Journal of Animal and Veterinary Advances 9 (19): 2477-2481, 2010
ISSN: 1680-5593

Severe Persistent Contagious Ecthyma Cases in Twin Goats

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Abstract: In the present study, severe persistent orf cases affecting only twin kids following an orf outbreak in a flock is reported to contribute the discussions on severe persistent form of orf. Although, orf lesions occurred only around the mouth of most of kids of the flock of 230 hair goats, 12 twin kids had lesions around mouth, feet, eye lids and anus area. A 2 month old kid suspected of orf was admitted to the clinics of Faculty of Veterinary Medicine University of Firat, Turkey. The kid was clinically examined and samples were collected for hematological and virological examinations. The results confimed the presence of severe persistent form of orf. In conclusion, the results may contribute to the discussions going on over the pathogenesis of the severe persistent form the disease.

Key words: Goat, contagious ecthyma, severe persistent orf, polymerase chain reaction, pathogenesis, virological examinations

INTRODUCTION

Contagious ecthyma, also known as orf, contagious pustular dermatitis, contagious pustular stomatitis, malignant aphta, soremouth or scabby mouth is a zoonotic viral disease affecting sheep, goat, wild ruminants as well as human being. An epitheliotrophic virus (genus parapoxvirus of the family Poxviridae) causes the disease (Michelsen and Smith, 2009; Reid, 2003). The disease is common all over the world and is transmitted through direct contact or indirectly through infected sheep coat, barn, pasture, shepherds and farm tools and devices (Aiello and Mays, 1998; Bilal, 2005; Gul, 2006; Reid, 2003).

The incubation period of the disease is 8-10 days (Gul, 2006). Classic form of contagious ecthyma is characterized by the appearance of vesicles, pustules, ulcers of the skin of nostril and lips.

In the severe form also known as generalized form, the lesions appear on the skin of the eye, feet, vulva and udder (Aiello and Mays, 1998; Gul, 2006; Radostits *et al*., 2008).

Although the disease is generally not fatal, it causes reluctance in feeding due to lesions of the animal and thus causes weight loss. In addition, secondary infections and/or sepsis may develop as a result of the reproduction of necrotic bacteria in ulcerative lesions (Bilal, 2005; Gul, 2006; Radostits *et al*., 2008; Reid, 2003). Contagious ecthyma occurs in animals of all ages however typical papillomatous lesions are mostly detected around nose and lips of especially lambs and kids. As clinical signs are is atypical in many cases, the laboratory diagnosis is required in some cases (Reid, 2003). Laboratory diagnosis of the disease is generally based on virus isolation, serology and electron microscopy (Berkin *et al*., 1985; Burgu and Toker, 1984; Cabalar *et al*., 1996; Gokce *et al*., 2005). However, each of these approaches also has disadvantages.

The sensitivity of serological tests is low as cross reaction with viruses belonging to the same family is common, virus isolation is time-consuming and difficult and electron microscopy is expensive and often unavailable in many laboratories. The Polymerase Chain Reaction (PCR) has been considered as a rapid, highly sensitive and specific method for the diagnosis of contagious ecthyma.

Therefore, PCR is widely used in the diagnosis of many viral diseases including contagious ecthyma (Gallina *et al*., 2006; Inoshima *et al*., 2000, 2001; Torfason and Guonadottir, 2002). Although a lot is known about the disease, pathogenesis of severe persistent orf has not been completely elucidated yet.

The occurrence of severe persistent forms of the disease has been explained with individual sensitivity and differences in molecular profiles of orf virus. In the present study, severe persistent orf cases affecting only twin kids following an orf outbreak in a flock is reported to contribute the discussions on severe persistent form of orf.

MATERIALS AND METHODS

Flock history and clinical cases: The flock consisted of 230 hair goats with no history of vaccination against Orf.

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Of the flock, 150 had given birth and 90 of these kids had similar lesions. The lesions were located around mouth and/or feet of the kids except for 12 twin where the lesions were also detected on the eyelids and anal region in addition to mouth and feet. The recovery was quite rapid in the flock except for 12 twin kids where the recovery period lasted about for 40 days. Some of the dams of infected kids also had lesion on the udder. A 2 months old kid suspected of orf was admitted to the clinics of Faculty of Veterinary Medicine, University of Firat, Turkey. The kid was clinically examined and samples were collected for hematological and virological examinations.

