



Detection of FMDV, BTV, PPRV, and ORFV by RT-PCR and DNA/RNA Sequencing



Yaping He¹, Qizhen He¹, Mengzhen Fan^{*}, Xinggang Xu^{*}
 College of Veterinary Medicine, Northwest A&F University, Yangling, Shaanxi 712100, China

ABSTRACT

Article history:
 Received 23 September 2016
 Received in final form 1 December 2016
 Accepted 14 January 2017
 Available online 19 January 2017

Keywords:
 Molecular epidemiology
 FMDV
 BTV
 PPRV
 SPPV
 GTPV
 ORFV

Molecular epidemiology of foot-and-mouth disease virus (FMDV), bluetongue virus (BTV), peste des petits ruminants virus (PPRV), and orf virus (ORFV) was investigated using RT-PCR and DNA/RNA sequencing. The results showed that FMDV, BTV, PPRV, and ORFV were detected in the samples. The genetic relationships between the detected viruses and those in other regions were analyzed. The results showed that the detected viruses were closely related to those in other regions. The results also showed that the detected viruses were highly specific for their respective hosts. The results further showed that the detected viruses were highly sensitive to heat and cold. The results finally showed that the detected viruses were highly resistant to disinfectants.

1. Introduction

Foot-and-mouth disease (FMD) is a highly contagious viral disease of animals caused by foot-and-mouth disease virus (FMDV). FMDV is a member of the Picornavirales order, Aphthoviridae family. FMDV is highly infectious and highly resistant to heat and cold. FMDV is highly sensitive to disinfectants. FMDV is highly resistant to heat and cold. FMDV is highly sensitive to disinfectants. FMDV is highly resistant to heat and cold. FMDV is highly sensitive to disinfectants.

BTV is a member of the Reovirales order, Reoviridae family. BTV is highly infectious and highly resistant to heat and cold. BTV is highly sensitive to disinfectants. BTV is highly resistant to heat and cold. BTV is highly sensitive to disinfectants. BTV is highly resistant to heat and cold. BTV is highly sensitive to disinfectants.

^{*} Corresponding author. E-mail address: ge2003@nwsuaf.edu.cn (X.G.X.).
¹ Both authors contributed equally to this work.

2010; Hfaea., 2008). Daga fhe fec a c ca e ga f ca fa e e e ce ba ed c ca a d e gca e h d he a . fCh a. (Haaea., 2009; Bhaaahe a., 2011). Iaae ha he eae a a e a ghe d eae c c, dffic dag ead de f hed ea e b c cadag ech ead e gca e h d. N c e cad ech e e PCR (I h a e a., 2002) a d ea e PCR e h d e e a ed dag e he d ea e fe e (B a e a., 2011; Ve a e a e a., 2012). H e e, he e ec a ech e a e ba ed he de ec f d ffe e a h ge g d ffe e PCR a a a ge g ece ec fic ge e b a e a ge be. The e a e e f he de ec a d d ffe e a f SPPV a d GTPV a b PCR e c f ag e e g h h (RFLP) (H a a e a., 2004; F z e e e a., 2006), PCR/ ea e PCR (O a e a., 2006; La e e a., 2011), CaPV a d ORFV b d e PCR (Zhe ge a., 2007), a da f a e de ec f BTV, FMDV, PPRV a d e c a a (VSV) (Q e a., 2015).

H e e, he f a ca he e f e PCR (PCR) f a e de ec a d d ffe e a f FMDV, BTV, PPRV, SPPV, GTPV, a d ORFV a ge bef a. The a f h d a de e he e RT PCR a d e PCR f a e de ec f hee a d g a DNA a d RNA e c ca ec e, c d g FMDV, BTV, PPRV, SPPV, GTPV, a d ORFV. F e, he e a de e ed h d a ec de ed be ef e h d f de f g e ec e f hee a d g a h e fec .

