Bulgarian Journal of Veterinary Medicine (2013), **16**, No 3, 159–169

GENETIC ANALYSIS OF THE VIRAL AGENTS CAUSING MUZZLE CRUST IN SMALL RUMINANTS OF SHIRAZ, IRAN

S. A. DAVARI, M. SAYYARI & A. MOHAMMADI

Department of Pathobiology, School of Veterinary Medicine,
Shiraz University, Shiraz, Iran

Summary

Davari, S. A., M. Sayyari & A. Mohammadi, 2013. Genetic analysis of the viral agents causing muzzle crust in small ruminants of Shiraz, Iran. *Bulg. J. Vet. Med.*, **16**, No 3, 159–169.

Orf virus (ORFV), the prototype of the genus parapoxvirus (PPV), is the cause of contagious ecthyma, a common viral skin disease of domestic and wild small ruminants with a worldwide distribution. This disease is endemic in Iran and it is sometimes difficult to differentiate it from similar diseases, especially sheep pox, goat pox and peste des petits ruminants (PPR). In this study 50 muzzle crusts were collected from involved sheep and goats from Shiraz suburb during 2011–2012 and PCR was carried out for molecular detection. Twenty-five (50%) of 50 scab specimens were orf positive, 4 (8%) were pox virus positive and 1 (2%) was positive for PPRV. The PCR products were sequenced for nucleotide analysis. Furthermore, orf virus phylogenetic analysis based on 045 gene demonstrated a high degree of identity with other ORFV strains, although there was a slight diversity among the nucleotide sequences. Our study showed that the role of ORFV was greater than the other two viruses for causing muzzle crusts. This is the first molecular study of ORFV in Iran.

Key words: capripox, contagious ecthyma, Iran, muzzle crust, PCR, PPR

INTRODUCTION

Contagious ecthyma, also called Orf, is a common viral disease which is caused by a parapox virus (Family Poxviridae, Subfamily Chordopoxvirinae) (Gallina *et al.*, 2008). Parapox viruses (PPVs) affect a wide range of species including sheep, goats, wild ruminants and less commonly, human beings, cattle and dogs (Guo *et al.*, 2003; Tryland *et al.*, 2005). The Orf virus has been commonly found throughout the world (Zhang *et al.*, 2010). Clinically, the disease is characterised by proliferative and self-resolving lesions around the muzzle and lips of involved animals (de la Concha-Bermejillo *et al.*, 2003; Zhao *et al.*, 2010). Cases of persistent contagious ecthyma with secondary bacterial infections or myasis have been reported (Guo *et al.*, 2003). Morbidity may be very high in the young animals but mortality is usually low (Lojkic *et al.*, 2010). Transmission typically occurs by direct contact or via handling contaminated animal products (Abrahao *et al.*, 2009). The disease has an economic impact on sheep and goats due to decreases in production, unwillingness to nurse, eat, or walk, failure to thrive and more susceptibility to adventitious bacterial infections due to immune suppression (Housawi *et al.*, 2008; Zhang *et al.*, 2010).

Sheeppox and goatpox are characterised by fever, generalised pock lesions in the skin, internal lesions and death. The

viruses belong to the genus Capripoxvirus in the family Poxviridae. (Chu *et al.*, 2011). A very high morbidity and mortality rate, especially in young animals was reported (Zhou *et al.*, 2012).

Peste des petites ruminants (PPR) is a contagious disease of sheep and goats caused by the PPR virus (PPRV), a member of the genus *Morbillivirus* in the family Paramyxoviridae (Luka *et al.*, 2012). PPR also leads to heavy morbidity and mortality and economic losses (Malik *et al.*, 2011).

The goal of this study was molecular detection and nucleotide analysis of orf, PPR, and capripox viruses causing muzzle crusts of small ruminants in Shiraz suburb.

MATERIALS AND METHODS

Clinical samples

The clinical signs and macroscopic lesions were observed in 50 suspected sheep and goats with muzzle crusts from different geographical locations of Fars province during 2011–2012. All infected animals had wart-like, scabby lesions on the muzzle, lips and oral mucosa to variable degrees. Tissue samples were collected and stored in a –70 ^{o}C freezer. The frozen tissue samples (100 mg) were ground to fine powder under liquid nitrogen. Fifty mg of this was used for DNA extraction.

DNA extraction

DNA was extracted from the samples using an AccuPrep Genomic DNA Extraction Kit (BIONEER, Korea), according to the manufacturer's instructions. A lyophilised freeze-dried live sheeppox vaccine was used for DNA extraction positive control. The extracted DNAs were stored at –70 ^{o}C.

RNA extraction

The frozen tissue sample powders (50 mg) were homogenised in 1 mL of RNX-PLUS solution (Cinnagen, Iran) and the procedure was carried out according to the manufacturer's instructions. A lyophilised freeze-dried live PPR vaccine was used as a reference virus in this stage. The extracted RNAs were kept at –70 ^{o}C until used.

