Chensue, 2001; Seet and McFadden, 2002), has been used for differential diagnosis of SPPV, GTPV, and LSDV (Le Goff et al., 2009; Lamien et al., 2011b). The *RPO30* gene, a homolog of the vaccinia virus *E4L* gene, encodes a 30-kDa DNA-dependent RNA polymerase subunit (Ahn et al., 1990; Tulman et al., 2002). Although the *RPO30* gene is conserved among capripoxviruses, it is still used to distinguish between SPPV, GTPV, and LSDV (Lamien et al., 2011a; Zhou et al., 2012).

In this study, we report 2 cases of SPPV in sheep in Gansu Province, China. We used *P32*, *GPCR*, and *RPO30* as molecular biomarkers for phylogenetic analysis.

MATERIAL AND METHODS

Treatment of samples

Papules or scabs were collected from ill sheep in the Zhangye and Huining counties of Gansu Province, China. In both cases, macules were observed on the lips, udders, and tails. Papules or scabs were first suspended in 0.01 M phosphate-buffered saline, pH 7.4. Next, the samples were homogenized in the lab for later use.

Propagation in cell culture

The homogenized samples were filtered using a 0.45-μm filter (Millipore, Billerica, MA, USA). Next, 2 mL filtrate was inoculated into lamb testis cell culture for virus isolation. When a cytopathic effect occurred, the cells were harvested 6 days post-inoculation by 3 cycles of alternating freezing and thawing.

Extraction of viral genomic DNA

After the lysate was centrifuged at 664 *g* for 10 min, the DNA was extracted according

to the instructions of the Axygen™ Viral DNA/RNA Miniprep kit (Axygen, Union City, CA, USA). The extracted DNA was stored at -20°C.

Amplification of *P32*, *GPCR*, and *RPO30*

The following specific primers for *P32*, *GPCR*, and *RPO30* genes were designed using primer 5.0 and synthesized by TaKaRa (Dalian, China):

P32-F: 5'-ATG GCA GAT ATC CCA TT-3'; *P32*-R: 5'-CTA AAC TAT ATA CGT AAA TAA CAT AC-3'; *GPCR*-F: 5'-TTT ATC AGC ACT AGG TCA TTA TCT-3'; *GPCR*-R: 5'-TAT CAC TCC CTT CCA TTT TTA T-3'; *RPO30*-F: 5'-CTC TGT TCC AAA CTA AAT CAT-3'; *RPO30*-R: 5'-TTT TTG TAT TAC CAA TTT CTG-3'; The polymerase chain reaction (PCR) system (50 μL total volume) included 1 μL extracted DNA, 5 μL 10X PCR buffer, 0.3 μL LA *Taq* DNA polymerase (5 U/μL) (TaKaRa), 4 μL 2.5 mM dNTPs, 38.2 μL nuclease-free water, and 0.75 μL of each primer (10 μM), and was performed in a Personal Thermal Cycler (Bio-Rad, Hercules, CA, USA) with the following conditions: denaturation for 4 min at 98°C, then 35 cycles of denaturation for 30 s at 95°C, annealing for 30 s at 52°C for *P32*, 49.5°C for *GPCR*, or 53°C for *RPO30*, extension for 120 s at 72°C, and a final elongation for 10 min at 72°C. The products were examined by 1% agarose gel electrophoresis with ethidium bromide staining. The products were then purified using the AxyPrep™ DNA Gel Extraction kit (Axygen). The samples were sequenced.

Phylogenetic analysis

The nucleotide sequences of the *P32*, *GPCR*, and *RPO30* genes of other capripoxvirus strains were retrieved from GenBank (Table 1). ClustalW was used to align these sequences. Phylogenetic analysis was performed using neighbor-joining, maximum parsimony, maximum-likelihood, and minimum evolution in MEGA 5.10 (McCormack and Clewley, 2002; Chakraborty et al., 2013). The topology of the trees was tested by bootstrap analysis with 1000 replicates (Sanderson and Wojciechowski, 2000).

