

## Sero-prevalence of Contagious Ecthyma in Lambs and Humans in Kars, Turkey

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**Abstract:** The sero-prevalence of contagious ecthyma (orf) in lambs and humans was studied in the Kars region of Turkey. For this purpose, serum samples were collected from 320 lambs from 5 different districts of the Kars region. Serum samples were also collected from 100 humans at risk of infection. The serum samples were tested for the presence of antibodies to orf virus by enzyme-linked immunosorbent assay (ELISA). The results showed that 52.81% and 5% of the serum samples collected from lambs and humans, respectively, were seropositive for orf virus. These results indicate that contagious ecthyma is a prevalent infection in lambs and that humans who are in regular contact with sheep are at risk of infection.

**Key Words:** Human, lamb, orf virus, contagious ecthyma, ELISA

### Kontagioz Ektima'nın Kars Yöresindeki Kuzu ve İnsanlardaki Sero-prevalansı

**Özet:** Bu çalışmada Türkiye'nin Kars yöresindeki kuzu ve insanlarda kontagioz ektimanın (orf) sero-prevalansı çalışıldı. Bu amaçla Kars'ın 5 farklı bölgesinde bulunan 320 kuzudan serum örnekleri toplandı. Ayrıca ektima'ya yakalanma riski olan 100 insandan da serum örnekleri toplandı. Bu serum örneklerinde orf virusuna karşı oluşmuş olan antikorlar ELISA yöntemi kullanılarak belirlendi. Çalışmada kuzulardan toplanan serumların % 52,81'inin ve insanlardan toplanan serumların ise %5'inin orf virus için sero-pozitif olduğu belirlendi. Sonuç olarak, kontagioz ektima Türkiye'nin Kars yöresinde kuzularda görülmektedir ve kuzularla sık olarak temas halinde olan insanlar bu enfeksiyona yakalanma riski altındadırlar.

**Anahtar Sözcükler:** İnsan, kuzu, orf virus, kontagioz ektima, ELISA

### Introduction

Contagious ecthyma (CE), also known as contagious pustular dermatitis or orf, is a specific skin disease of sheep and goats, caused by a parapox virus of the family poxviridae (1-3). The disease is commonest in young animals between 3 and 6 months of age, but a few adult animals may also be affected (3,4). It is worldwide in its distribution and is found particularly in sheep and goat breeding countries (2,5-7). CE occurs at any time of the year but is more common during spring and summer mainly amongst lambs and kids (2). Lesions are most commonly localised in and around the mouth and nostrils. The lesions progress through the clinical stages of erythematous macula, papule, vesicle, pustule and scab

formation (2,4). In uncomplicated CE, natural recovery takes 3 to 6 weeks, with shedding of scab material rich in the virus (2,4). Prolongation of the infection and an increase in its severity are nearly always associated with secondary bacterial infections (3,4,8). Some infected animals become carriers and shed the virus for a long period. The virus persists in the environment and remains infective to susceptible animals and humans for years (2,9,10).

The economic importance of CE can be considerable. The morbidity of the disease can be very high, approaching 100%, but the mortality rate in uncomplicated cases rarely exceeds 1%. Secondary, usually staphylococcal, infection is a frequent occurrence

and mortality rates may range from 20% to 50% in these lambs (2,4). Death occurs, especially in young suckling lambs, due to a combination of dehydration and starvation, as the pain and distortion of the lips and mouth preclude the lamb from sucking. Lambs affected with the mouth form or with strawberry footrot exhibit considerably reduced growth rates. Outbreaks in neonatal lambs lead not only to loss of lambs, but also to vastly increased labour costs as endeavours are made to keep lambs alive by artificial feeding.

Human CE occurs mainly in relatively well-defined “at risk” populations such as veterinary surgeons, shepherds, farm workers and abattoir workers, for whom it is an occupational hazard (11,12). The infection usually appears as discrete lesions on the hands, fingers or face. While extremely painful and often taking many weeks to heal, the condition is more of an inconvenience than a danger (4,11,12).

