Case Report: Contagious Ecthyma - Deviations in the Anatomical Appearance of Lesions in an Outbreak in Lambs in Israel

Bouznach, A.,[1] Hahn, S.,[1] Stram, Y.,[2] Menasherov, S.,[2] Edery, N.,[1] Shicaht, N.,[1] Kenigswald, G.[3] and Perl, S.[1]

[1] Department of Pathology, Kimron Veterinary Institute, 50250 Bet Dagan, Israel.
[2] Department of Virology, Kimron Veterinary Institute, 50250 Bet Dagan, Israel.
[3] "Hachaklait" Veterinary Services POB 3039, 38900 Caesaria, Israel.

* **Corresponding author:** Prof. S. Perl, Department of Pathology, Kimron Veterinary Institute, 50250 Bet Dagan, Israel. Email: perls@moag.gov.il.

ABSTRACT

Contagious ecthyma (orf) is a highly contagious viral disease of small domestic and wild ruminants usually affecting young animals with economic and zoonotic implications. The disease is characterized by the formation of vesiculo-proliferative lesions on the lips, nostrils and around the eyes, also on the udder of nursing ewes of affected lambs. Rarely the lesions can be seen in the oral cavity and gastrointestinal tract. In this case 2 lambs of 4 month of age where submitted to the Kimron Veterinary Institute for post mortem examination. This report describes findings of proliferations consistent with orf found on gingival mucosa and ruminal epithelium which are relatively rare, however without external lesions. The diagnosis of ecthyma was confirmed by PCR.

Keywords: Contagious Ecthyma, Orf, Rumen, Lambs, PCR.

INTRODUCTION

Contagious ecthyma (orf, contagious pustular dermatitis) is a viral disease of sheep, goats and many species of wild ruminants (1, 2). Orf is also a zoonotic disease affecting humans, usually those working with small ruminants (1, 2, 3) and rarely other species like dogs and rabbits (1, 2). Orf is distributed worldwide and is endemic in the Mediterranean region and primarily affects young sheep and goats but can be present at any age (4). Contagious ecthyma is caused by orf virus of the genus parapoxvirus – epitheliotropic parapoxvirus (2, 3) of the family poxviridae.

The orf lesions appear 6-7 days after infection of abrasions and wounds. The disease is characterized by the formation of crusty vesiculo-proliferative lesions, papules, pustules, scabs covering ulceration, and granulation tissue (1, 2). In lambs, lesions are usually located around the mouth on lips and nostrils. Lesions can also be found within the buccal cavity and occasionally in the osphagus and abomasum (5). Rarely lesions can be seen in the rumen mucosa (1, 3, 6).

The infection initiates in scar tissue and the virus can first be detected in newly formed epidermis (1). Swelling and vacuolization of the keratinocytes and eosinophilic cytoplasmic inclusion bodies occasionally can be seen in histological section of squamous epithelial cells (7).

Diagnostic confirmation of the disease is made by virus identification using PCR. This unusual case report describes the rare appearance of orf lesions in the in gingival and rumen mucosa of lambs.

CASE HISTORY

Two lambs of 4 months of age were submitted to the Kimron Veterinary Institute for post mortem examination. The lambs were of mixed Merino and Romanov breeds which were established more than 10 years ago and for the last 5 years bred with Afek Asaf rams for higher fecundity.

The herd consisted of 750 ewes in intensive breading with an average of 2.2 lambs per parturition. Ninety percent of the lambs were fed by their mother with a neonatal mortality rate

was 12%. In 2010 cases of bluetongue where diagnosed. The herd has often been diagnosed with ecthyma with an average morbidity of 30% annually.

The lambs were treated with the antibiotic penicillin (Norocillin Norbrook Laboratories, Ltd., Newry North Ireland 30 mg/kg SID for 5 days) and the non-steroidal anti-inflammatory drug flunixin (Norbrook Laboratories LTD Newry North Ireland 2 mg/kg SID for 3 days). The oral lesions were disinfected with an iodine spray (Iodine Spray, CTC Spray 3.21g/ml. Eurovet, Badel, The Netherlands).