RESULTS AND DISCUSSION

In both cases, macules were observed on the lips, udders, and tails (Figure 1). Compared with other capripoxvirus isolates, the Huining and Zhangye isolates shared 97.9-99.9% nucleotide identity and 72.3-99.7% amino acid identity for *P32* (Table 2), 95.0-99.7 and 93.0-100% for *GPCR* (Table 3), and 96.2-100 and 97.4-100% for *RPO30,* respectively (Table 4). Based on the results of amino acid sequence comparison of *P32*, *GPCR*, and *RPO30*, the 2 isolates showed no unique amino acid substitutions compared with other members. The alignment of the partial amino acid sequences of the *P32* genes showed that our strains (HN and ZYGT) had the highest similarity to other SPPV and LSDV strains (Figure 2). Moreover, the putative amino acid sequences of Huining and Zhangye isolates contained an aspartic acid at the 55th position, which is absent in GTPV (Hosamani et al., 2004). Combined with the alignment results for *GPCR* and *RPO30* (data not shown), the 2 isolates were SPPV. Similar topological trees were obtained using the 3 genes above (Figures 3-5). In the phylogenetic trees based on the *P32*, *GPCR*, and *RPO30* sequences, the viruses were clearly segregated into 3 major groups: SPPV, GTPV, and LSDV. The 2 isolates were clustered with the SPPV branch, and clustered together with the Asia isolates.

Table 1. Capripoxvirus strains used for phylogenetic analysis.

Gene	Virus designation	Host	Accession No.	Species of capripoxvirus	Country of isolation	Reference
P32	Huining isolate	Sheep		SPPV	Huining (China)	This study
P32	Zhangye isolate	Sheep		SPPV	Zhangye (China)	This study
P32	China-EF522177.1-GTPV	Goat	EF522177.1	GTPV	China	Unpublished
P32	China-EF522180.1-GTPV	Goat	EF522180.1	GTPV	China	Unpublished
P32	China-EF522176.1-GTPV	Goat	EF522176.1	GTPV	China	Unpublished
P32	Vietnam-EU625263.1-GTPV	Goat	EU625263.1	GTPV	Vietnam	(Babiuk et al., 2009)
P32	China-HM572329.1-GTPV	Goat	HM572329.1	GTPV	China	Unpublished
P32	China-EF514890.1-GTPV	Goat	EF514890.1	GTPV	China	Unpublished
P32	China-AY773088.1-GTPV	Goat	AY773088.1	GTPV	China	Unpublished
P32	China-EF514892.1-GTPV	Goat	EF514892.1	GTPV	China	Unpublished
P32	India-FJ748488.1-GTPV	Goat	FJ748488.1	GTPV	India	Unpublished
P32	China-JN602370.1-GTPV	Goat	JN602370.1	GTPV	China	(Zhou et al., 2012)
P32	India-AY159333.1-GTPV	Goat	AY159333.1	GTPV	India	(Hosamani et al., 2004)
P32	China-AY881707.1-GTPV	Goat	AY881707.1	GTPV	China	NA
P32	India-AY382869.1-GTPV	Goat	AY382869.1	GTPV	India	(Hosamani et al., 2004)
P32	China-EF514889.1-GTPV	Goat	EF514889.1	GTPV	China	Unpublished
P32	China-EF522178.1-GTPV	Goat	EF522178.1	GTPV	China	Unpublished
P32	China-JN596275.1-GTPV	Goat	JN596275.1	GTPV	China	(Zhou et al., 2012)
P32	China-EF514891.1-GTPV	Goat	EF514891.1	GTPV	China	Unpublished
P32	Yemen-EU625262.1-GTPV	Goat	EU625262.1	GTPV	Yemen	(Babiuk et al., 2009)
P32	India-DQ153219.1-SPPV	Sheep	DQ153219.1	SPPV	India	Unpublished
P32	India-DQ153224.1-SPPV	Sheep	DQ153224.1	SPPV	India	Unpublished