In the Kars region, sheep farming is common practice. Sheep are reared in this region and moved to other parts of Turkey. It is well known that some of the orf virus infected animals become symptomless carriers and continue shedding the virus (2,3,10). The determination of carrier or orf virus-free animals is very important if the disease is to be eliminated and flocks kept free of the virus. Human populations such as veterinary surgeons, shepherds, farm workers and abattoir workers are also at risk of the infection. Therefore, it is essential to conduct serological tests in a situation where there is a risk of introducing infection into new flocks through replacement sheep from unknown premises.

There are few published reports on CE in other parts of Turkey indicating the occurrence of the disease in both lambs (5,7) and humans (11). While clinical CE is commonly observed, there are no publications on the prevalence of CE in sheep and humans in the Kars region.

The aim of this study was to determine the sero-prevalence of CE among sheep and humans in this major sheep breeding region of Turkey by enzyme-linked immunosorbent assay (ELISA).

**Materials and Methods**

**Lambs**

Three hundred and twenty 3- to 4-month old Morkaraman breed lambs with a history of regular occurrence of CE were randomly selected from 5 different districts of the Kars region. All the lambs had been reared in the region and they had not been vaccinated against any pathogen when the study commenced. The number of lambs used in the study from each farm is shown in Table. Twenty-five clinically healthy 4-month-old Morkaraman breed lambs were provided from the farms of the faculty of Veterinary Medicine, University of Kafkas, Kars, and used as negative controls. These lambs had not been vaccinated against either orf or smallpox viruses.

**Humans**

A hundred serum samples were also collected from humans of different ages and occupations: veterinary surgeons (n = 14), veterinary technicians (n = 15), shepherds (n = 20) and farm workers (n = 51). Twenty serum samples from humans with no history of vaccination against smallpox were collected and previously tested for anti-orf virus antibodies and found negative by ELISA. These serum samples were used as controls. Routine smallpox vaccination has not been practised since 1978 in the region. However, some people were over 20 years of age and it is not known whether or not they had been injected with smallpox vaccine.

Table. Numbers and percentages of lambs seropositive and clinically positive for orf virus.

District	Number of lambs	Number seropositive	Percentage seropositive (with 95% CI)	Number of lambs with clinical signs
Kars (Centre)	100	61	61 (51.4-70.6)	17 (17%)
Başgedikler	50	15	30 (17.3-42.7)	10 (20%)
Duraklı	70	45	64.28 (53.1-75.5)	55 (78.57%)
Akçalar	50	29	58 (44.3-71.7)	13 (26%)
Arpaçay	50	19	38 (24.5-51.5)	18 (36%)
Kars region	320	169	52.81 (47.3-58.3)	113 (35.31%)

### Clinical investigation

Routine clinical examination was carried out and the lesions of CE were recorded in lambs and humans.

### Serum samples

Peripheral blood samples were collected from both lambs and humans into plain vacutainers and used to prepare serum samples. These samples were kept at  $-20^{\circ}\text{C}$  until being used for ELISA.

### Antigen source

A cell-adapted strain of orf virus (ST22), kindly provided by Dr. P.F. Nettleton, Moredun Research Institute, Edinburgh, UK, was grown in lamb testis cell (LTC) culture and used to prepare the viral antigen (Cell Culture Laboratory, Faculty of Veterinary Medicine, Liverpool, UK).