2. Materials

2.1. Viruses and cells

The CVCC AV41 acc e a f GTPV (Sha d g L d B ech e C., Ca. . 151824003), HCE acc e a f ORFV (Sha d g Ta Fe g B gca P d c C., Ca. . 151784013), Nge a/75/1 acc e a f PPRV (X ag Ta Ka g B ech e C., Ca. . 2007291), BTV (Ch a e e a c e c ec a age e ce e, Ca. . CVCC AV47), SPPV (Ch a e e a c ec ec a age e ce e, Ca. . CVCC AV1011), a d FMDV e O (Ch a e e a c ec ec a age e ce e, Ca. . CVCC AV100) e e cha ed. B e a da hea e (BVDV) Shaa a a ded d b P fe J g Y Wa g, C ege f Ve e a Med c e, N h e A&F U e. The e e e e da a da d e f he e PCR a d a da -80°C e g. B e e ce (BTC) a de c bed e (T a e a., 2013). BHK 21 ce (Sha gha Ga g B gca C., Ca. N. CMT 013), a d Ve ce e (ATCC . CCL 81) e e ab a c. BTC, BHK 21 ce, a d Ve ce e, *Escherichia coli* (Ch a e e a c ec ec a age e ce e, Ca. N. CVCC3798) a e a BVDV e e a ed he ec fic a a. GTPV, SPPV, a d ORFV e e aga ed he B e e ce, BTV a d PPRV e e aga ed he Ve ce a d FMDV a aga ed he BHK 21 ce. T e a a e he effice c f he e ac e h d a d a e he e PCR, a e f ce fec ed h each f FMDV, BTV, PPRV, SPPV, GTPV ORFV e e a ed b e PCR.

2.2. Clinical specimens

D g he e d f Dece be 2014 Dece be 2015, 43 c ca ec e c g f cab, he e, b d, ab, g, ee, h de a d e e ec ec ed f hee a d g a. The e a e e e he c ec ed f fie d bea b he d ea e e ga g ea b ed ab a f

c ca e ga f ca fa e e e ce fCh a.

2.3. Extraction of RNA and DNA

V a ge c DNA a d RNA e e e ac ed f ce c e fec ed h each a d c ca ec e g he A ge RNA/DNA M K (A ge, Sa Fa c c, USA) acc d g he a fac d c. A ge c e c ac de ac c a ad ed f he a e e ac f b h RNA a d DNA e. C ca a e h ge zed a 10% e g h ha eb ffe a e e e ed f e ac f a c e c ac da d ed a -20°C e. The fec ed ce a d c ca a e e e f ee ze ha ed hee e bef e bec g f ge ce ac. The a c e c ac da e e e e ac ed f 500 μ e f e e. The e ac ed a c e c ac da e a ed a -80°C e.

2.4. Designing the mPCR primers

The e e ce f e a ed a f he 3D ge e f FMDV e e he a ea e e (Q e a., 2015). The e e ce f e a ed a f he NS3 ge e f BTV e e he a ea e e (Fe ge a., 2014). The e e ce f e a ed a f he N ge e f PPRV e e he a ea e e (Ma e a., 2010). P e f a f g SPPV, GTPV ORFV e e de g ed g PRIMER PREMIER 5.0 f a e. S a f e e e he zed b I ge T ad g C. (Sha gha, Ch a). PCR e a f each a ge ge ea d Ge Ba acce be h each a ge ed ge ea d he e ec ed ze f PCR d c e e a zed Tab e 1.

2.5. Reverse transcription

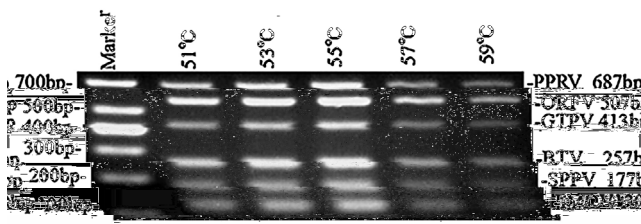
The e e e a c (RT) eac a e f ed a 20 μ e, h ch c a 5 μ e a a e f he a c e c ac d a e, 5 μ 4× FQ RT S e M (Fa Q a RT E z e, RNA e h b, Ra d e, O g d TP e, d NTP M e, eac B ffe, Ta ge, TIANGEN B ech, Be g, Ch a), a d 10 μ DEPC a e. The eac c a e: he ef c ba a 42°C f 15 a d e a ed he eac b hea ga 95°C f 3. The d c e e e 4°C f e a d e PCR.