MATERIALS AND METHODS

Pathology

The lambs were submitted for post mortem examination with the history of sudden death after showing signs of weakness, fever, anorexia and dyspnea. Tissue samples of the gingiva, rumen, intestine, lungs, heart, liver, spleen, pancreas, kidney and several lymph nodes where taken in 10% formaldehyde. The fixed material was embedded in paraffin wax, sectioned at 3μ and stained with Hematoxylin and Eosin (H&E).

PCR

DNA was extracted from the skin using Viral Gen-Spine kit (iNtRON, Kyunggi-do, Korea) according to the manufacturer's instructions. The extracted DNA was denatured at 98°C for 5 min., and chilled immediately at 4°C. The forward primer was TATGAGTCCTACGCCAACTT at position 260 of the viral polyemerase gene, and the reverse primer was GTTCCCGTAGCCGATGAG at position 554 (acc. OVU33419). The assay was accomplished by using maxim PCR-premix (iNtRON, Kyunggi-do, Korea) for 35 cycles, and according to the manufacturer's instructions was set to 60°C annealing temperature. PCR products were visualized on 1.5 Ethedium bromide stained agrose gel.

In an attempt to compare the 2010 Israeli isolate to other isolates a phylogenetic analysis was performed using a 580 bp fragment of B2L gene. Viruses from Greece, Turkey, India, Brazil, Finland, China, Japan, Taiwan and Korea and 4 Israeli isolates were included in the analysis (Table 1).

P1 and P4 primers (8, 9) were used to amplify the isolated viruses' DNA and Pairwise distance and Phylogenetic and analysis were conducted using MEGA version 5.1 (10).

Table 1: Sequences studied

Sequences	Accession No.
ISR-2010	Submitted
ISR-2012-1	Submitted
ISR-2012-2	Submitted
ISR-2013	Submitted
GRE-1-2003	JN368482
GRE-2-2004	JN368483
Turkey-TR-ORF-S-2011	JQ936990
Brazil A-2011	JN088053
India-Assama/10-2010	JQ040300
India-59/5-2005	DQ263304
India-67/04-2004	DQ263305
Japan-Jilin-2008	FJ808074
Brazil MT-05	JN613809
Brazil-SV27/12-2012	JX485995
FinlandF07.810R-1999	JF773699
Finland-F07.748S-2007	JF773702
Finland-F92.849-1992	JF773697
Brazil-MT05-2005	FJ665818
China-Shanxi-2011	JN565696
Brazil-PA-2011	JQ349520
China-CS/YT-2012	JQ904798
China-SD/DY 2012	JQ904794
Japan-Ena-1995	AB521175
Japan-Wamura-1999	AB521172
Japan-Matasumo-1999	AB521174
Japan-R-1-1999	AB521167
Taiwan-2007	EU327506
Korea-2010	JX968990

PATHOLOGICAL AND HISTOPATHOLOGICAL FINDINGS

At necropsy cauliflower like proliferations were observed bilaterally on the gingival buccal mucosa near the molar teeth of the maxilla (Figures 1 and 2). Multifocal proliferations of ruminal epithelium were also present (Figures 3 and 4). No additional changes where seen in any other organs.

Histological sections of the gingival mucosa revealed multifocal proliferations of the squamous epithelium with vesicular degeneration of squamous epithelial cells (Figures 5 and 6). In some areas multifocal necrosis with secondary neutrophilic and histiocytic infiltrations were present. The ruminal epithelium showed multifocal proliferations of the squamous epithelium and secondary infiltration with inflammatory cells extending into the submucosa (Figure 5). In some areas vacuolization (ballooning degeneration) of the squamous epithelium cells were present. A few intracytoplasmic inclusion bodies were observed (Figure 6).