P32	India-DQ153223.1-SPPV	Sheep	DQ153223.1	SPPV	India	Unpublished
P32	India-DQ153225.1-SPPV	Sheep	DQ153225.1	SPPV	India	Unpublished
P32	India-DQ153220.1-SPPV	Sheep	DQ153220.1	SPPV	India	Unpublished
P32	India-DQ153221.1-SPPV	Sheep	DQ153221.1	SPPV	India	Unpublished
P32	India-DQ153226.1-SPPV	Sheep	DQ153226.1	SPPV	India	Unpublished
P32	India-AY368684.1-SPPV	Sheep	AY368684.1	SPPV	India	Unpublished
P32	China-JN596274.1-SPPV	Sheep	JN596274.1	SPPV	China	(Zhou et al., 2012)
P32	India-FJ882029.1-SPPV	Goat	FJ882029.1	SPPV	India	Unpublished
P32	India-FJ748487.1-SPPV	Goat	FJ748487.1	SPPV	India	Unpublished
P32	China-HQ607368.1-SPPV	Sheep	HQ607368.1	SPPV	China	NA
P32	South Africa-AF409137.1-LSDV	Cattle	AF409137.1	LSDV	South Africa	(Kara et al., 2003)
P32	Kenya-AF325528.1-LSDV	Cattle	AF325528.1	LSDV	Kenya	(Tulman et al., 2001)
P32	Kenya-NC003027.1-LSDV	Cattle	NC003027.1	LSDV	Kenya	(Tulman et al., 2001)
GPCR	Huining isolate	Sheep		SPPV	Huining (China)	This study
GPCR	Zhangye isolate	Sheep		SPPV	Zhangye (China)	This study
GPCR	China-JQ310667.1-GTPV	Goat	JQ310667.1	GTPV	China	(Zhou et al., 2012)
GPCR	Bangladesh-FJ869355.1-GTPV	Goat	FJ869355.1	GTPV	Bangladesh	(Le Goff et al., 2009)
GPCR	China-JQ310672.1-GTPV	Goat	JQ310672.1	GTPV	China	(Zhou et al., 2012)
GPCR	India-FJ869358.1-GTPV	Goat	FJ869358.1	GTPV	India	(Le Goff et al., 2009)
GPCR	Oman-FJ869359.1-GTPV	Goat	FJ869359.1	GTPV	Oman	(Le Goff et al., 2009)
GPCR	Chad-FJ869392.1-GTPV	Goat	FJ869392.1	GTPV	Chad	(Le Goff et al., 2009)
GPCR	Chad-FJ869363.1-GTPV	Goat	FJ869363.1	GTPV	Chad	(Le Goff et al., 2009)
GPCR	Burkina Faso-FJ869353.1-GTPV	Goat	FJ869353.1	GTPV	Burkina Faso	(Le Goff et al., 2009)
GPCR	Yemen-FJ869362.1-GTPV	Goat	FJ869362.1	GTPV	Yemen	(Le Goff et al., 2009)
GPCR	Turkey-FJ869356.1-GTPV	Goat	FJ869356.1	GTPV	Turkey	(Le Goff et al., 2009)
GPCR	Iraq-FJ869357.1-GTPV	Goat	FJ869357.1	GTPV	Iraq	(Le Goff et al., 2009)
GPCR	Sudan-FJ869369.1-LSDV	Cattle	FJ869369.1	LSDV	Sudan	(Le Goff et al., 2009)
GPCR	Egypt-FJ869377.1-LSDV	Cattle	FJ869377.1	LSDV	Egypt	(Le Goff et al., 2009)
GPCR	South Africa-FJ869375.1-LSDV	Cattle	FJ869375.1	LSDV	South Africa	(Le Goff et al., 2009)
GPCR	South Africa-FJ869373.1-LSDV	Cattle	FJ869373.1	LSDV	South Africa	(Le Goff et al., 2009)
GPCR	South Africa-FJ869372.1-LSDV	Cattle	FJ869372.1	LSDV	South Africa	(Le Goff et al., 2009)
GPCR	Nigeria-FJ869368.1-LSDV	Cattle	FJ869368.1	LSDV	Nigeria	(Le Goff et al., 2009)
GPCR	Sudan-FJ869367.1-LSDV	Cattle	FJ869367.1	LSDV	Sudan	(Le Goff et al., 2009)
GPCR	Niger-FJ869366.1-LSDV	Cattle	FJ869366.1	LSDV	Niger	(Le Goff et al., 2009)
GPCR	Burkina Faso-FJ869352.1-LSDV	Cattle	FJ869352.1	LSDV	Burkina Faso	(Le Goff et al., 2009)
GPCR	South Africa-FJ869374.1-LSDV	Cattle	FJ869374.1	LSDV	South Africa	(Le Goff et al., 2009)
GPCR	South Africa-FJ869371.1-LSDV	Cattle	FJ869371.1	LSDV	South Africa	(Le Goff et al., 2009)
GPCR	South Africa-FJ869370.1-LSDV	Cattle	FJ869370.1	LSDV	South Africa	(Le Goff et al., 2009)
GPCR	Niger-FJ869365.1-LSDV	Cattle	FJ869365.1	LSDV	Niger	(Le Goff et al., 2009)