2.6. The uniplex PCR

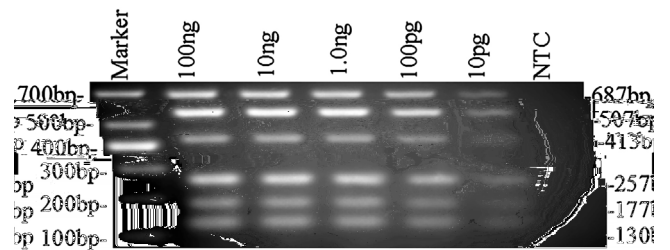
The e PCR eac f FMDV, BTV, PPRV, SPPV, GTPV, a d ORFV a ca ed a 25 μ e c a g 12.5 μ 2× P e Ta™ (20 M T HC H 8.3, 100 M KC, Ta DNA e a e 1.25 U/25 μ, 3 M MgC₂, 0.4 M each dNTP), 1 μ f each 10 /μ e (Tab e 1), 100 g f DNA a d cDNA e a e, a d he added h d ed a e 25 μ e a. The d ed a e a da a e ga ec. The a fica e e e f ed a The C ce K960 (Hea F ce, Sha gha, Ch a) a fie g he f g : af e a da e a a 95°C f 5, 30 c ce a 95°C f 30, 57°C f 30 a d 72°C f 45, f ed b a fi a e e a 72°C f 10. A c e e e a a zed b

Table 1
 Specific primers for each genotype.

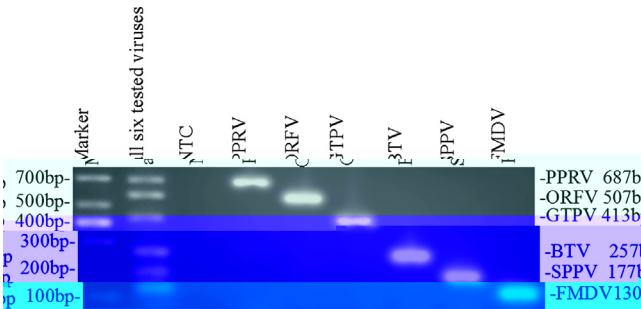
Virus	Primer	Sequence (5'–3')	Position	Product size	Accession	Accession (Sra)
DNA SPPV	SPPVF	ACTAAACTTGTTACATTGTGA	119555–119576	177 bp	ORF112	AY077832.1 (TU V02127)
GTPV	SPPVR	AACTTCTCCATCAATACATGA	119731–119711			



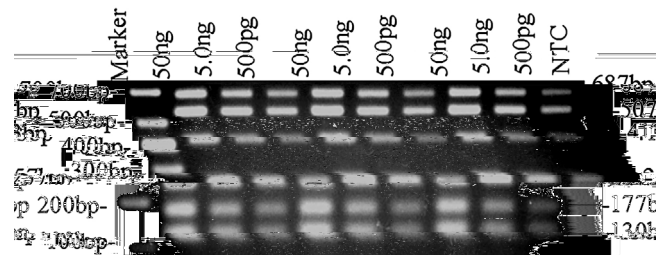
F . 1. Agarose gel electrophoresis of the PCR products (130bp for FMDV, 257bp for BTV, 687bp for PPRV, 177bp for SPPV, 413bp for GTPV and 507bp for ORFV) generated from the extracted RNA. Lane: DL1000 DNA added as a negative control.



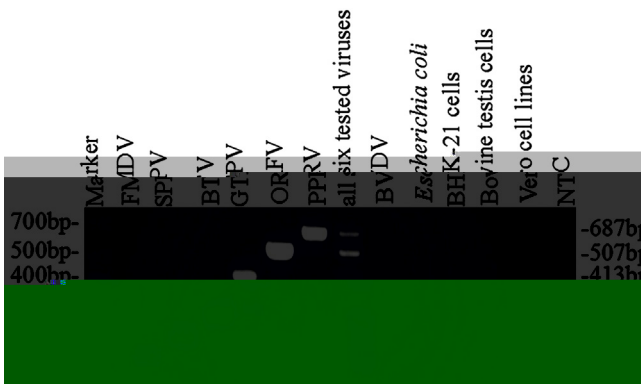
F . 4. Specificity of the PCR. Specificity of the PCR for FMDV, BTV, PPRV, SPPV, GTPV, and ORFV. Lane: DL1000 DNA added as a negative control (ddH₂O); and the extracted RNA of FMDV, BTV, PPRV, SPPV, GTPV, and ORFV.