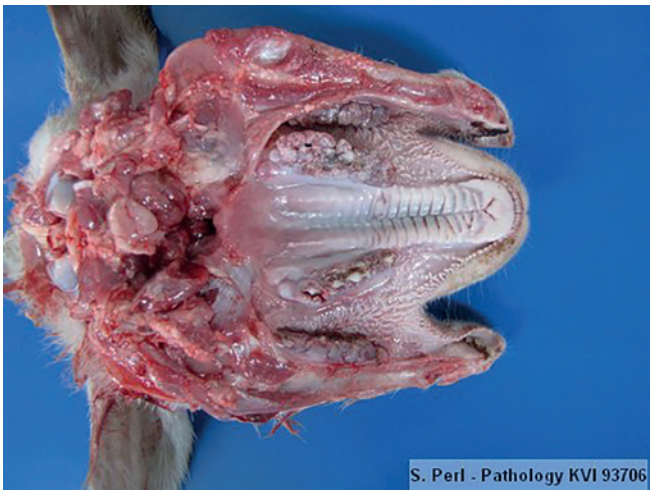

Figure 1: Bilateral proliferations of the mucosa in the molar region.

Figure 2: The molar tooth are imbedded in the proliferative lesions of the buccal mucosa.

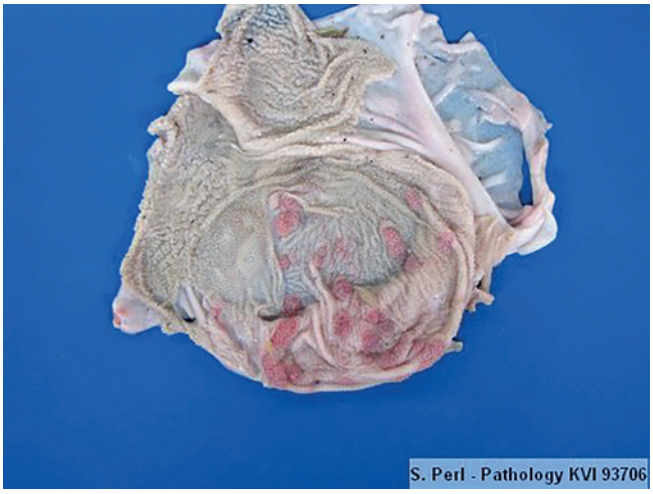

Figure 3: Rumen with multifocal proliferations of the mucosa.

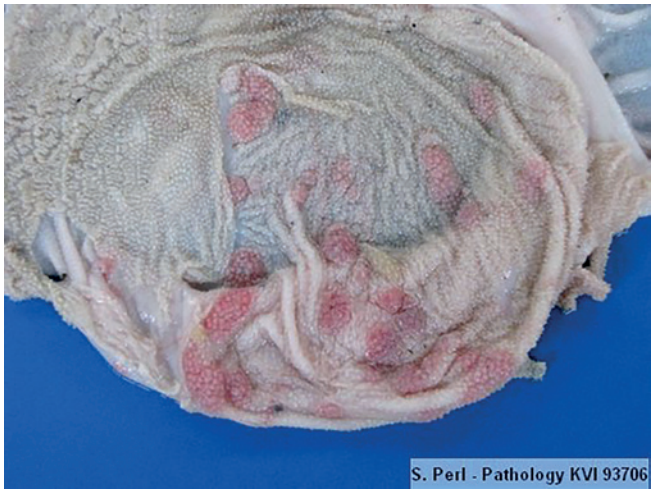

Figure 4: Rumen with multifocal proliferations of the mucosa.

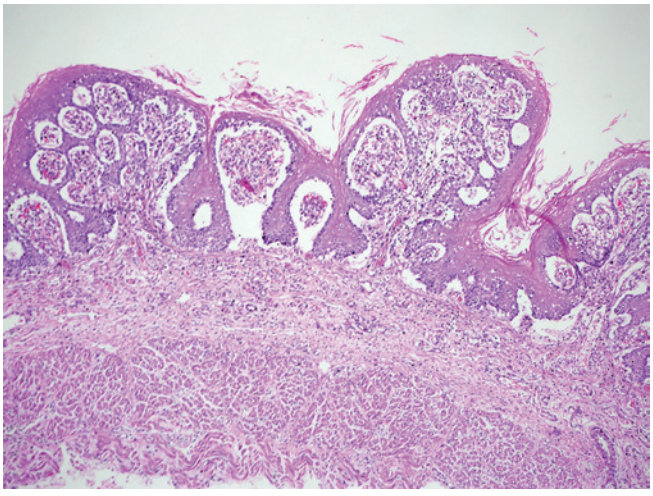

Figure 5: Rumen, proliferation of the squamous epithelium with mild hyperkarathosis and mild infiltration with inflammatory cells in the submucosa (×4, H&E).