PCR for orf virus detection

A partial sequence of the 045 gene was amplified by PCR using the primers 045F-045R (Table 1) (Kottaridi *et al.*, 2006). PCR was performed in 20 μL volume containing 5 μL extracted DNA, 1× reaction buffer, 0.2 mM of dNTPs, 2 mM of $MgCl_2$, 0.5 pmol/μL of each primer and 1U of Taq DNA polymerase. The programme consisted of an initial denaturation at 95 ^{o}C for 5 min, followed by 39 cycles at 95 ^{o}C for 15 s, 47 ^{o}C for 15 s and 74 ^{o}C for 15 s; the amplification was completed with a final extension in 78 ^{o}C for 15 min.

PCR for poxvirus detection

The capripoxvirus fusion gene was amplified using CPVF and CPVR primers (Table 1) with the following programme: an initial cycle at 94 ^{o}C for 5 min, 50 ^{o}C for 30 s, 72 ^{o}C for 1 min followed by 34 cycles at 94 ^{o}C for 1 min, 55 ^{o}C for 40 s, 72 ^{o}C for 1 min and a final elongation step at 72 ^{o}C for 5 min (Ireland & Binepal, 1998). The reaction mixture (20 μL) contained 1× PCR buffer, 1.5 mM of $MgCl_2$, 0.2 mM of dNTPs, 0.5 pmol/μL of each primer, 1 unit of Taq DNA polymerase and 5 μL of template DNA.

Table 1. The oligonucleotide sequences were used in different PCRs.

Name	Purposes	Target	Sequence (5` – 3`)
045	Forward	045 gene	CCTACTTCTCGGAGTTCAGC
045	Reverse	045 gene	GCAGCACTTCTCCTCGTAG
NP_3	Forward	NP gene	TCTCGGAAATCGCCTCACAGACTG
NP_4	Reverse	NP gene	CCTCCTCCTGGTCCTCCAGAATCT
CPVF	Forward	Fusion gene	ATGGACAGAGCTTTATCA
CPVR	Reverse	Fusion gene	TCATAGTGTTGTACTTCG

Single stranded cDNA synthesis and PCR for PPRV detection

Five µL of extracted RNA was subjected for cDNA synthesis using BIONEER AccuPower^{TM} RT PreMix kit (Korea). The DNA fragment of 353 bp targeting the nucleo-protein (NP) gene was amplified by PCR with the set of primers NP3 and NP4 (Table 1). The reaction was carried out in a volume of 25 µL containing 1× PCR buffer, 2.5 mM of $MgCl_2$, 0.2 mM of dNTPs, 0.4 pmol/µL of each primer, 1U of Taq DNA polymerase and 5 µL of cDNA template. The programme consisted of an initial denaturation at 95 ^oC for 5 min followed by 34 cycles of denaturation at 94 ^oC for 30 s, annealing at 62.5 ^oC for 30 s, extension at 72^oC for 30 s and termination by a final extension of 2 min at 72 ^oC. All PCR amplicons were analysed by electrophoresis on 1% agarose stained with ethidium bromide. Amplified product was visualised under UV light.

Sequencing

The positive PCR products of CPV and PPRV as well as 5 samples of ORFV were sequenced (BIONEER, Korea). The sequence identities of nucleotides (nt) were analysed using the Clustal W method (Thompson *et al.*, 1994). Comparison of the ORFV, PPRV and CPV sequences with those available in the GenBank database was separately performed for each virus using the online BLAST programme (http: //www.ncbi.nlm.nih.gov/blast/Blast. cgi). The accession numbers of sequences that have been submitted to GenBank are: KC534486 (Orf-Shiraz1), KC534487 (Orf-Shiraz2), KC534488 (Orf-Shiraz3), KC534489 (Orf-Shiraz4), KC534490 (Orf-Shiraz5), KC607874 (Pox-Shiraz1), KC534492 (PPR-Shiraz1). Phylogenetic tree derived from nt sequences was constructed for orf virus using the neighbour-joining (NJ) method of MEGA4 software (Tamura *et al.*, 2007).

RESULTS

Twenty-five (50%) of the 50 scab specimens were positive for ORFV. The PCR amplicons of approximately 393 bp were obtained from the reactions (Fig. 1). The target gene (viral fusion gene) of poxvirus was amplified by PCR which produced an amplicon of 446 bp in size only in 4 (8%) of the samples. Of these, 2 samples were also orf virus positive (Fig. 2). Finally, just one (2%) sample produced the expected amplification product of 353 bp with the NP3/NP4 primers for PPRV (Fig. 3).