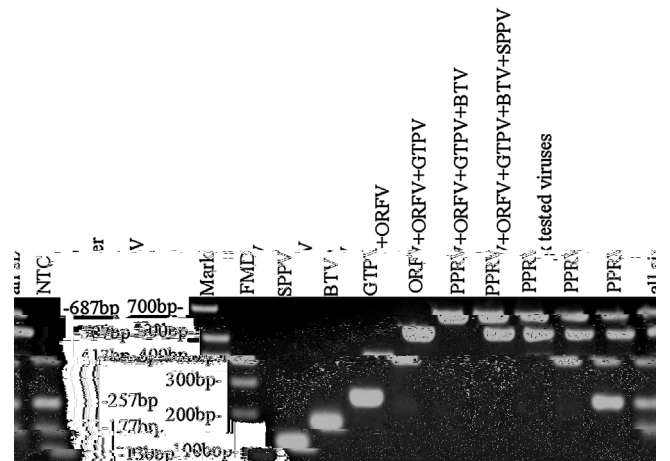
F . 2. Agarose gel electrophoresis of the PCR products (130bp for FMDV, 257bp for BTV, 687bp for PPRV, 177bp for SPPV, 413bp for GTPV, and 507bp for ORFV) generated from the extracted RNA. Lane: DL1000 DNA added as a negative control (ddH₂O); and the extracted RNA of FMDV, BTV, PPRV, SPPV, GTPV, and ORFV.



F . 5. The detection limit of the PCR. The gel shows the detection limit of the PCR for FMDV, BTV, PPRV, SPPV, GTPV, and ORFV. Lane: DL1000 DNA added as a negative control (ddH₂O); and the extracted RNA of FMDV, BTV, PPRV, SPPV, GTPV, and ORFV.



F . 3. Specificity of the PCR. The gel shows the specificity of the PCR for FMDV, BTV, PPRV, SPPV, GTPV, and ORFV. Lane: DL1000 DNA added as a negative control (ddH₂O); and the extracted RNA of FMDV, BTV, PPRV, SPPV, GTPV, and ORFV.



F . 6. Agarose gel electrophoresis of the PCR products (130bp for FMDV, 257bp for BTV, 687bp for PPRV, 177bp for SPPV, 413bp for GTPV, and 507bp for ORFV) generated from the extracted RNA. Lane: DL1000 DNA added as a negative control (ddH₂O); and the extracted RNA of FMDV, BTV, PPRV, SPPV, GTPV, and ORFV.

3.3. The sensitivity and reproducibility of the multiplex PCR

To determine the sensitivity of the multiplex PCR, the extracted RNA (100 µg) of each virus was tested by the multiplex PCR. The sensitivity of the multiplex PCR was 100 µg of the extracted RNA (Fig. 4). In the detection limit of the multiplex PCR, the extracted RNA was tested by the multiplex PCR (Fig. 5).

3.4. Multiplex PCR in positive samples

The extracted RNA of each virus was tested by the multiplex PCR. The extracted RNA of each virus was tested by the multiplex PCR. The extracted RNA of each virus was tested by the multiplex PCR.

3.5. Screening of clinical specimens by the uniplex and multiplex PCR

The extracted RNA of each virus was tested by the multiplex PCR. The extracted RNA of each virus was tested by the multiplex PCR.