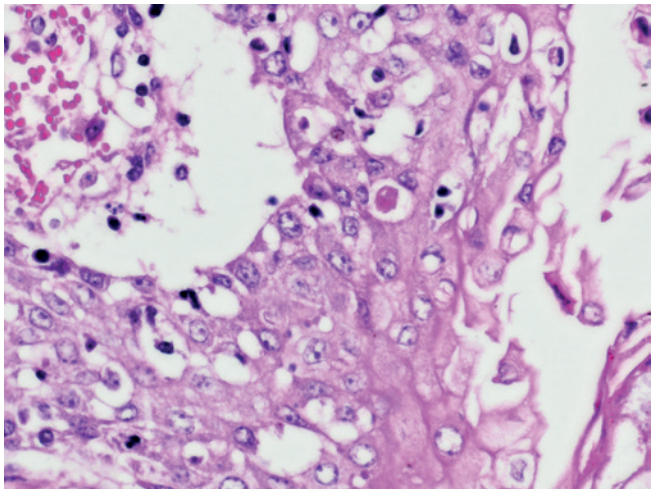

Figure 6: Vacuolization of the squamous epithelium with eosinophilic cytoplasmic inclusion bodies (×20, H&E).

PCR TEST RESULTS

The results of the phylogenetic study presented in figure 7 shows that there is no significant differences among all the tested sequences. In the analysis is included sequences from viruses isolated from Asiaand the Mediterranean area countries such as Israel (ISR 2010-2013) Greece (Gre 2003, 2004) and Turkey (TR-ORF-S). Far East countries like India (India 59-5, 67-04) and China (Shanxi-2011) were also studies (Figure 7). From Figure 7 it can be seen that the percentage nucleotide differences among all isolates as well as from various time points ranged was at a low level of 5%. The maximum nucleotide divergence amongst the isolate was 3.8% (between Israel-12-2 and China-CS-YT-2012). The nucleotide variation among the 3 Israeli isolates has been shown to be between 2.5-2.7%. (Table 2) which is not much different compared to other isolates (Table 1).

The results present in this analysis and others (10) indicate that there are no significant differences among the different orf isolates throughout the world based on the viral B2L gene.