### Antigen preparation for ELISA

Orf virus was grown in LTC culture and harvested by 2 cycles of freezing and thawing 5 days after inoculation when there were extensive cytopathic effects (13,14). These infected materials were then used to prepare ELISA antigen as described by Gökçe (13) and Azwai et al. (14). Briefly, the virus suspension was sonicated (Vibra-Cell, Sonics & Materials Inc., Danbury, USA) intermittently on ice for 30 s with 15 s of rest, for a total sonication time of 2 min. After centrifugation at  $1000 \times g$  for 10 min, supernates were collected. The virus was pelleted by ultracentrifugation (OTD Combi ultracentrifuge, Du Pont, USA) at  $45,000 \times g$  for 60 min at  $4^{\circ}\text{C}$  and the pellet was resuspended in PBS. The viral suspension was stirred and NaCl was added to 330 mM, followed by polyethylene glycol (PEG, mol. wt. 6000; BDH) to a concentration of 7%, followed by stirring at  $4^{\circ}\text{C}$  overnight. The suspension was centrifuged at  $3000 \times g$  for 10 min at  $4^{\circ}\text{C}$  and the pellet washed twice in 15 mM NaCl. Viral proteins were obtained by disruption with freezing and thawing in 15 mM NaCl and stripping of the surface proteins by 1% non-ionic detergent (Nonidet P40; Sigma) treatment at  $37^{\circ}\text{C}$  for 3 h, followed by freezing and thawing, and centrifugation at  $3000 \times g$  for 10 min at  $4^{\circ}\text{C}$ . The supernatant (containing viral antigen) was collected and extensively dialysed against several changes of PBS. The protein content of the antigen preparation was determined using the Lowry method (15). Various

concentrations of the antigen preparation were tested and  $10 \mu\text{g/ml}$  was found to be optimal for ELISA (8). Aliquots were then prepared and stored at  $-70^{\circ}\text{C}$  until being used for ELISA.

### Enzyme-linked immunosorbent assay (ELISA)

The ELISA for anti-orf virus antibodies was performed as described by Gökçe and Woldehiwet (8) and Azwai et al. (14). Briefly, ELISA plates (Flow Laboratories, Rickmansworth, Herts, UK) were coated with  $50 \mu\text{l}$  of viral antigen ( $10 \mu\text{g/ml}$ ) in carbonate-bicarbonate buffer at pH 9.6 by overnight incubation at  $4^{\circ}\text{C}$ , followed by incubation in a moist chamber for 30 min at  $37^{\circ}\text{C}$ . After washing, non-specific binding sites were blocked by adding  $100 \mu\text{l}$  of 2% foetal calf serum (FCS, Sigma, Taufkirchen, Germany). The plates were then incubated for 30 min in a moist chamber at  $37^{\circ}\text{C}$  and washed. Fifty microlitres of 1:50 dilutions of lamb or human test serum samples were added to each well in triplicate. Dilutions of known negative and positive control sera (kindly provided by Dr. P.F. Nettleton) were also added in triplicate. After incubation for 60 min at  $37^{\circ}\text{C}$  and washing,  $50 \mu\text{l}$  of an optimum dilution of anti-sheep (Sigma) or anti-human IgG conjugated with horseradish peroxidase (Sigma) was added. The plates were incubated further for 60 min at  $37^{\circ}\text{C}$  and then washed. A freshly prepared substrate solution ( $100 \mu\text{l}$  of ortho-phenylenediamine, Sigma) was added and the plates were left in the dark (15 min) at room temperature until colour had developed in the positive control wells. The reaction was then stopped by adding  $50 \mu\text{l}$  of 2.5 M  $\text{H}_2\text{SO}_4$  and the optical density (OD) of each well was determined with a micro-ELISA plate reader (Tecan-Spectra, Austria) at a test wavelength of 490 nm. The machine was blanked with substrate controls. The mean OD of the control serum samples collected from 25 lambs or from 20 humans known to be negative for anti-orf virus antibodies plus 3 times the standard deviation was regarded as the cut-off value for the assays. OD values of the test serum samples greater than the cut-off value were considered positive (8,16).

### Statistical analysis

The results were expressed as the percentage of seropositive lambs with their respective 95% confidence intervals (CI) for each district.

## Results

### Clinical findings

*Lambs:* Information provided by the farmers indicated that the flocks moved to the pastures in June and grazed until September. The pastures contained thistles, hard stubble and similar plants causing the scarification of the external layer of the skin. There was a good correlation between the outbreak of CE which occurred in July and the timing of the grazing on the pasture.