Hematological analysis: Blood samples were taken from jugular vein into EDTA treated tubes. Haematocryte, total leukocyte and erythrocyte counts and hemoglobin concentration were determined (Schalm *et al.*, 1975) immediately after blood collection.

Polymerase chain reaction: DNA was extracted from the wart-like lesion around mouth using a commercial extraction kit (Wizard Genomic DNA Extraction System) as instructed by manufacturer (Promega Corp., Madison, WI). The DNA pellets were dissolved in 50 μL distilled water and were stored at -20°C until used. Semi-nested PCR method was used for the diagnosis of orf as previously reported by Inoshima *et al.* (2000). In this study, PPP-1 (5′-gtc gtc cac gat gag cag ct-3′), PPP-4 (5′-tac gtg gga agc gcc tcg ct-3′) and PPP-3 (5′-gcg agt ccg aga aga ata cg-3) primers were used. First stage PCR reaction was carried out in a total of 50 μL PCR mixture including 5 μL of template DNA, 5 μL 10 × PCR Buffer (670 mM Tris-HCl, pH: 8,8, 0.1% Tween-20, 160 mM $(NH_4)_2SO_4$, 25 mM Magnesium chloride), 2 mM from each of 4 deoxynucleotides, 1U Taq DNA polymerase (Bioron) and 0.2 μM primers (PPP-1 and PPP-4). Amplifications were performed with the following cycling profile: an initial step of 5 min at 95°C, followed by 25 cycles of 1 min/94°C, 1 min/50 °C, 1 min/72°C and a final extension step of 5 min/ 72°C. After that second stage PCR was applied using PP3 -PP4 primers and 3 μL of PCR products taken from the first round PCR under the same conditions. About 5 μL of PCR products were analyzed by electrophoresis in 2% agarose gel at constant voltage (90 V) for approximately 45 min, stained with ethidium bromide (0.5 μg mL^{-1}) and visualized under UV light.

Sequencing: The PCR product taken from first round of semi-nested PCR was purified using DNA purification system (Promega). Then, purified DNA sample were sequenced using ABI 310 Genetic Analysis System (Iontec Co., Istanbul, Turkey). Comparison of these sequence results with the present genome sequences in Gene databank was performed using BLAST program.

RESULTS AND DISCUSSION

Clinical and hematological findings: On the clinical examination, twin kids had severe multi-focal, proliferative lesions around lips, eye lids (Fig. 1), gums, extremities, foot and anal region (Fig. 2). A slight enlargement of submandibular lymph node was noted on palpation. Body temperature was 39.5°C, heart rate was 160 min^{-1} and respiratory rate was 48 min^{-1}. Although, most of the kids recovered spontaneously within 2-3 weeks, the recovery was quite long about 3 months in the twin kids. Hematocryte 36%, total leukocyte count was 4.8 $\times 10^9$ L^{-1}, erythrocyte count was 5.94 $\times 10^{12}$ L^{-1} and hemoglobin level was 11.8 g dL^{-1}.

Polymerase chain reaction: The semi-nested PCR revaled a 235 bp specific band indicating a positive result for orf virus (Fig. 3).

Fig. 1: Severe multi-focal, proliferative lesions were observed in the lips, eye lids

Fig. 2: Severe multi-focal, proliferative lesions were observed in anus area

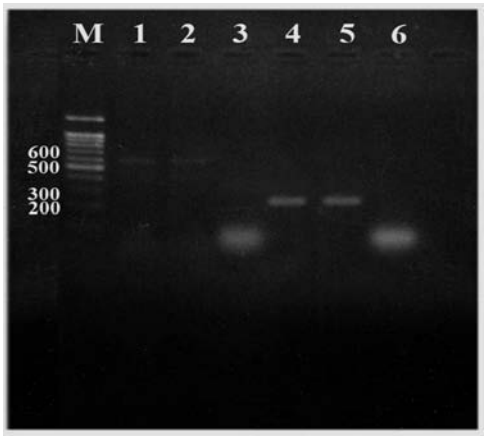

Fig. 3: Agarose gel electrophoresis of PCR products amplified with semi-nested PCR. M; 100 bp DNA ladder, Lane 1, 2; first stage PCR products of the clinical sample taken in the lip and anus areas, Lane 3; first stage PCR products of negative control sample (sterile dH_2O), Lane 4, 5; second stage PCR products of the clinical samples taken in the lip and anus areas, Lane 3; first stage PCR products of negative control samples, Lane 6; second stage PCR products of negative control sample

Sequence findings: Sequencing of the PCR product taken from the first round of semi-nested PCR confirmed the presence of orf virus in clinical sample (Fig. 4).