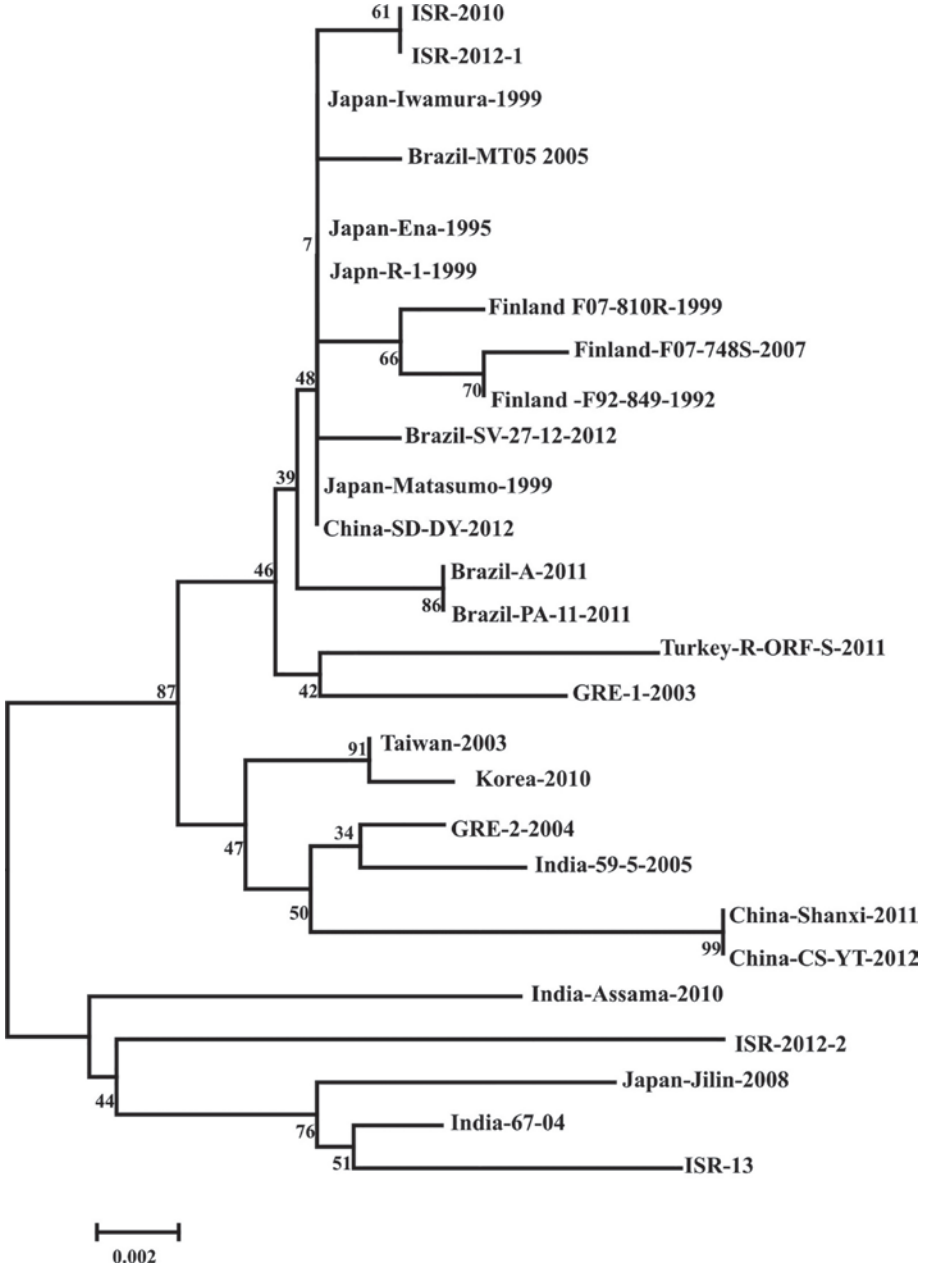

Figure 7: Phylogenetic tree of Israeli isolates from 2010 using Neighbor-Joining method in MEGA 5.1.

Table 2: Pair-wise distances of the of the nucleotide sequences of the isolates.