In each of the 5 different districts studied, there were clinically positive lambs showing characteristic signs of CE, with lesions observed in 113 (35.31%) of the 320 randomly selected lambs (Table). On clinical examination, the lesions were found to be proliferative on the lips, nostrils and gingiva but were not pruritic (Figure 1a). In most of the lambs the lesions were moderate unless

complicated by other pathogens. However, in one district, Duraklı, there was an outbreak of CE and 78.57% of the lambs had severe lesions. The lesions over the coronets in particular were thick and wart-like, and when the scabs were removed a bleeding, fleshy mass surrounded by a shallow ulcer was revealed. These lesions become ulcerative and necrotic without scab formation, healing time was delayed and there was continual rubbing (Figure 1b). The lesions were covered with purulent materials indicating the presence of secondary bacterial contamination. Many of the affected lambs developed lameness and lost their hooves. The lambs did not appear systemically ill and their appetite was normal. However, they were not able to eat because of the presence of painful lesions on the lips and gingiva. According to the farmers, recovery took more than 2 months and the lesions appeared repeatedly in the lambs of Duraklı



Figure 1. Appearance of proliferative lesions on the lips (a) and bleeding shallow ulcer over the coronets (b) of a lamb infected with orf virus.

district, whereas the lambs from all the other districts completely healed within 4 to 5 weeks. Nine lambs (2.81%) were found dead in flocks from 3 districts, namely Duraklı (6 lambs), Akçalı (1 lamb) and Arpaçay (2 lambs).

*Humans:* Characteristic clinical signs of CE were observed in 2 people. One person was a 17-year-old shepherd whose lambs were severely infected with orf virus. His work on the farm included cleaning barns, feeding the lambs, treating the wounds of the infected lambs and shearing their wool. He had proliferative lesions and scabs on his hands. The other person was the 10-year-old daughter of a farmer whose lambs had severe contagious ecthyma. She was known to have had contact with the infected lambs and to have played around the barn where the infected lambs were kept. This girl had proliferative and pustular lesions on her face and hands. All the lesions observed in humans healed completely within 4 to 5 weeks of their onset. Unfortunately, the orf virus and other bacterial pathogens could not be isolated either from lambs or humans in the study.

#### Serological findings

*Lambs:* The cut-off value for the test samples obtained from lambs was  $0.44 \pm 0.02$ . Therefore, the OD values of the test samples over the cut-off value were considered positive. In the present study, 52.81% of the lambs were seropositive to orf virus as measured by ELISA. The percentage of seropositive lambs with their respective 95% CI is between 42% and 63% for the Kars region. No orf virus-specific antibodies were detected in serum samples obtained from control lambs. The numbers and percentages of positive serum samples for the districts are shown in the Table.

*Humans:* The cut-off value for the test samples obtained from humans was  $0.42 \pm 0.02$ . OD values greater than this were considered positive. Five per cent (with 95% CI; 0.7%-9.3%) of serum samples obtained from humans were also seropositive to orf virus. Three of the seropositive humans (23-25 years of age) were veterinary technicians who had a history of using orf virus vaccine but showed no clinical signs of the disease. The other 2 had clinical lesions of CE and also developed a detectable level of antibodies to orf virus.

#### Discussion

Orf virus replicates within the surface layers of the skin as well as the mucosa of the mouth and oesophagus. The non-woolly areas of the skin provide the primary sites for the lesions. Microabrasions or scarification of the external layer of the skin is necessary before lesions or disease can occur (2,4,17). Such abrasions can be caused by thistles, hard stubble or similar plants, which are common in the Kars region where the lambs graze.