Contagious ecthyma was described first in the last century, 1923 (Robinson and Balassu, 1981). Now, the disease is widespread all over the world including Turkey (Bilal, 2005; Burgu and Toker, 1984; Cabalar *et al*., 1996; Gokce *et al*., 2005; Gul, 2006; Gumbrell and Mcgregor, 1997; Mazur and Machado, 1989; Reid, 2003).

Contagious ecthyma is a common disease in Turkey and affects many small ruminants during spring and fall. In addition, severe persistent form of orf has frequently been observed in twins even if it sometimes occurs in young animals on the clinical observations. However, these cases have not been reported so far due to lack of virological confirmation.

The objective of the present study was to virogically confirm the orf in twin goat kids.Contagious ecthyma lesions have commonly been found around the lips and mouth of the infected animals (labial form) (De La Concha-Bermejillo *et al*., 2003; Gul, 2006; Michelsen and Smith, 2009; Radostits *et al*., 2008; Reid, 2003; Robinson and Balassu, 1981) and therefore, it is widely known only through this form (De La Concha-Bermejillo *et al*., 2003). The cauliflower like proliferative ecthyma lesions observed in the extremity, foot and anus area in this report are consistent with the literature (Abu and Housawi, 1997; Bilal, 2005; Gul, 2006; Guo *et al*., 2003; Michelsen and Smith, 2009) and suggesting the occurrence of pedal and genital forms in addition to the most common labial.

De La Concha-Bermejillo *et al*. (2003) reported that along with the labial form, multifocal, acute, proliferative dermatitis were observed in 16 Boer or Boer hybrid goat kids younger than 1 year of age.

Baipoledi *et al*. (2002) also severe clinical symptoms of contagious ecthyma (swelling in lips and submandibular lymph node, gingivitis, ulceration of lips and gum mucosa and crustacean formation on ulcerative areas) in Tswana goats but no mortality occurred and no other kind of lesions were observed in other parts of the body.

In an orf epidemic occurred in Saudi Arabia between 1987 and 1989, sheep and goats of all ages were affected, the morbidity ratio was 70-80% and the mortality ratio was 5-15%.

In this epidemic, the lesions occurred only around the mouth and lips of the affected animals and no leasions were observed on the other parts of the body (Housawi *et al*., 1991).

All three forms of contagious ecthyma were observed in the same the flock but severe persistent orf cases were only diagnosed in only twin goat kids in the present study. Labial and pedal forms of the disease were observed in the goat kids and the genital form of the disease was observed in their dam.

Severe persistent form of orf can be observed in goats depending on individual sensitivity as suggested previously (De La Concha-bermejillo *et al*., 2003) and this the generalized form may be attributed to the virus which may have a different pathogenicity transmitted from the mother and also to the effect of twin birth.

Presence of different forms of the disease may be related to different genotype of the virus. However, the relationship between different forms of the disease and different genotype of the virus was not investigated in this study. Presence of different form the disease may not be attributed to at least for this outbreak, genetic differences of the virus as severe form and less severe forms occurred at the same time in the flock. However, the occurrence of the different forms may be speculated to be due to the weakness of immune parameters in twins. This may well be the case as the level of maternal ntibodies in twins would be expected to be lower when compared to the animals borne single because maternal antibodies

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OV-TR08          GTCGTCCACGATGAGCAGCTTGGTGTTGTTCGCAGCGTCGTCCCGCCCGGGCACCACGAA
AY386264.1       GTCGTCCACGATGAGCAGCTTGGTGTTGTTCGCAGCGTCGTCCCGCCCGGGCACCACGAA
AY386263.1       GTCGTCCACGATGAGCAGCTTAGTGTTGTTCGCGGCGTCGTCCCGGCCGGGTACCACGAA
AY278209.1       GTCGTCCACGATGAGCAGCTTAGTGTTGTTCGCGGCGTCGTCCCGGCCGGGTACCACGAA
AY424970.1       GTCGTCCACGATGAGCAGCTTAGTGTTGTTCGCGGCGTCGTCCCGGCCGGGTACCACGAA