ISR-2010	
ISR-2012-1	0.000
Turkey-TR-ORF-S	0.012 0.012
GRE-1-2003	0.010 0.010 0.014
Brazil-27-12	0.004 0.004 0.012 0.010
Japan-Ena-1995	0.002 0.002 0.010 0.008 0.002
Japan-Iwamura-1999	0.002 0.002 0.010 0.008 0.002 0.000
Japan-Matasumo-1999	0.002 0.002 0.010 0.008 0.002 0.000 0.000
Finland-F07.748S	0.008 0.008 0.016 0.014 0.008 0.006 0.006 0.006
Finland-F92.849	0.006 0.006 0.014 0.012 0.006 0.004 0.004 0.004 0.002
Finland-F07.810R	0.006 0.006 0.014 0.012 0.006 0.004 0.004 0.004 0.006 0.004
Japan-R-1-1999	0.002 0.002 0.010 0.008 0.002 0.000 0.000 0.000 0.006 0.004 0.004
ChinaSD-DY-2012	0.002 0.002 0.010 0.008 0.002 0.000 0.000 0.000 0.006 0.004 0.004 0.000
Brazil-MT-05	0.004 0.004 0.012 0.010 0.004 0.002 0.002 0.002 0.008 0.006 0.006 0.002 0.002
Brazil-A	0.006 0.006 0.014 0.012 0.006 0.004 0.004 0.004 0.010 0.008 0.008 0.004 0.004 0.006
Brazil-PA-11	0.006 0.006 0.014 0.012 0.006 0.004 0.004 0.004 0.010 0.008 0.008 0.004 0.004 0.006 0.000
GRE-2-2004	0.012 0.012 0.020 0.010 0.012 0.010 0.010 0.010 0.016 0.014 0.014 0.010 0.010 0.012 0.014 0.014
India-59-5	0.014 0.014 0.020 0.014 0.014 0.012 0.012 0.012 0.018 0.016 0.016 0.012 0.012 0.014 0.016 0.016 0.006
China-Shanxi-2011	0.018 0.018 0.025 0.023 0.018 0.016 0.016 0.016 0.023 0.021 0.021 0.016 0.016 0.018 0.021 0.021 0.014 0.014
China-CS-YT-2012	0.018 0.018 0.025 0.023 0.018 0.016 0.016 0.016 0.023 0.021 0.021 0.016 0.016 0.018 0.021 0.021 0.014 0.014 0.000
Taiwan-2007	0.010 0.010 0.018 0.012 0.010 0.008 0.008 0.008 0.014 0.012 0.012 0.008 0.008 0.010 0.012 0.012 0.006 0.008 0.016 0.016
Korea-2010	0.012 0.012 0.020 0.014 0.012 0.010 0.010 0.010 0.016 0.014 0.014 0.010 0.010 0.012 0.014 0.014 0.008 0.010 0.018 0.018 0.002
India-Assama/10	0.022 0.022 0.029 0.025 0.022 0.020 0.020 0.020 0.027 0.025 0.025 0.020 0.020 0.022 0.020 0.020 0.022 0.025 0.033 0.033 0.020 0.022
India-67-04	0.020 0.020 0.029 0.027 0.020 0.018 0.018 0.018 0.025 0.023 0.023 0.018 0.018 0.020 0.018 0.018 0.020 0.023 0.027 0.027 0.018 0.020 0.020
Japan-Jilin	0.025 0.025 0.033 0.031 0.025 0.023 0.023 0.023 0.029 0.027 0.027 0.023 0.023 0.025 0.023 0.023 0.025 0.027 0.031 0.031 0.023 0.025 0.022 0.010
ISR-2012-2	0.027 0.027 0.033 0.033 0.027 0.025 0.025 0.025 0.031 0.029 0.029 0.025 0.025 0.027 0.025 0.025 0.031 0.033 0.038 0.038 0.025 0.027 0.027 0.023 0.029
ISR-2013	0.027 0.027 0.033 0.033 0.027 0.025 0.025 0.025 0.031 0.029 0.029 0.025 0.025 0.027 0.025 0.025 0.027 0.029 0.031 0.031 0.025 0.027 0.023 0.010 0.016 0.025

DISCUSSION

Contagious ecthyma is a common widespread disease which is endemic in the Mediterranean region usually affecting animals of 3-6 months of age. Animals as young as 10-12 days of age as well as adults may also be affected. (1, 2). The morbidity rate can be very high reaching up to 100% (1, 3, 8); the mortality is between 5%-15% (6). The clinical signs last between 1-4 weeks (3) and the lesions heal without scaring (1, 3, 8).

The virus is very resistant in the environment especially in dry conditions and can survive on the premises in scabs from lesions, for as long as 15 years (1, 3) making the eradication of the disease very difficult. Wild ruminants and chronically infected animals may also serve as a source of infection.

Vaccination has a limited effect and usually only decreases the severity and duration of the disease. Vaccination of flocks free of the disease is not recommended (1, 2, 3).

Ecthyma causes severe economic losses due to young animals loosing condition, slower growth rate, trading restrictions and death of lambs (1).Contagious ecthyma is not difficult to diagnose clinically or pathologically when the lesions are present in the typical locations such as lips, muzzle and teats. The clinical diagnosis may become complex, such as in the case described in this report where lesions are concealed in the molar area of the maxilla or on the rumen mucosa. In this case the lesions will only be observed incidentally following a complete necropsy. Differential diagnosis should include neoplasia, warts, ulcerative dermatosis and chorioptic mange (4).