Continued on next page

Table 1. Continued.

Gene	Virus designation	Host	Accession No.	Species of capripoxvirus	Country of isolation	Reference
GPCR	China-JQ310675.1-SPPV	Sheep	JQ310675.1	SPPV	China	(Zhou et al., 2012)
GPCR	China-JQ310668.1-SPPV	Sheep	JQ310668.1	SPPV	China	(Zhou et al., 2012)
GPCR	Turkey-FJ869389.1-SPPV	Sheep	FJ869389.1	SPPV	Turkey	(Le Goff et al., 2009)
GPCR	China-JQ310666.1-SPPV	Sheep	JQ310666.1	SPPV	China	(Zhou et al., 2012)
GPCR	Tunisia-FJ869347.1-SPPV	Sheep	FJ869347.1	SPPV	Tunisia	(Le Goff et al., 2009)
GPCR	Tunisia-FJ869349.1-SPPV	Sheep	FJ869349.1	SPPV	Tunisia	(Le Goff et al., 2009)
GPCR	Morocco-FJ869378.1-SPPV	Sheep	FJ869378.1	SPPV	Morocco	(Le Goff et al., 2009)
GPCR	Niger-FJ869379.1-SPPV	Sheep	FJ869379.1	SPPV	Niger	(Le Goff et al., 2009)
GPCR	Tunisia-FJ869350.1-SPPV	Sheep	FJ869350.1	SPPV	Tunisia	(Le Goff et al., 2009)
GPCR	Tunisia-FJ869351.1-SPPV	Sheep	FJ869351.1	SPPV	Tunisia	(Le Goff et al., 2009)
GPCR	Nigeria-FJ869381.1-SPPV	Sheep	FJ869381.1	SPPV	Nigeria	(Le Goff et al., 2009)
GPCR	Nigeria-FJ869387.1-SPPV	Sheep	FJ869387.1	SPPV	Nigeria	(Le Goff et al., 2009)
GPCR	Algeria-FJ869385.1-SPPV	Sheep	FJ869385.1	SPPV	Algeria	(Le Goff et al., 2009)
GPCR	Algeria-FJ869386.1-SPPV	Sheep	FJ869386.1	SPPV	Algeria	(Le Goff et al., 2009)
GPCR	Tunisia-FJ869348.1-SPPV	Sheep	FJ869348.1	SPPV	Tunisia	(Le Goff et al., 2009)
GPCR	Tunisia-FJ869346.1-SPPV	Sheep	FJ869346.1	SPPV	Tunisia	(Le Goff et al., 2009)
GPCR	Tunisia-FJ869345.1-SPPV	Sheep	FJ869345.1	SPPV	Tunisia	(Le Goff et al., 2009)
GPCR	Turkey-FJ869388.1-SPPV	Sheep	FJ869388.1	SPPV	Turkey	(Le Goff et al., 2009)
GPCR	Turkey-FJ869384.1-SPPV	Sheep	FJ869384.1	SPPV	Turkey	(Le Goff et al., 2009)
GPCR	Turkey-FJ869382.1-SPPV	Sheep	FJ869382.1	SPPV	Turkey	(Le Goff et al., 2009)
GPCR	Senegal-FJ869380.1-SPPV	Sheep	FJ869380.1	SPPV	Senegal	(Le Goff et al., 2009)
GPCR	Turkey-FJ869383.1-SPPV	Sheep	FJ869383.1	SPPV	Turkey	(Le Goff et al., 2009)
RPO30	Huining isolate	Sheep		SPPV	Huining (China)	This study
RPO30	Zhangye isolate	Sheep		SPPV	Zhangye (China)	This study
RPO30	Iraq-GU119942.1-GTPV	Goat	GU119942.1	GTPV	Iraq	(Lamien et al., 2011a)
RPO30	Turkey-GU119940.1-GTPV	Goat	GU119940.1	GTPV	Turkey	(Lamien et al., 2011a)
RPO30	Yemen-GU119927.1-GTPV	Goat	GU119927.1	GTPV	Yemen	(Lamien et al., 2011a)
RPO30	Chad-GU119931.1-GTPV	Goat	GU119931.1	GTPV	Chad	(Lamien et al., 2011a)
RPO30	Chad-GU119930.1-GTPV	Goat	GU119930.1	GTPV	Chad	(Lamien et al., 2011a)
RPO30	Burkina Faso-GU119939.1-GTPV	Goat	GU119939.1	GTPV	Burkina Faso	(Lamien et al., 2011a)
RPO30	Ghana-GU119935.1-GTPV	Goat	GU119935.1	GTPV	Ghana	(Lamien et al., 2011a)
RPO30	China-JQ310674.1-GTPV	Goat	JQ310674.1	GTPV	China	(Zhou et al., 2012)
RPO30	Bangladesh-GU119949.1-GTPV	Goat	GU119949.1	GTPV	Bangladesh	(Lamien et al., 2011a)
RPO30	India-GU119936.1-GTPV	Goat	GU119936.1	GTPV	India	(Lamien et al., 2011a)
RPO30	Oman-GU119933.1-GTPV	Goat	GU119933.1	GTPV	Oman	(Lamien et al., 2011a)
RPO30	South Africa-GU119945.1-LSDV	Cattle	GU119945.1	LSDV	South Africa	(Lamien et al., 2011a)
RPO30	Egypt-GU119947.1-LSDV	Cattle	GU119947.1	LSDV	Egypt	(Lamien et al., 2011a)
RPO30	South Africa-GU119951.1-LSDV	Springbok	GU119951.1	LSDV	South Africa	(Lamien et al., 2011a)
RPO30	South Africa-GU119950.1-LSDV	Cattle	GU119950.1	LSDV	South Africa	(Lamien et al., 2011a)
RPO30	Niger-GU119952.1-LSDV	Cattle	GU119952.1	LSDV	Niger	(Lamien et al., 2011a)
RPO30	South Africa-GU119948.1-LSDV	Springbok	GU119948.1	LSDV	South Africa	(Lamien et al., 2011a)
RPO30	Burkina Faso-GU119946.1-LSDV	Cattle	GU119946.1	LSDV	Burkina Faso	(Lamien et al., 2011a)
RPO30	South Africa-GU119943.1-LSDV	Cattle	GU119943.1	LSDV	South Africa	(Lamien et al., 2011a)
RPO30	Sudan-GU119944.1-LSDV	Cattle	GU119944.1	LSDV	Sudan	(Lamien et al., 2011a)
RPO30	South Africa-GU119937.1-LSDV	Cattle	GU119937.1	LSDV	South Africa	(Lamien et al., 2011a)
RPO30	Turkey-GU119916.1-SPPV	Sheep	GU119916.1	SPPV	Turkey	(Lamien et al., 2011a)
RPO30	Senegal-GU119926.1-SPPV	Sheep	GU119926.1	SPPV	Senegal	(Lamien et al., 2011a)
RPO30	China-JQ310671.1-SPPV	Sheep	JQ310671.1	SPPV	China	(Zhou et al., 2012)
RPO30	China-JQ310673.1-SPPV	Sheep	JQ310673.1	SPPV	China	(Zhou et al., 2012)
RPO30	China-JQ310670.1-SPPV	Sheep	JQ310670.1	SPPV	China	(Zhou et al., 2012)
RPO30	Algeria-GU119920.1-SPPV	Sheep	GU119920.1	SPPV	Algeria	(Lamien et al., 2011a)
RPO30	Nigeria-GU119924.1-SPPV	Sheep	GU119924.1	SPPV	Nigeria	(Lamien et al., 2011a)
RPO30	Turkey-GU119923.1-SPPV	Sheep	GU119923.1	SPPV	Turkey	(Lamien et al., 2011a)
RPO30	Algeria-GU119921.1-SPPV	Sheep	GU119921.1	SPPV	Algeria	(Lamien et al., 2011a)
RPO30	Turkey-GU119919.1-SPPV	Sheep	GU119919.1	SPPV	Turkey	(Lamien et al., 2011a)
RPO30	Turkey-GU119918.1-SPPV	Sheep	GU119918.1	SPPV	Turkey	(Lamien et al., 2011a)
RPO30	Turkey-GU119917.1-SPPV	Sheep	GU119917.1	SPPV	Turkey	(Lamien et al., 2011a)
RPO30	Niger-GU119922.1-SPPV	Sheep	GU119922.1	SPPV	Niger	(Lamien et al., 2011a)
RPO30	China-JQ310669.1-GTPV	Goat	JQ310669.1	GTPV	China	(Zhou et al., 2012)