The partial sequences (393 bp) of the 045 gene of orf virus were compared with the 9 sequences representing ORFV, Pseudocowpox virus (PCPV) and Bovine Pa-

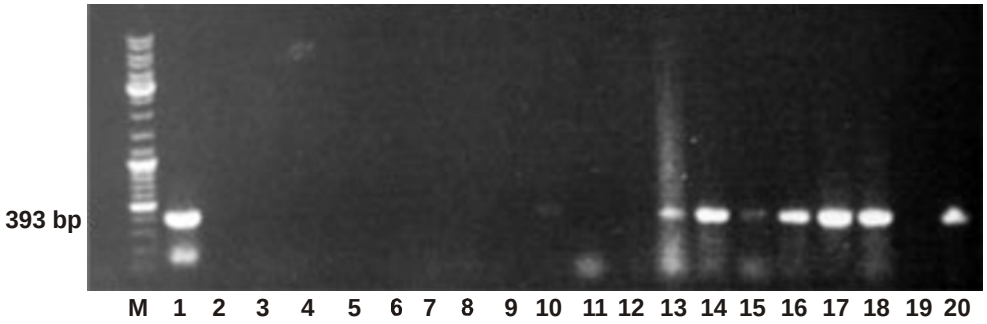

Fig. 1. Amplification of ORFV 045 gene by PCR. Lane M: 100 bp DNA ladder; lane 1: positive control, lane 2: blank; lanes 3–9, 11, 12 & 19: negative samples; lanes 10, 13-18, 20: positive samples.

pular Stomatitis Virus (BPSV) available in the GenBank database. The percentage of nucleotide sequences identity between all strains/isolates was in the range of 89–99% and the phylogenetic relationship is presented in Fig. 4. Besides, the comparison of the orf virus sequences demonstrated a high degree of identity among our isolates and others (Table 2) and identity at the nucleotide level ranged from 98% to 99%.

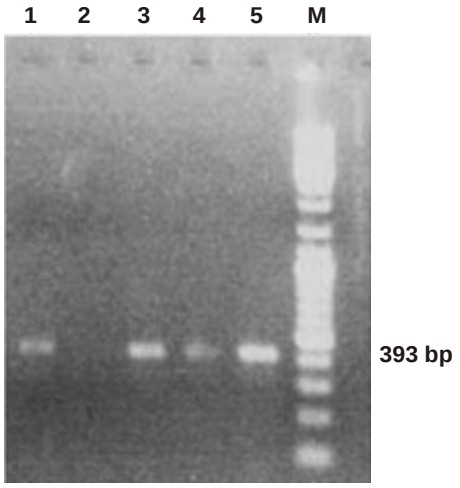

Fig. 2. Amplification of capripoxvirus fusion gene by PCR. Lane M: 100 bp DNA ladder; lanes 1, 3 & 4: positive samples; lane 2: blank; lane 5: positive control.

The phylogenetic analysis based on the 393 bp nucleotide sequences of 045 genes has revealed that two strains, DSM 1 including the isolates Shiraz 1,3,4,5 and DSM 2 including Shiraz-2 isolate were obtained. The grouping pattern of all parapoxviruses (ORFV, PCPV and BPSV) formed separate clusters. Our strains (DSM 1 and 2) were grouped with ORFV strains from NEW Zealand, the USA, Egypt and Germany (Fig. 4). The partial CPV gene of the amplified positive specimens were sequenced (KC607874) and subjected to similarity analysis. The results shared a close relationship with other capripoxvirus isolates from different regions (Table 3) and Blast program showed a maximum of 98% identity with goatpox virus strain G20-LKV (AY077836). Sequencing of the partial NP gene (353 bp) of PPRV (KC534492) revealed 100% homology with PPRV strain Nigeria/75/I (HQ197753) (Table 4).

DISCUSSION

Contagious ecthyma is endemic all over the world but is rarely reported in the literature because of its low morbidity and

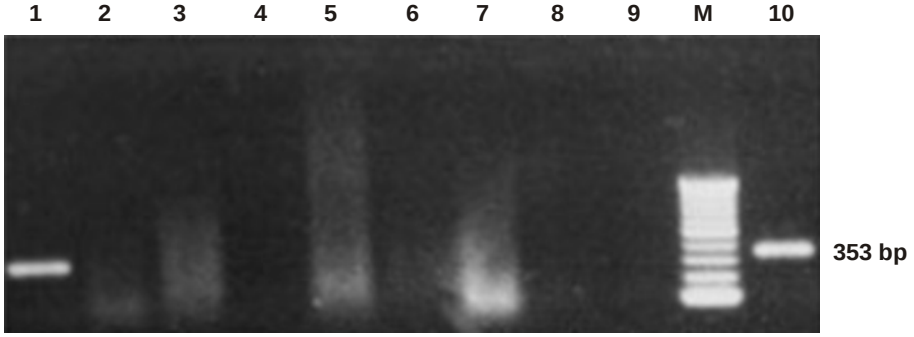

Fig. 3. Amplification of PPRV NP gene by RT-PCR. Lane M: 100 bp DNA ladder; lane 1: positive sample, lanes 2–8: negative samples, lane 9: blank; lane 10: positive control.