From Figure 7 and Table 2 the differences in the nucleotide sequences among all the viruses and does not exceeds 3.8% between the viruses showing the highest distances (between ISR-2012-2 and China-Shanxi-2011). The data presented in this communication and others (10) give the impression that there is no significant diversification among the virus from different parts of the world (as demonstrated in our data) and probably among all contagious ecthyma isolates throughout the world. The fact that virus isolated from distant locations such as Brazil, Israel, Greece, China, Korea and Taiwan have such small differences probably pursue a strong evolutionary pressure against a high mutation rate in this viral gene. Another explanation for this phenomenon

may be found in the DNA polymerase proof reading activity (9) resulting in an extremely low mutation rate. Both activities, low mutation rates and evolutionary pressure probably contributes to the low level of sequence changes even in isolates from different continents and different periods of time.

In conclusion, this report describes the presence of lesions of contagious ecthyma in lambs as proliferations on the gingival mucosa and ruminal epithelium, the location of which is relatively rare. The genetic characterization of the etiological agent of contagious ecthyma was found to be very similar to viruses characterized both in Israel and throughout the world indicating that the unusual distribution of the lesions is not due to the presence of a another strain of orf.

REFERENCES

1. Radotits, O.M., Guy, C.C., Hinchcliff, K.W. and Constable, D.D.: Veterinary Medicine. 10th edition, 1418 –1421, 2007.
2. De la Concha Bermejillo, A., Guo, J., Zhang, Z. and Waldron. D.: Severe persistent orf in young goats. Vet. Diagn. Invest. 15:423-431, 2003.
3. Meynink, S.E., Lackson, P.G.G. and Platt, D.: Treatment of intraoral orf lesions in lambs using diathermy and cryosurgery. Vet. Rec. 121: 594, 1987.
4. Yeruham, I., Perl, S. and Abraham, A.: Orf infection in four sheep flocks. The Vet. J. 160: 74-76, 2000.
5. Billinis, C., Mavrogianni, V.S., Spyrou, V. and Fthenakis, G.C.: Phylogenetic analysis of strains of *Orf virus* isolated from two outbreaks of the disease in sheep in Greece. Virol. J. 9: 24, 2012. doi: 10.1186/1743-422X-9-24.
6. Ginn, P.E., Mansell, J.E.K.L., Rakich, P.M.: Skin and appendages. In: Maxie MG (ed.), Jubb, Kennedy and Palmer's Pathology of DomesticAnimals, pp. 665-667, 2007.
7. Gumbrell, R.C. and McGregor, D.A.: Outbreak of severe fatal orf in lambs Vet. Rec. 141: 150-151, 1997.
8. Schmidt, C., Cargnelutti, J.F., Brum, M.C., Traesel, C.K., Weiblen, R. and Flores, E.F.: Partial sequence analysis of B2L gene of Brazilian orf viruses from sheep and goats. Vet. Microbiol. 162: 245–253, 2013.
9. Gammon, D.B. and Evans, D.H.: The 3'-to-5' exonuclease activity of vaccinia virus DNA polymerase is essential and plays a role in promoting virus genetic recombination. J. Virol. 83: 4236-4250, 2009.
10. Tamura, K., Peterson, D., Peterson, N, Stecher. G., Nei, M. and Kumar, S.: MEGA5. Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol. Biol.Evol. 28: 2731-2739, 2011
11. Morales, A.G. and Von Kruningen, H.J.: Contagious ovine ecthyma with primary lesions of rumen and concurrent phycomycosis: a case report. Amer. J. Vet. Res. 32: 163-166, 1971.
12. Zamri-Saad, M., Karim, S., Al-ajeeli, A. and Ibrahim, L.A.: A severe outbreak of orf involving the buccal cavity of goats. Trop. Anim. Health Prod. 24: 177-178, 1992.