NA, not available.

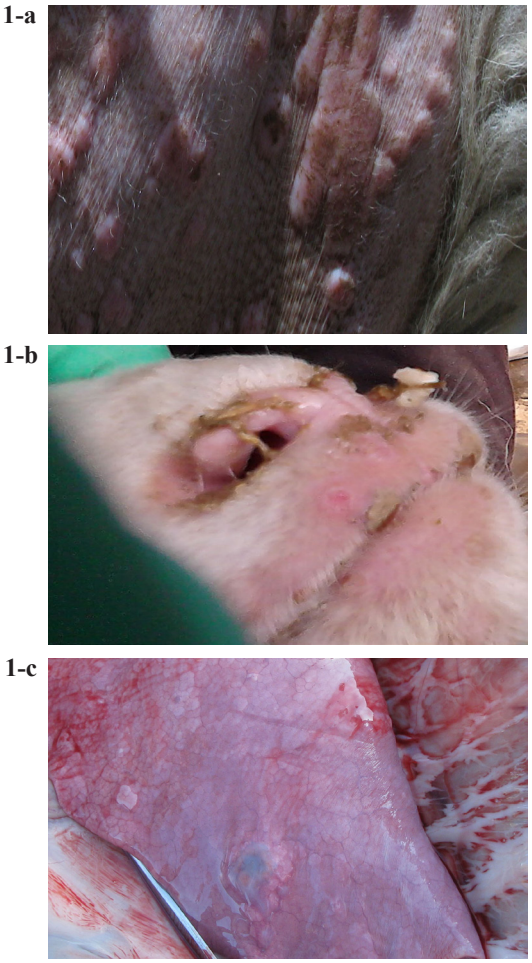

Figure 1. Representative clinical cases in Huining sheep flocks. **1-a.** papules under the tail; **1-b.** scabs on the lips; **1-c.** pox in the liver.