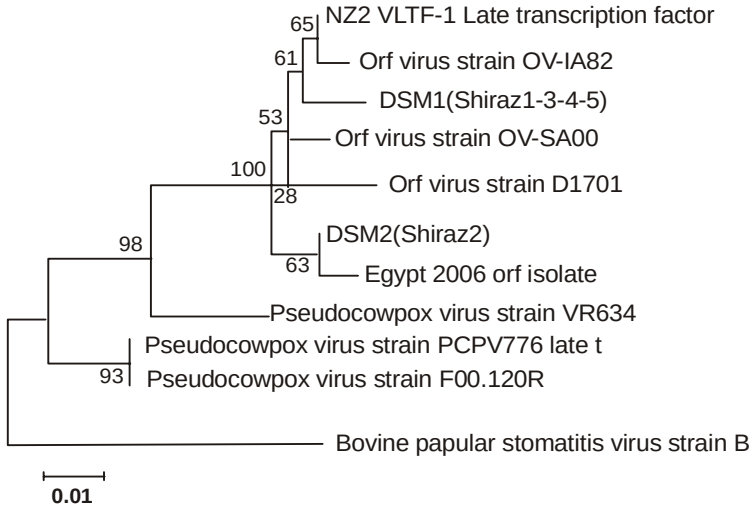

Fig. 4. Phylogenetic tree of different parapoxvirus strains based on the nucleotide sequences of 045 genes by using the neighbour-joining methods in Mega4 software.

minimal economic consequences (Zhao *et al.*, 2010). In Fars province, more cases of ecthyma in sheep and goat populations has been observed over the past several years, but no specific vaccination has been implemented to control this disease. Contagious ecthyma may become a serious problem in young, stressed, immunosuppressed or overcrowded animals (Buttner & Rziha, 2002). The differential diagnosis of the diseases causing crusts in small ruminants like contagious ecthyma, pox and PPR which have, in some cases, similar clinical symptoms may be a problem. With the development of molecular biology, the PCR technique has met the demands for specific and sensitive diagnosis of orf virus infection in the field specimens from affected animals (Lojkic *et al.*, 2010; Zhao *et al.*, 2010). The pre-

Table 2. Nucleotide sequence of the 045 ORFV gene of 5 isolates and comparison with the same sequences from others available in GenBank. Nucleotides identical with the Orf-Shiraz1 isolate are indicated with dots; hyphens stand for nucleotides absent in certain isolates.

```
                1                                                   50
Orf-Shiraz1     TCCTACTTCT CGGAGTTCAG CGAGGAGGAA TCGGTGAACG TGGACGTCGG
Orf-Shiraz4     .......... .......... .........C .......... ..........
Orf-Shiraz5     .......... .......... .........C .......... ..........
Orf-Shiraz2     .......... .......... ........TC .......... ..........
    OV-SA00     -......... .......... ........TC .......... ..........
Orf-Shiraz3     .......... .......... ........T. .......... .......T..
        NZ2     ---....... .......... ........TC .......... ..........
                51                                                  100
Orf-Shiraz1     CGATGGACTC ATGTACATAT TCGCGGCGCT GGGCGGGTCC GTGAACATCT
Orf-Shiraz4     .......... .......... .......... .......... ..........
Orf-Shiraz5     .......... .......... .......... .......... ..........
Orf-Shiraz2     .......... .......... .......... .........T ..........
    OV-SA00     .......... .......... .......A.. .......... ..........
Orf-Shiraz3     .......... .......... .......... .......... ..........
        NZ2     ......C... .......... .......... .......... ..........
                101                                                 150
Orf-Shiraz1     GGACCATCGT GCCGCTCAGC GCGAGCGTGG TATACGACGG CGATGTCAGC
Orf-Shiraz4     .......... .......... .......... .......... ..........
Orf-Shiraz5     .......... .......... .......... .......... ..........
Orf-Shiraz2     .......... .......... .......... .......... ..........
    OV-SA00     .......... .......... .......... .......... ..........
Orf-Shiraz3     .......... .......... ........T. .......... ..........
        NZ2     .......... .......... .......... .......... ..........
                151                                                 200
Orf-Shiraz1     CGCGTGTTCA ACCTGCCCGT GCTCAAGGTG AAGGCCTGTC TGTGCAGCTT
Orf-Shiraz4     .......... .......... .......... .......... ..........
Orf-Shiraz5     .......... .......... .......... .......... ..........
Orf-Shiraz2     .......... .......... .......... .......... ..........
    OV-SA00     .......... .......... .......... .......... ..........
Orf-Shiraz3     .......... .......... .......... .......... ..........
        NZ2     .......... .......... .......... .......... ..........
              201                                                   250
Orf-Shiraz1     CCACCCCGAC TCGGTGGTGA GCCTGGAGCC CGACCTCGAG GACAACGTGG
Orf-Shiraz4     .......... .......... .......... .......... ..........
Orf-Shiraz5     .......... .......... .......... .......... ..........
Orf-Shiraz2     .......... .......... .......... .......... ..........
    OV-SA00     .......... .......... .......... .......... ..........
Orf-Shiraz3     .......... .......... .......... .......... ..........
        NZ2     .......... .......... .......... .......... ..........
                251                                                 300
Orf-Shiraz1     TGCGGCTCTC GAGCCACCAC GTGGTCAGCG TGGACTGCGA CAACGAGCCC
Orf-Shiraz4     .......... .......... .......... .......... ..........
Orf-Shiraz5     .......... .......... .......... .......... ..........
Orf-Shiraz2     .......... .......... .......... .......... ..........
    OV-SA00     .......... .......... .......... .......... ..........
Orf-Shiraz3     .......... .......... .......... .......... ..........
        NZ2     .......... .......... .......... .......... ..........
                301                                                 350
Orf-Shiraz1     GTGGCGCACC GCACGAACAC CGCCATCTGC CTGGGCATTA ACCAGCGCAA
Orf-Shiraz4     .......... .......... .......... .......... ..........
Orf-Shiraz5     .......... .......... .......... .......... ..........
Orf-Shiraz2     .......... .......... .......... ........C. ..........
    OV-SA00     .......... .......... .......... ........C. ..........
Orf-Shiraz3     .......... .......... .......... .......... ..........
        NZ2     .......... .......... .......... .......... ..........
```