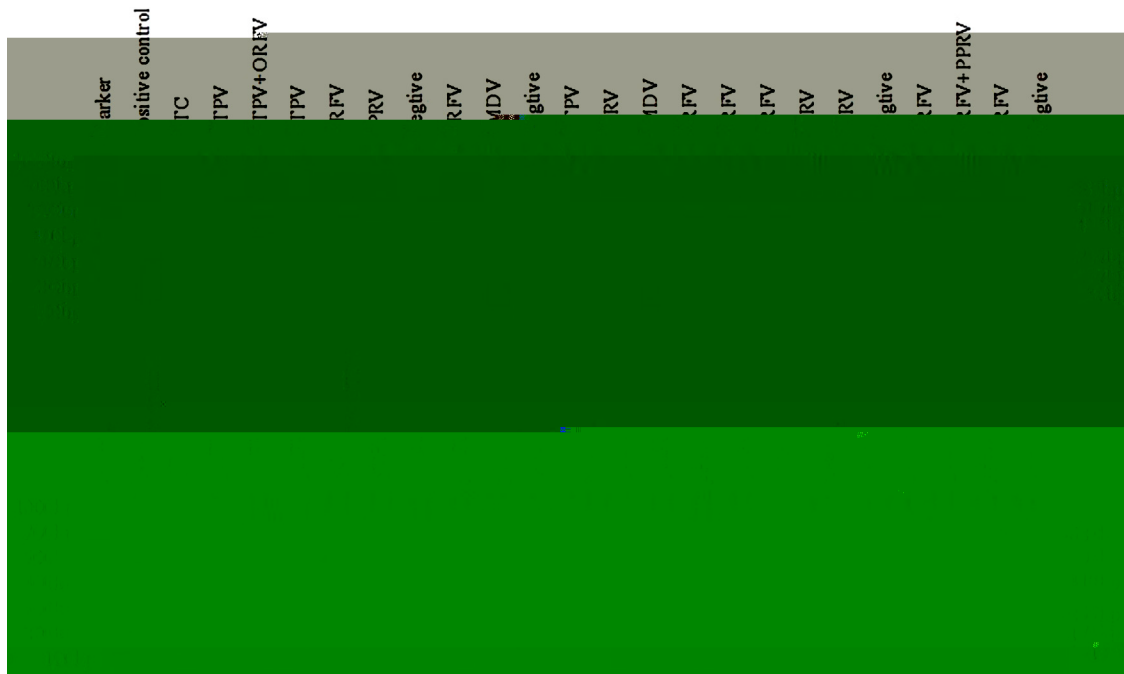


Fig. 7. Agarose gel electrophoresis of 43 samples of PCR products. Each lane represents a different sample. Lane 1, Marker, DL 1000. Lane 2, Positive control. Lane 3, FMDV+ORFV; Lane 4, FMDV+SPPV; Lane 5, FMDV+ORFV+SPPV; Lane 6, FMDV+ORFV+PPRV; Lane 7, FMDV+SPPV+ORFV; Lane 8, FMDV+SPPV+ORFV+PPRV; Lane 9, FMDV+SPPV+ORFV+PPRV; Lane 10, FMDV+SPPV+ORFV+PPRV; Lane 11, FMDV+SPPV+ORFV+PPRV; Lane 12, FMDV+SPPV+ORFV+PPRV; Lane 13, FMDV+SPPV+ORFV+PPRV; Lane 14, FMDV+SPPV+ORFV+PPRV; Lane 15, FMDV+SPPV+ORFV+PPRV; Lane 16, FMDV+SPPV+ORFV+PPRV; Lane 17, FMDV+SPPV+ORFV+PPRV; Lane 18, FMDV+SPPV+ORFV+PPRV; Lane 19, Negative control. Lane 20, DNA ladder.

Table 2
Detection of 43 samples of PCR products.

Sample	Virus					
	FMDV	SPPV	BTV	GTPV	ORFV	PPRV
Ultrasonic PCR/RT-PCR	3	0	0	8	22	7
Manual PCR	3	0	0	8	22	7

Table 3
Detection of 43 samples of PCR products.

Virus	Number of samples	Percentage (%)
FMDV	3	6.98
SPPV	0	0
BTV	0	0
GTPV	6	13.95
ORFV	19	44.19
PPRV	6	13.95
GTPV+ORFV	2	4.65
ORFV+PPRV	1	2.33

de a d e e e c e c e d f h e e e e c e f C h a a d d e c e d b e a d e PCR (Fig. 7). The e e e d e c a h e a c h h e (Table 2). A PCR d c e e e e c e d a d a e h e e c f i c f h e e a d e PCR. A g 43 c c a e c e , 37 e c e e e e b e e PCR g e PCR a a . C f e c h e e a d e a e d 3 a e (6.98%). C f e c h e h e e e e a d e c e d h e e c c a a e (Table 2 a d 3). The e b a e d b h e e PCR e e c e h e b d f e a e e , c d g h e PCR f SPPV, GTPV a d ORFV (V e a e a e a ., 2014; X a e a ., 2012), a d a e PCR f BTV, FMDV, PPRV (Q e a ., 2015).