In the study, characteristic signs of CE were observed in both lambs (35.31%) and humans (2%). In one district, Duraklı, where there was an outbreak of CE, there were severe and complicated lesions on the lips, nostrils and gingiva and over the coronates. Lambs from other districts were moderately affected and complication by other pathogens was occasionally observed. Animals infected with orf virus normally recover in 3 to 6 weeks without treatment (2,4,8). In this study, the lambs completely healed within 4 to 5 weeks except for those from Duraklı district, whose recovery took more than 2 months possibly due to the development of severe lesions complicated by other pathogens. It is known that orf virus infection may cause immunosuppression, which results in severe secondary bacterial infection and delays recovery (3). Some researchers have suggested that orf is an opportunist virus and only produces clinical infection when there is increased host susceptibility due to one or more factors (8,18,19). Experimental studies have indicated that when animals are concurrently infected with orf virus and *Ehrlichia phagocytophila* (8) or *Dermatophilus congolensis* (18), the humoral immune response to orf virus is reduced, the severity of CE is increased and the shedding of the virus is prolonged. These studies suggest that orf virus acts synergistically with other pathogens to produce severe generalised lesions (8,18), which may explain the extended recovery period observed in the lambs from Duraklı district.

In our study, characteristic lesions of CE were observed in 35.31% of the lambs, while 52.81% of the lambs were seropositive to orf virus. The difference between the percentage of seropositive and clinically positive lambs appears to be due to the fact that some of the seropositive lambs exposed to the virus either recovered or became symptomless carriers. In Duraklı district, in contrast, 64.28% of the lambs were seropositive but 78.57% of the lambs were clinically positive. A study has shown that the clinical signs of CE

are first detected 4 to 5 days after the application of orf virus to lambs, while the levels of orf virus specific antibodies first become detectable 8 to 10 days after application (8). Since there was an outbreak of CE in Duraklı at the time of serum collection and observation, there may have been insufficient time for these lambs to develop detectable levels of anti-orf virus antibodies.

In the present study, all the veterinary technicians had a history of vaccinating ewes with orf virus vaccine and 3 of them were positive by ELISA. The vaccinal virus is a live mild field strain which can cause both the local as well as the systemic condition in humans. It also shares serologically cross-reacting epitopes with orf virus (4). It is not known whether these persons were injected with the vaccinal virus accidentally or exposed to the virus through contact with contaminated objects. Routine vaccination against smallpox has not been practised since 1978. It is also not known whether or not the seropositive persons (over 20 years of age) without clinical signs had the smallpox vaccine, which may give positive results with orf virus antigen. On the other hand, 2 other seropositive persons with no history of smallpox vaccination were also clinically positive to CE. They were known to have had contact with contaminated objects and with the infected lambs, indicating naturally occurring CE in humans.

It has long been accepted that orf virus infections of sheep provide no long-lasting immunity, and annual outbreaks in flocks are relatively common (2,4,9). Orf virus-rich scabs shed by the previous year's infected animals and cutaneous or sporadic shedding of the virus from previously infected carrier animals play an important role in the spread of CE (2,3,10). Thus, sheep

with no clinical signs of CE can transmit orf virus to susceptible animals (10). In addition, the vaccinal virus can contaminate houses and pastures due to shed virus-rich scabs, causing widespread mild clinical CE in sheep. These vaccines do not produce strong long-lasting immunity and hence no protection is transferred to the lambs by the ewe's colostrum (2,4). Therefore, a vaccine against orf should never be used in a flock which has not experienced clinical orf previously. On the other hand, vaccination of already affected lambs has been found to reduce the course and severity of the disease (2). Our results indicated that orf virus infection is widespread in the areas of Kars studied. It is, therefore, recommended that vaccination should be carried out on a regular basis.

In conclusion, this study indicates that CE occurs in lambs and humans in the Kars region and causes considerable economic losses in lambs. Humans with regular contact with sheep appear to be at great risk of infection. Sheep are reared in the Kars region and moved to other parts of Turkey. Therefore, it is essential to conduct serological tests in a situation where there is a risk of introducing infection into new flocks through replacement sheep from unknown premises. On the basis of our findings, it is also recommended that lambs in the region should be regularly vaccinated to reduce the severity of CE and its consequent economic impacts.

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