Table 2 (cont'd). Nucleotide sequence of the 045 ORFV gene of 5 isolates and comparison with the same sequences from others available in GenBank. Nucleotides identical with the Orf-Shiraz1 isolate are indicated with dots; hyphens stand for nucleotides absent in certain isolates.

```
                  351                                                393
Orf-Shiraz1  GTCCTACGTG TTCAACTTCC GGCGCTACGA GGAGAAGTGC TGC
         Orf-Shiraz4  .......... .......... .......... .......... ...
         Orf-Shiraz5  ........G. .......... .......... .......... ...
         Orf-Shiraz2  .......... .......... .......... .......... ...
             OV-SA00  .......... .......... .......... .......... ...
         Orf-Shiraz3  .......... .......... .......... .......... ...
                 NZ2  .......... .......... .......... .......... ...
```

Table 3. Comparison of nucleotide sequences of the CPV gene of 3 capripoxvirus isolates. Nucleotides identical with the Pox-Shiraz1 isolate are indicated with dots; hyphens stand for nucleotides absent in NISKHI strain.

```
                  1                                                    50
         Pox-Shiraz1  ATGGACAGAG CTTTATCAAT CTTTCCAGGC GACGATGATG AAACCAATGA
             G20-LKV  .......... .......C.. .......... .......... ..........
              NISKHI  .......... .G........ .......... .......... ..........
                  51                                                   100
         Pox-Shiraz1  AAGAAATATA AATCACAGAG AGAAAACTAG TAATGATCAC GGTCATTATG
             G20-LKV  .......... .......T.. .......... .GG....... ..G.......
              NISKHI  .......... .......... .......... .......... ..........
                  101                                                  150
         Pox-Shiraz1  AAGATAATCT TTTGGAATTA AGCGATGAAG AACCCAATAT GATAAAAATA
             G20-LKV  .......A.. ......C... .......................... ..........
              NISKHI  .......... .......... .......... .......... ..........
                  151                                                  200
         Pox-Shiraz1  AAAAATGATA TTAAGAAAAT AATTAATGAA AGATATAGCA GTTATATTTC
             G20-LKV  .......... .......... .......... .......... ..........
              NISKHI  .......... ....T..... .......... .......... A.........
                  201                                                  250
         Pox-Shiraz1  TATCAACGAC GATGAAATTT CTAACATTTT AAAAGATTCG TTCATTAGTA
             G20-LKV  .......... .......... .......... .......... ..........
              NISKHI  .......... .......... .......... .......... ..........
                  251                                                  300
         Pox-Shiraz1  ACGAAGAAAT GCAAATAAAA GATTTTGTTT TAAGACTGTT AGTATTAGAA
             G20-LKV  .......... .......... .......... .......... ..........
              NISKHI  .......... A......... .......... .......TG. T.........
                  301                                                  350
         Pox-Shiraz1  AAACTATTTC AAACGTCAGT AAAAGAATGC AATTCACTAA AAAATATTAT
             G20-LKV  .......... .......... .......... .......... ..........
              NISKHI  .......... ....A..... .......... .......... ..........
                  351                                                  400
         Pox-Shiraz1  TAAAAGATTA GAAAATCATA TAGAAACTAT TAGAAAAAAT ATGATTGTTT
             G20-LKV  .......... .......... .......... .......... ..........
              NISKHI  .......... .......... ....G..... .......... ..........
                  401                                                  447
         Pox-Shiraz1  TAACAAAAAA GGTAGATTTT CAAACAGG.C GAAGTACAAC ACTATGA
             G20-LKV  .......... .......... ........A. .......... .......
              NISKHI  .......... .......... ........AA ...T...... ....---
```