Table 2. Percent identities of the *P32* genes of Huining and Zhangye isolates with other members of the genus Capripoxvirus.

	Huining isolate		Zhangye isolate	
	Nucleotide	Amino acid	Nucleotide	Amino acid
China isolates of capripoxvirus	98.0-99.9	96.9-99.7	97.9-99.8	96.3-99.4
China isolates of SPPV	99.1-99.8	97.2-99.4	99.0-99.7	96.9-99.4
China isolates of GTPV	98.0-98.5	96.6-97.5	97.9-98.3	96.6-97.2
Asia isolates of capripoxvirus	98.0-99.9	72.3-99.7	97.9-99.8	72.3-99.7
Asia isolates of SPPV	98.0-99.9	72.3-99.7	97.9-99.8	72.3-99.7
Asia isolates of GTPV	98.0-98.5	96.3-97.5	97.9-98.3	96.3-97.5
Worldwide isolates of capripoxvirus	98.0-99.9	72.3-99.7	97.9-99.8	72.3-99.7
Worldwide isolates of SPPV	99.1-99.9	72.3-99.7	97.9-99.8	72.3-99.7
Worldwide isolates of GTPV	98.0-98.5	96.6-97.5	97.9-98.3	96.6-97.2
LSDV	98.0-99.8	72.3-97.5	97.9-99.7	72.3-97.2
Huining isolate	100	100	99.9	99.7
Zhangye isolate	99.9	99.7	100	100

Table 3. Percent identities of the *GPCR* genes of Huining and Zhangye isolates with other members of the genus Capripoxvirus.

	Huining isolate		Zhangye isolate	
	Nucleotide	Amino acid	Nucleotide	Amino acid
China isolates of capripoxvirus	95.2-99.7	93.9-99.5	95.2-99.7	93.6-99.2
China isolates of SPPV	99.5-99.7	99.5-99.7	99.5-99.7	99.2-99.7
China isolates of GTPV	95.2-95.4	93.9	95.2-95.4	93.6
Asia isolates of capripoxvirus	95.2-99.7	93.3-100	95.2-99.7	93.0-99.7
Asia isolates of SPPV	99.5-99.7	99.5-100	99.5-99.7	99.2-100
Asia isolates of GTPV	95.2-99.6	93.3-99.5	95.2-99.6	93.0-99.2
Worldwide isolates of capripoxvirus	95.0-99.7	93.3-100	95.0-99.7	93.5-99.7
Worldwide isolates of SPPV	99.5-99.7	99.2-100	99.5-99.7	98.9-99.7
Worldwide isolates of GTPV	95.2-99.6	93.9-99.5	95.2-99.6	93.0-99.2
LSDV	95.0-95.7	93.8-94.7	95.0-95.7	93.5-94.4
Huining isolate	100	100	99.8	99.7
Zhangye isolate	99.8	99.7	100	100

Table 4. Percent identities of the *RPO30* genes of Huining and Zhangye isolates with other members of the genus Capripoxvirus.

	Huining isolate		Zhangye isolate	
	Nucleotide	Amino acid	Nucleotide	Amino acid
China isolates of capripoxvirus	97.1-100	98.5-100	97.1-100	98.5-100
China isolates of SPPV	99.8-100	99.5-100	99.8-100	99.5-100
China isolates of GTPV	96.9-97.1	98.5	96.9-97.1	98.5
Asia isolates of capripoxvirus	96.4-100	97.4-100	96.4-100	97.4-100
Asia isolates of SPPV	99.8-100	99.5-100	99.8-100	99.5-100
Asia isolates of GTPV	96.4-97.1	97.4-98.5	96.4-97.1	97.4-98.5
Worldwide isolates of capripoxvirus	96.2-100	97.4-100	96.2-100	97.4-100
Worldwide isolates of SPPV	99.5-100	99.5-100	99.5-100	99.5-100
Worldwide isolates of GTPV	96.4-99.5	97.4-99.5	96.4-99.5	97.4-99.5
LSDV	96.2-96.6	97.4-98	96.2-96.6	97.4-98
Huining isolate	100	100	100	100
Zhangye isolate	100	100	100	100