OV-TR08          CTTGCGCACGGACATGTCCACGCTGCCGACGCCAAAGTCGTCGAGGCTGCGCGCGGCCGA
AY386264.1       CTTGCGCACGGACATGTCCACGCTGCCGACGCCAAAGTCGTCGAGGCTGCGCGCGGCCGA
AY386263.1       CTTGCGCACGGACATGTCCACGCTGCCGACGCCAAAGTCGTCGAGGCTGCGCGCGGCCGA
AY278209.1       CTTGCGCACGGACATGTCCACGCTGCCGACGCCAAAGTCGTCGAGGCTGCGCGCGGCCGA
AY424970.1       CTTGCGCACGGACATGTCCACGCTGCCGACGCCAAAGTCGTCGAGGCTGCGCGCGGCCGA

OV-TR08          GCACCGACAGCGGGTCCGCGTTCTTCCACTCGGTGATGATTACGCGCACGCGCACGCCGC
AY386264.1       G-ACCGACAGCGGGTCCGCGTTCTTCCACTCGGTGATGATCACGCGCACGCGCACGCCGC
AY386263.1       G-ACCGACAGCGGGTCCGCGTTCTTCCACTCGGTAATGATCACGCGCACGCGCACGCCGC
AY278209.1       G-ACCGACAGCGGGTCCGCGTTCTTCCACTCGGTAATGATCACGCGCACGCGCACGCCGC
AY424970.1       G-ACCGACAGCGGGTCCGCGTTCTTCCACTCGGTAATGATCACGCGCACGCGCACGCCGC

OV-TR08          GGTCGATGGCCGCGCGCAACAACGCGTCTATGATCCGCGGCCAGTACTCCACGGCGCCGG
AY386264.1       GGTTGATGGCCGCGCGCAGCAGCGCGTCTATGATCCGAGGCCAGTACTCCACGGCGCCGG
AY386263.1       GGTCGATGGCCGCGCGCAGCAGCGCGTCTATGATCCGCGGCCAGTACTCCACGGCGCTGG
AY278209.1       GGTCGATGGCCGCGCGCAGCAGCGCGTCTATGATCTGCGGCCAGTACTCCACGGCGCTGG
AY424970.1       GGTCGATGGCCGCGCGCAGCAGTGCGTCTATGATCCGCGGCCAGTACTCCACGGCGCTTG

OV-TR08          CGTGCTTGATCACCGGCACCATCGAGAGCAGCGAGAGGTCGATGCTGTTCTTGGCGTTCT
AY386264.1       CGTGCTTGATCACCGGCACCATCGAGAGCAGCGAGAGGTCGATGCTGTTCTTGGCGTTCT
AY386263.1       CGTGCTTGATCACCGGCACCATAGAGAGCAGCGAGAGGTCGATGCTGTTCTTGGCGTTCT
AY278209.1       CGTGCTTGATCACCGGCACCATAGAGAGCAGCGAGAGGTCGATGCTGTTCTTGGCGTTCT
AY424970.1       CGTGCTTGATCACCGGCACCATCGAGAGCAGCGAGAGGTCGATGCTGTTCTTGGCGTTCT

OV-TR08          CGATGCGGTGCAGCACGAGGTCCTCGTCGAGCGTGCGGTAGAAGCCCAGGAAGCGCTCCG
AY386264.1       CGATGCGGTGCAGCACGAGGTCCTCGTCGAGCGTGCGGTAGAAGCCCAGGAAGCGCTCCG
AY386263.1       CGATGCGGTGCAGCACGAGGTCCTCGTCGAGCGTGCGGTAGAAGCCTAGGAAGCGCTCCG
AY278209.1       CGATGCGGTGCAGCACGAGGTCCTCGTCGAGCGTGCGGTAGAAGCCTAGGAAGCGCTCCG
AY424970.1       CGATGCGGTGCAGCACGAGGTCCTCGTCGAGCGTGCGGTAGAAGCCTAGGAAGCGCTCCG