Table 4. Comparison of nucleotide sequences of the NP gene of 4 PPRV isolates. Nucleotides identical with the PPR-Shiraz1 isolate are indicated with dots; hyphens stand for nucleotides absent in certain isolates.

```
              1                                                    50
 PPR-Shiraz1  TTCTCGGAAA TCGCCTCACA GACTGGGGAT GAACGAACCG TTAGAGGGAC
Nigeria/75/1  -......... .......... .......... .......... ..........
   Shiraz101  -......... .TCT.GG.A. T........C ...A...... C.........
  PRADESH95N  ---------- ---------- --........C ...A...... C.........
              51                                                  100
 PPR-Shiraz1  TGGGCCTCGA CAGGCGCAGG TCTCCTTCCT CCAGCATAAA ACAGATGAGG
Nigeria/75/1  .......... .......... .......... .......... ..........
   Shiraz101  .......... .......... .......... ......C... ....GA....
  PRADESH95N  .......... .......... .......... ......C... ....GA....
              101                                                 150
 PPR-Shiraz1  GAGAGTCGCC TACACCAGCG ACCAGAGAAG AAGTCAAAGC TGCGATCCCA
Nigeria/75/1  .......... .......... .......... .......... ..........
   Shiraz101  ........T. CG........ .......... GG........ .........G
  PRADESH95N  ........T. CG........ .......... GG.....G.. ..........
              151                                                 200
 PPR-Shiraz1  AATGGGTCCG AAGGAAGGGA CACAAAGCGA ACACGCTCAG GAAAGCCCAG
Nigeria/75/1  .......... .......... .......... .......... ..........
   Shiraz101  ..C..A..T. ...AG..... ..G.....A. ......C... ...G......
  PRADESH95N  ..C..A..T. ...AG..... ..G.....A. ......C... ...G......
              201                                                 250
 PPR-Shiraz1  AGGAGAAACT CCCGGCCCAC TGCTTCCGGA GATCATGCAA GAGGATGAAC
Nigeria/75/1  .......... .......... .......... .......... ..........
   Shiraz101  ......G..C .......A.. ....C.T... T.......C. .---------
  PRADESH95N  ......G..C .......A.. ....C.T... A.......C. ........GG
              251                                                 300
 PPR-Shiraz1  TCTCGCGAGA GTCTAGTCAA AACCCTCGTG AGGCTCAAAG ATCGGCTGAG
Nigeria/75/1  .......... .......... .......... .......... ..........
   Shiraz101  -......... ....G..... .......... .......... ......C...
  PRADESH95N  .......... ....G.C... .......... .......... ......C...
              301                                                 350
 PPR-Shiraz1  GCACTCTTCA GGCTGCAGGC CATGGCCAAG ATTCTGGAGG ACCAGGAGGA
Nigeria/75/1  .......... .......... .......... .......... ..........
   Shiraz101  .......... .......... .......... .......... ..........
  PRADESH95N  .......... .......... .......... .......... ..........
              351
 PPR-Shiraz1  GGA
Nigeria/75/1  ..-
   Shiraz101  ..-
  PRADESH95N  ..-
   Consensus  ..-
```

sent study has demonstrated for the first time the characteristics of sheep and goat contagious ecthyma in Fars province, Iran. The only study of contagious ecthyma carried out in Iran was performed as a pathological case report and reviewed by Nourani & Maleki (2006). We have analysed 50 suspected clinical samples for the presence of ORFV DNA by PCR method and the results showed 50% positivity. Conducting NCBI nucleotide BLAST on 5 orf virus DNA sequences to search for highly similar DNA sequences revealed 99% homology with ORFV strain NZ2, ORFV OV-SA00 and the strain OV-IA 82. This homology was more significant than with other PPV members, including PCPV (94–97%) and BPSV (89–90%). On the basis of derived phylogenetic tree, two ORFV strains (DSM1 and DSM2) were obtained. There was a high degree of identity among

DSM1 strain and NZ2, OV-IA82, OV-SA00 and D1701. Furthermore, DSM2 was more homologous to Egypt 2006 orf isolate. The similarity of ORFV strains to PCPV is higher than that to the BPSV strain (Fig. 4). To prevent the spread of the disease, both infected animals and new ones should be quarantined.