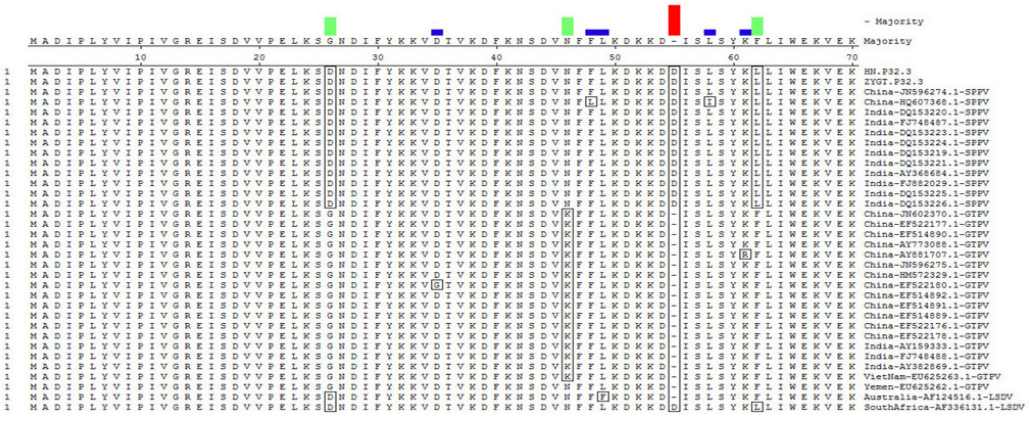

Figure 2. Alignment of the partial amino acid sequences of *P32* genes from several SPPV and GTPV strains. HN: Huining isolate; ZYGT: Zhangye isolate.

Figure 3. Phylogenetic analysis of different capripoxviruses based on the nucleotide sequences of the *P32* genes.

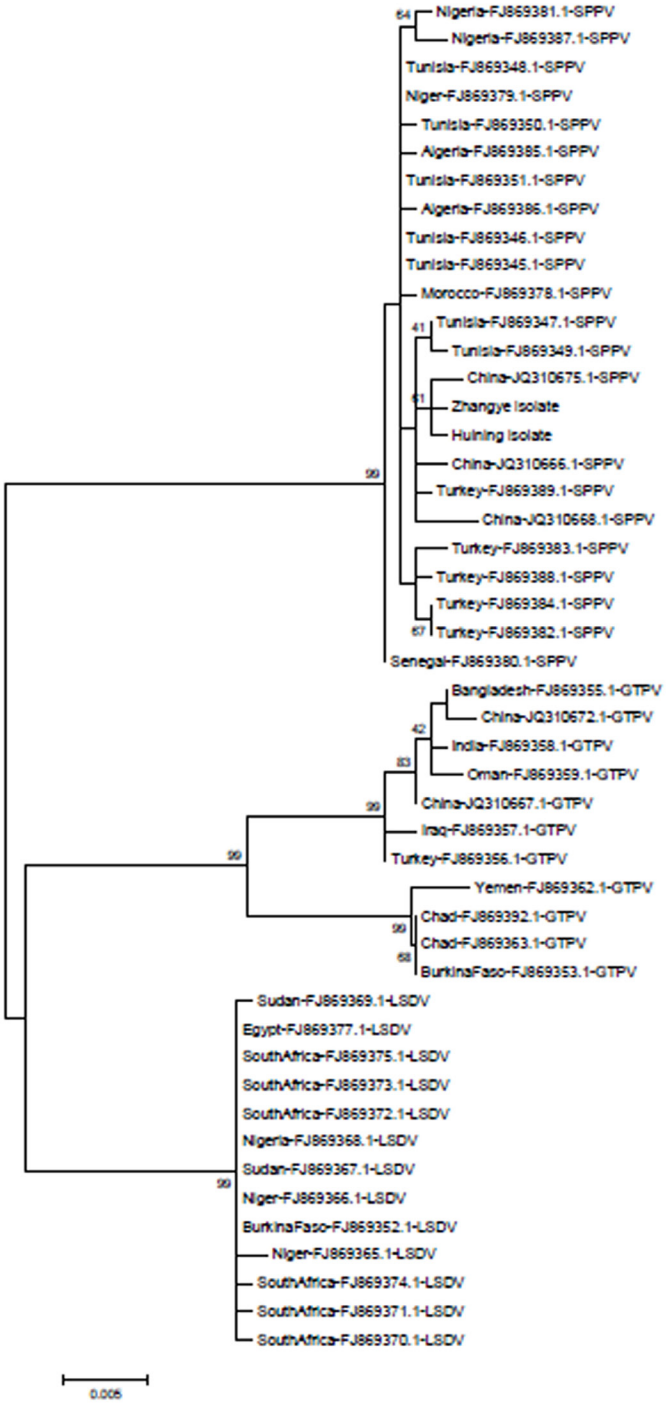

Figure 4. Phylogenetic analysis of different capripoxviruses based on the nucleotide sequences of the *GPCR* genes.