OV-TR08          GCGAGTCCGAGAAGAATACGCCGCCCCCGGAGTGGTTGAGGTGGAAGTTCGTGGCCGTGG
AY386264.1       GCGAGTCCGAGAAGAATACGCCGCCCCCGGAGTGGTTGAGGTGGAAGTTCGTGGCCGTGG
AY386263.1       GCGAGTCCGAGAAGAATACGCCGCCCCCGGAGTGGTCGAGGTGGAAGTTCGTGGCCGTGG
AY278209.1       GCGAGTCCGAGAAGAATACGCCGCCCCCGGAGTGGTCGAGGTGGAAGTTCGTGGCCGTGG
AY424970.1       GCGAGTCCGAGAAGAATACGCCGCCCCCGGAGTGGTCGAGGTGGAAGTTCGTGGCCGTGG

OV-TR08          GTGTGACGACGGCGCAACAGAGCCGCGTGAACGGCACCTTCGGCTCTACGATCATGGAGT
AY386264.1       GCGTGACGACGGCGCAGCAGAGCCGCGTGAACGGCACCTTCGGCTCCACGATCATGGAGT
AY386263.1       GCGTGACGACGGCGCAGCAGAGCCGCGTGAACGGCACCTTCGGCTCCACGATCATGGAGT
AY278209.1       GCGTGACGACGGCGCAGCAGAGCCGCGTGAACGGCACCTTCGGCTCCACGATCATGGAGT
AY424970.1       GCGTGACGACGGCGCAGCAGAGCCGCGTGAACGGCACCTTCGGCTCCACGATCATGGAGT

OV-TR08          AGAAGGTGTTGTAGCGGTTCATGAGGTCCCAGGCCAGGTGCTTGTTGGGGGAAGTACAGC
AY386264.1       AGAAGGTGTTGTAGCGGTTCATGAGGTCCCAGGCCAGGTGCTTGTTGGTGG-AGTAGAGC
AY386263.1       AGAAGGTGTTGTAGCGGTTCATGAGGTCCCAGGCCAGGTGCTTGTTGGTGG-AGTAGAGC
AY278209.1       AGAAGGTGTTGTAGCGGTTCATGAGGTCCCAGGCCAGGTGCTTGTTGGTGG-AGTAGAGC
AY424970.1       AAAAGGTGTTGTAGCGGTTCATGAGGTCCCAGGCCAGGTGCTTGTTGGTTG-AGTAGAGC

OV-TR08          CCGAGGTTCTTGATGGTGGACACTGACCCGCCCGTGAGCGAGGCGCTGCCCACGTA
AY386264.1       CCGAGGTTCTTGATGGTGGACACGGACCCGCCCGTGAGCGAGGCGCTGCCCACGTA
AY386263.1       CCGAGGTTCTTGATGGTGGACACGGACCCGCCCGTGAGCGAGGCGCTGCCCACGTA
AY278209.1       CCGAGGTTCTTGATGGTGGACACGGACCCGCCCGTGAGCGAGGCGCTGCCCACGTA
AY424970.1       CCGAGGTTCTTGATGGTGGACACGGACCCGCCCGTGAGCGAGGCGCTGCCCACGTA
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Fig. 4: Alignment of nucleotide sequences of the PCR product with the 594 base pair long of Turkey OV (OV-TR08) with the nucleotide sequences available from the database of different orf viruses

had to be shared between twins. However, this suggestion warrants a confirmation by further studies.

CONCLUSION

The results may contribute to the discussions on the pathogenesis of the severe persistent form of orf disease.

REFERENCES

Abu, E.E. and F.M. Housawi, 1997. Severe long-lasting Contagious ecthyma infection in a goat's kid. Zentralbl. Veterinarmed. Reihe. B., 44: 561-564.

Aiello, S.E. and A. Mays, 1998. The Merck Veterinary Manuel. 8th Edn., National Publishing, Philadelphia.

Baipoledi, E.K., J.F. Nyange and J.M. Hyera, 2002. A severe case of contagious ecthyma in Tswana goats. J. S. Afr. Vet. Assoc., 73: 86-87.