Goat and sheep pox are endemic in Iran, giving rise to economic losses such as abortion, decreasing milk production and damage to wool and hides. These diseases are classified as a notifiable problem by the World Organization for Animal Health (2000). There is no specialised vaccination for contagious ecthyma in Iran. In vaccinated or previously infected animals, reinfection may occur several months later (Mazur *et al.*, 2000). Reinfection and remaining dormant agents within the soil and environment for at least 12 years makes eradicating the virus from the flocks difficult (Wilson & Mcfarlane, 2012). Because of cross serological reaction between capripox virus and parapox virus, an unequivocal differentiation should be implemented with molecular techniques. Hence, PCR is used as a simple, rapid, specific and sensitive assay (Varshovi *et al.*, 2009). In this study, a PCR assay based on amplifying a fragment of fusion gene was applied to identify capripoxvirus in the samples. Four of 50 (8%) samples were positive. Of these, 2 samples were also orf virus positive. Sequence analysis of the Pox-Shiraz1 (KC607874) showed 98% identity compared to G20-LKV strain from the USA. Vaccination and minimizing transportation stress are some of the major control measures to reduce losses due to sheep and goat pox (Ireland & Binepal., 1998). In Iran, goat pox vaccine is free and is part of governmental plans. This attenuated live vaccine along with Anthrax vaccine is impregnated simultaneously on goats and lambs which are older than 6 months once a year. Nevertheless, prevention of the disease would be difficult because Capripox virus remains viable on the wool or hair of previously infected animals for several months. Moreover, the infectivity is resistant to the environmental conditions (Garner *et al.*, 2000, Mohammed Ali *et al.*, 2004).

PPR is another disease that is endemic in Iran with huge economic losses and rapidly fatal in young animals (Abdollahpour *et al.*, 2006). Furthermore, it has been identified as an immunosuppressive disease. So regarding high productivity losses in small ruminants, outbreaks of the disease should be carefully monitored. This disease is clinically similar to orf infection, so a reliable and rapid differential diagnosis based on molecular assays has to be made between these two diseases. In the present study a PCR method using NP3-NP4 primers was carried out (Couacy-Hymann *et al.*, 2002) to amplify NP gene for detection of PPRV. Only one sample (2%) was positive. PPRV sequence identities of nucleotides were analysed by the Clustal W method. It was found that our isolate (Shiraz1) had 100% homology with the strain Nigeria/75/1 (HQ197753). PPR vaccination in Iran is practiced where the disease is established and it provides good immunity. Because of the close relationship of PPR virus to rinderpest virus, the attenuated rinderpest virus was used as a vaccine, but with the current efforts to eradicate rinderpest worldwide, it is no longer used.

In conclusion, ORFV, CPV and PPRV have an important role on forming muzzle crusts in small ruminants of Fars province, Iran, although ORFV has the more considerable role in this matter. Moreover, phylogenetic analysis had revealed that the sequence diversity of the gene 045 of ORFV deserves attention. Further,

struggle to detect the other causative agents of muzzle crusts like bluetongue virus would be necessary.

ACKNOWLEDGMENTS

The authors wish to thank the School of Veterinary Medicine, Shiraz University, for providing the necessary facilities to carry out this work.

REFERENCES

Abdollahpour, G., A. Raoofi, J. Najafi, F. Sasani & E. Sakhaie, 2006. Clinical and para-clinical findings of a recent outbreaks of peste des petits ruminants in Iran. *Journal of Veterinary Medicine B,* **53**, 14–16.

Abrahao, J. S., R. K. Campos, G. S. Trindade, M. IM. Guedes, Z. IP. Lobato, C. Mazur, P. CP. Ferreira, C. A. Bonjardim & E. G. Kroon, 2009. Detection and phylogenetic analysis of orf virus from sheep in Brazil: a case report. *Virology Journal*, **6**, 47–50.

Buttner, M. & H. J. Rziha, 2002. Parapoxviruses: From the lesion to the viral genome. *Journal of Veterinary Medicine B*, **49**, 7–16.

Chu, Y., X. Yan, P. Gao, P. Zhao, Y. He, J. Liu & Z. Lu, 2011. Molecular detection of a mixed infection of goatpox virus, orf virus, and *Mycoplasma capricolum* subsp. *Capripneumoniae* in goats. *Journal of Veterinary Diagnostic Investigation*, **23**, 786–789.

Couacy-Hymann, E., F. Roger, C. Hurard, J. P. Guillou, G. Libeau & A. Diallo, 2002. Rapid and sensitive detection of peste des petits ruminants virus by a polymerase chain reaction assay. *Journal of Virological Methods*, **100**, 17–25.

de la Concha-Bermejillo, A., J. Guo, Z. Zhang & D. Waldron, 2003. Severe persistent orf in young goats. *Journal of Veterinary Diagnostic Investigation*, **15**, 423–431.

Gallina, L., L. Scagliarini, C. J. McInnes, A. Guercio, G. Purpari, S. Prosperi & A. Scagliarini, 2008. Parapoxvirus in goats: experimental infection and genomic analysis. *Veterinary Research Communications*, **32**, S203–S205.

Garner, M. G., S. D. Sawarkar, E. K. Brett, J. R. Edwards, V. B. Kulkarni, D. B. Boyle & S. N. Singh, 2000. The extent and impact of sheep pox and goat pox in the state of Maharashtra, India. *Tropical Animal Health and Production*, **32**, 205–223.