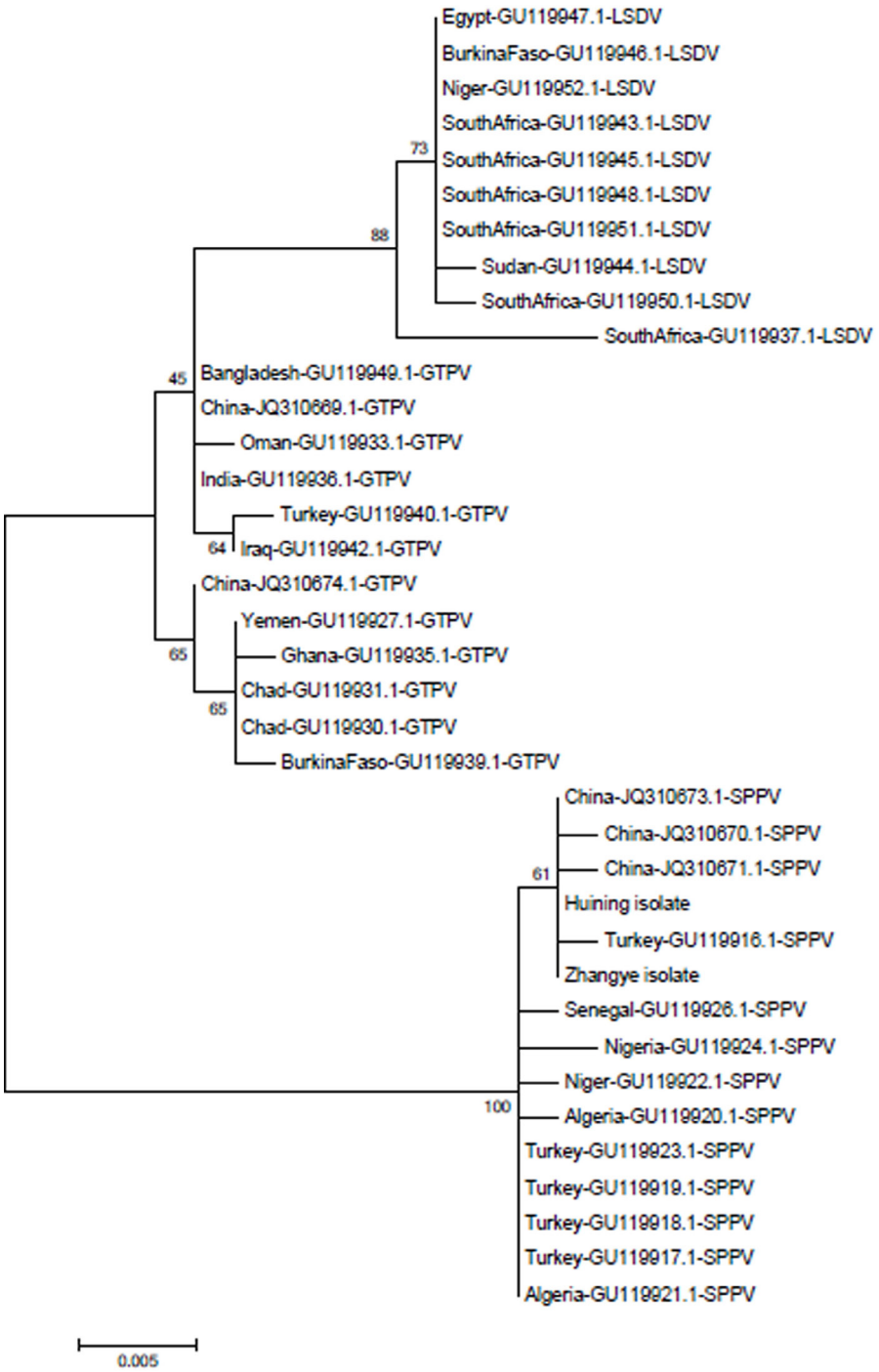

Figure 5. Phylogenetic analysis of different capripoxviruses based on the nucleotide sequences of the *RPO30* genes.

Based on the phylogenetic analysis of the *P32*, *GPCR*, and *RPO30* genes, the 2 strains were segregated into the SPPV group consistently in the 3 phylogenetic trees. There is a close relationship between the 2 isolates and Chinese SPPVs as they are clustered together. Because these ill sheep were imported from Jingyuan, another county of Gansu Province, our study strongly suggests the importance of veterinary surveillance prior to transportation.

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