4. D

The e PCR a d RT PCR c e e d e e d h d f h e a e d e c f g e a e a e d f e c h e e / g a . R e a a d e d c e d e a e , h c h a e a c c a g , c a e e e e c c e h e e / g a . FMDV, BTV, PPRV, SPPV, GTPV, a d ORFV e e c e e c a e d h e e d e a e . F h e e , e f e c h a h g e a e c h e h e e / g a d d e . The e f e , a a d a d e c e d a g c d e e c f FMDV, BTV, PPRV, SPPV, GTPV, a d ORFV e e a f e a d e c , e a c e , a d e e f e a d f d e a e . T d a e , a e d h a d e c b e d h e d e c f h e e DNA e a d RNA e , d c a g h a e PCR/RT PCR h a h g h e a d e c f i c (Q e a ., 2015; F e g e a ., 2014; M a e a ., 2010; X a e a ., 2012; Z h e a ., 2007; X a g e a ., 2011). I h e e e d , h e a g e c DNA a d RNA a e a c e d a e a d b e c e d h e e PCR a e a c . The e f e , a e c e f f e c e a d e a g e a f d a g , c e e g , a d e a c e a f e a d e e d h . Th e PCR d e c a e a 100 g f e e d e . A h g h , h e e f h e e PCR a e 5 10 f d e h a e a b h e d e PCR (Q e a ., 2015; F e g e a ., 2014; M a e a ., 2010; X a e a ., 2012; Z h e a ., 2007; X a g e a ., 2011), h e e e f e a g d c e a e f h e d c . N a f i c a a b a e d f h e a h g e a d c e , c h a BVDV, Escherichia coli, BHK 21 c e , B e e c e , a d V e c e e , d c a g h a h e d e e d e PCR h a h g h e c f i c . The e a a f 43 c c a a e b e PCR d c a e d h a 6.98% f h e a e e e c f e c e d h e e . C f e c h e h e e e e a d e c e d h e e c c a a e . B e c a e h e e c c a a e a e e e e a e f c c a a e g e a e e e c e f C h a , h e e d c a e h a h e e a c a d e a e h d b e a e h e e d e c f FMD, GP, ORF a d PPR.

I a , he de e ed a a a f a d, ec fic, a d e de ec a d e a ce f e a fec hee /g a a df fi a f he e e e e f c ca a cab .

A

Th a a ed b g a f he a d a a cha ec f Shaa P ce (2016KTZDNY02 06), he ba c e ea ch a d e a ge e e f N h e A&F U e (G a N .Z109021427) a d he a ec f ag ech g f Shaa P ce, Ch a (N .2016NY 092).

R

Ba a ga , V., Se , A., Sa a a a , P., S gh, R.P., S gh, R.K., Ra , T.J., Ba d adh a , S.K., 2006. O e e e RT PCR a a f he de ec f e e de e a c ca a e .Ve .Re .C .30, 655 666.

Bha a a h, V., H a a , M., S gh, R.K., 2011. P ec fc a d e ad ca fca f hel da bc e :a e ec e.A a Re .91, 225 232.

B a, D.P., Ve a e a , G., Bha a a h, V., Ba a ga , V., P abh , M., S a a a , M.S., Y g Fa adh a, R., 2011. Ta Ma ea e PCR a a ba ed DNA e a e ge ef a d de ec f O f fec .J.V .Me h d 178, 249 252.

Ch , Y., Ya , X., Ga , P., Zha , P., He, Y., L , J., L , Z., 2011. M ec a de ec fa ed fec fga f , a d M c a aca c b .ca e ae ga .J.Ve .D ag .I e .23, 786 789.

Dha , P., S ee a a, B.P., Ba e , T., C e , M., S gh, R.P., Ba d adh a , S.K., 2002. Rece e de g f e de e a ().Ve .M c b .88, 153 159.

Fe g, Y.F., Zha , G.H., X.U, Q.Y., Ya g, T., S , E.C., L , J.P., W , D.L., 2014. De e e f e e c e h df he de ec fb e ge .Ch .J.P e .Ve .Med.36, 712 714.

F ze e, S., S gh, R.K., H a a , M., M da , B., Yada , M.P., 2006. E a a f d e PCR a d PCR RFLP f dag f hee a d ga .I .J.T .Med.1, 66 70.

H f a , M.A., Re z , S., Made , M., Cha g a , V., W a, G., Th e , B., 2008. Ge e c cha ace za f gge b g b a e b e ge , f g a , S ze a d.I e g.I fec .D .14, 1855 1861.

H a a , M., M da , B., Te bh e, P.A., Ba d adh a , S.K., S gh, R.K., Ra , T.J., 2004. Dffe e a f hee a d ga e b e e cea a a d c fl f 32 ge e.V Ge e 29, 73 80.

H a a , M., Scag a , A., Bha a a h, V., Mcl e , C.J., S gh, R.K., 2009. O f : a da e c e e ea ch a df e e ec e .E e Re .A I fec .The .7, 879 893.