Guo, J., Z. Zhang, J. F. Edwards, R. W. Ermel, C. Taylor, Jr. & A. de la Concha-Bermejillo, 2003. Characterization of a North American orf virus isolated from a goat with persistent, proliferative dermatitis. *Virus Research*, **93**, 169–179.

Housawi, F., E. T. Abu Elzein, A. M. Al-Naeem, A. Gameel & A. Q. Homaida, 2008. Induced udder orf infection in sheep and goats. *Veterinarski Arhiv*, **78**, 217–225.

Ireland, D. C. & Y. S. Binepal, 1998. Improved detection of capripoxvirus in biopsy samples by PCR. *Journal of Virological Methods*, **74**, 1–7.

Kottaridi, C., K. Nomikou, R. Lelli, P. Markoulatos & O. Mangana, 2006. Laboratory diagnosis of contagious ecthyma: Comparison of different PCR protocols with virus isolation in cell culture*. Journal of Virological Methods*, **134**, 119–124.

Lojkic, I., Z. Cac, A. Beck, T. Bedekovic, Z. Cvetnic & B. Sostaric, 2010. Phylogenetic analysis of Croatian orf viruses isolated from sheep and goats. *Virology Journal*, **7**, 314–320.

Luka, P. D., C. Ayebazibwe, D. Shamaki, F. N. Mwiine & J. Erume, 2012. Sample type is vital for diagnosing infection with peste des petits ruminants virus by reverse transcription PCR. *Journal of Veterinary Science*, **13**, 323–325.

Malik, Y. S., D. Singh, K. M. Chandrashekar, S. Shukla, K. Sharma, N. Vaid & S. Chakravarti, 2011. Occurrence of dual infection of peste-des-petits-ruminants and goatpox in indigenous goats of central India. *Transboundary and Emerging Diseases*, **58**, 268–273.

Mazur, C., I. I. Ferreira, F. B. Rangel Filho & R. Galler, 2000. Molecular characterization of Brazilian isolates of orf virus. *Veterinary Microbiology*, **73**, 253–259.

Mohammed Ali, S., M. E. Hamad, B. H. Ali & A. S. Ali, 2004. Alterations in some epidemiological patterns and virus heterogeneity recently observed in sheeppox outbreaks in the Sudan. *Veterinarski Arhiv*, **74**, 341–350.

Nourani, H. & M. Maleki, 2006. Contagious ecthyma: Case report and review. *Pakistan Journal of Biological Sciences*, **9**, 2543–2545.

Tamura, K., J. Dudley, M. Nei & S. Kumar, 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, **24**, 1596–1599.

Thompson, J. D., D. G. Higgins & T. J. Gibson, 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, **22**, 4673–4680.

Tryland, M., J. Klein, E. S. Nordoy & A. S. Blix, 2005. Isolation and partial characterisation of a parapoxvirus isolated from a skin lesion of a Weddell seal. *Virus Research*, **108**, 83–87.

Varshovi, H. R., H. Keyvanfar, K. Aghaiypour, S. A. Pourbakhsh, A. H. Shooshtari & M. Aghaebrahimian, 2009. Capripoxvirus identification by PCR based on P32 gene. *Archives of Razi Institute*, **64**, 19–25.

Wilson, D. J. & L. Mcfarlane, 2012. Contagious ecthyma in a Rocky Mountain bighorn sheep from Utah. *Human-Wildlife interactions*, **6**, 7–11.

World Organization for Animal Health, 2010. Sheep pox and goat pox. In: Manual of diagnostic tests and vaccines for terrestrial animals. OIE, Paris, France. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.07.14_S_POX_G_POX.pdf. 1-12 (May 2010, date last accessed).

Zhang, K., Z. Lu, Y. Shang, H. Zheng, Y. Jin, J. He & X. Liu, 2010. Diagnosis and phylogenetic analysis of orf virus from goats in China: A case report. *Virology Journal*, **7**, 78–82.

Zhao, K., D. Song, W. He, H. Lu, B. Zhang, C. Li, K. Chen, F. Gao, 2010. Identification and phylogenetic analysis of an orf virus isolated from an outbreak in sheep in the Jilin province of China. *Veterinary Microbiology*, **142**, 408–415.

Zhou, T., H. Jia, G. Chen, X. He, Y. Fang, X. Wang, Q. Guan, S. Zeng, Q. Cui & Z. Jing, 2012. Phylogenetic analysis of Chinese sheeppox and goatpox virus isolates. *Virology Journal*, **9**, 25–32.

Paper received 11.03.2013; accepted for publication 21.06.2013

Correspondence:

A. Mohammadi,
Department of Pathobiology,
School of Veterinary Medicine,
P.O.Box 71345-1731,
Shiraz University, Shiraz, Iran,
Phone: +987116138677;
Fax: +987112286940;
e-mail: mohammad@shirazu.ac.ir