Berkin, S., M. Kahraman, R. Haziroglu, M. Izgur and A.D. Yonguc, 1985. Enzootik ecthyma contagiosum ovis-necrobacillosis. Ankara Univ. Vet. Fak. Derg., 32: 157-171.

Bilal, T., 2005. Viral Hastaliklar. In: Koyun-Kecilerin Ic Hastaliklari ve Beslenmesi, Bilal, T. and T. Bilal (Eds.). Istanbul Universitesi Basim ve Yayinevi Mudurlugu, Istanbul, pp: 129-204.

Burgu, I. and A. Toker, 1984. Isolation of ecthyma contagiosum virus (orf) from the gingiva of a lamb. Ankara Univ. Vet. Fak. Derg., 32: 230-239.

Cabalar, M., H. Voyvoda and S. Sekin, 1996. The case of ecthyma contagiosum (Orf) in a sheep flock in van. Ankara Univ. Vet. Fak. Derg., 43: 45-51.

De La Concha-Bermejillo, A., J. Guo, Z. Zhang and D. Waldron, 2003. Severe persistent orf in young goats. J. Vet. Diagn. Invest., 15: 423-431.

Gallina, L., F. dal Pozzo, C.J. McInnes, G. Cardeti and A. Guercio *et al*., 2006. A real time PCR assay for the detection and quantification of orf virus. J. Virol. Methods, 134: 140-145.

Gokce, H.I., O. Genc and G. Gokce, 2005. Sero-prevalence of contagious ecthyma in lambs and humans in Kars, Turkey. Turk. J. Vet. Anim. Sci., 29: 95-101.

Gul, Y., 2006. Gevis Getiren Hayvanlarin Ic Hastaliklari (Sigir, Koyun-Keci). 2nd Edn., Medipres Matbaacilik Yayincilik Ltd., Sti, Malatya, pp: 129-165.

Gumbrell, R.C. and D.A. Mcgregor, 1997. Outbreak of severe fatal orf in lambs. Vet. Rec., 141: 150-151.

Guo, J., Z. Zhang, J.F. Edwards, R.W. Ermal, C. Taylor and A. de la Concha-Bermejillo, 2003. Characterization of a North American orf virus isolated from a goat with persistent, proliferative dermatitis. Virus Res., 93: 169-179.

Housawi, F.M.T., E.M.E. Abu-Elzein, M.M. Amin and A.I. Al-Afaleq, 1991. Contagious pustular dermatitis (orf) infection in sheep and goats in Saudi Arabia. Vet. Rec., 128: 550-551.

Inoshima, Y., A. Morooka and H. Sentsui, 2000. Detection and diagnosis of parapoxvirus by the polymerase chain reaction. J. Virol. Methods, 84: 201-208.

Inoshima, Y., K. Murakami, T. Yokoyama and H. Sentsui, 2001. Genetic heterogeneity among parapoxviruses isolated from sheep, cattle and Japanese serows (*Capricornis crispus*). J. Gen. Virol., 82: 1215-1220.

Mazur, C. and R.D. Machado, 1989. Detection of contagious pustular dermatitis virus of goats in a severe outbreak. Vet. Rec., 125: 419-420.

Michelsen, P.G.E. and B.P. Smith, 2009. Contagious Ecthyma (Sore Mouth, Orf, Contagious Pustular Dermatitis, Scabby Mouth). In: Large Animal Internal Medicine, Smith, B.P. (Ed.). 4th Edn., Mosby Elsevier, USA., pp: 789-790.

Radostits, O.M., C.C. Gay, K.W. Hinchcliff and P.D. Constable, 2008. Veterinary Medicine, A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats. 10th Edn., W.B. Sounders, London, New York, Oxford.

Reid, H.W., 2003. Orf. In: Disease of Sheep, Martin, W.B. and I.D. Aitken (Eds.). 3rd Edn., Blackwell, USA., pp: 261-266.

Robinson, A.J. and T.C. Balassu, 1981. Contagious pustular dermatitis (orf). Vet. Bull., 51: 771-782.

Schalm, O.W., N.C. Jain and G.H. Carroll, 1975. Veterinary Hematology. 3rd Edn., Lea and Febiger, Philadelphia.

Torfason, E.G. and S. Guonadottir, 2002. Polymerase chain reaction for laboratory diagnosis of orf virus infections. J. Clin. Virol., 24: 79-84.