I h a, Y., M a a , K., W , D., Se , H., 2002. Cha ac e za f aa e c c a ga g dJa a e e e (Ca c c).M c b .I .46, 583 587.

Ja g, T., La g, Z., Re , W., Che , J., Zh , X., Q , G., e a ., 2011. De e e a d a da fa a e a fl a a gc da g df he de fica f e e ec ficf a d h d ea e , a a da a l .J.V .Me h d .171, 74 80.

La e , C.E., Le G ff, C., S be , R., Wa ace, D.B., G az, V., T a e , E., Mada , H., Ca f , P., Ada , T., E Ha a , M., L c , A.G., A b a, E., Da , A., 2011. U e f he Ca h g e f acc a 30 Da RNA e a e b (RPO30) ge ea a e dag ca dge g a ge : de e e fa ca ca PCR e h d dffe e a e ga f hee .Ve .M c b .149, 30 39.

Le, G.C., La e , C.E., Fa hfa h, E., Chade a , A., Aba Ad gba, E., L bea , G., T a e , E., Wa ace, D.B., Ada , T., S be , R., G az, V., Mada , H., Ca f , P., Ha a , S., Da , A., A b a, E., 2009. Ca G e c ed che e ece :a h a ge ge e ab ef a a g d c a .J.Ge .V .90, 1967 1977.

MacLach a , N.J., 2010. G ba ca f he e ce e e ge ce fb e ge e e .Ve .C .N h A .F d A .P ac .26, 163 171.

Mac ach a , N.J., 2011. B e ge e h g ba e de g , a d a h ge e .P e .Ve .Med.102, 107 111.

Ma , L., D , Y.X., Zha , J.J., Wa g, Y.C., G g, W., Ca , X.P., 2010. De e e f RT PCR f de ec f e e de e a .Ch .Ve .Sc .40, 593 597.

OIE, 2016. OIE L ed D ea e , I fec a d I fe a F ce 2016.

O a, E.S., Shche ba a, A.V., D e , V.I., Za Fa , V.M., 2006. Dffe e a f ca e ce a d a b e a e cha eac .M .B .40, 158 164.

Q , M., Z , F.C., Ya , Y.Q., Zh , H., N e, F.P., Wa g, H., Ya g, J., Ye, L.L., Zh , X.L., A, J., 2015. De e e f e PCR e h df de ec f BTv, FMDV, PPRV a d VSV. P g .Ve .Med.36, 18 22.

Ta , T.T., L , J., Ya , Y.L., L , Q.R., X , J.Y., Zha , Y.Q., Che , D.K., 2013. P e a a f f ac a ed acc ea da e e f e effec .P g .Ve .Med.34, 17 20.

Ve a e a , G., Bha a a h, V., Ba a ga , V., B a, D.P., P abh , M., Y g ha adh a, R., Pa de , A.B., 2012. Ra d de ec a d a fica f O f f fec ed cab a e a f hee a d ga .Ac a V .56, 81 83.

Ve a e a , G., Ba a ga , V., Bha a a h, V., 2014. Ta a ba ed ea e d e c f a e de ec a d a a fca a d f ge e c ca a e .J.V .Me h d 201, 44 50.

Xa g, Z.L., Che g, Z.T., Zh , J.H., Zh , B.J., O , D.Y., XIAN, S.M., L , F., RAN, G.X., 2011. E ab h e a d d e a ca fd e PCR f ga a d f .Ch .A .H b .Ve .Med.2011 (38), 88 91.

Xa , W., N e, F.P., Wa g, Y., X a , J.W., Zh , X.M., D g, S., Zh , Q., L , Y.G., L , L., 2012. Dffe e a f hee a d ga b d e PCR. Ch .J.P e .Ve .Med.34, 551 554.

Ya , X., W , G., L , J., Zh , H., Zha g, Q., 2010. The a e de c a f ca ch a.Ch .Ag c.Sc.B .26, 6 9.

Zhe g, M., L , Q., J , N., G , J., H a g, X., L , H., Zh , W., X g, Y., 2007. Ad e PCR a f a e de ec a d dffe e a f Ca a d O f .M .Ce .21, 276 281.

Zh , W., X g, Y., L , Q., G , J.G., L , H.M., 2007. De e e f e PCR f a d dffe e a f ga f e h .Ch .J.P e .Ve .Med.29, 394 396.