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## **Lamb mortality due to bronchopneumonia secondary to orf infection and control by vaccination**

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## **Θνησιμότητα αμνών λόγω δευτερογενούς βρογχοπνευμονίας σε λοιμώδες έκθυμα και έλεγχος της κατάστασης με εμβολιασμό**

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**ABSTRACT.** Newborn lambs of a sheep flock in Greece showed clinical signs of bronchopneumonia with high perinatal mortality. All different therapeutic protocols that were applied were ineffective. Clinical examination of 4 lambs confirmed that the animals exposed signs of bronchopneumonia and contagious ecthyma. Haematological parameters of all the 4 lambs were within normal limits, while gross pathologic findings were compatible with bronchopneumonia. Lung and scab specimens were taken from all 4 animals for microbiological examination. Orf virus was isolated from all scab specimens, while lung specimens were found negative. In contrast, all lung specimens yielded colonies of *Pasteurella multocida*, *Manheimia haemolytica* and *Mycoplasma* spp. All the remaining pregnant ewes of the flock were vaccinated against orf virus with a commercial live attenuated vaccine. It resulted in diminishment of bronchopneumonia cases in the newborn lambs. In conclusion, it is supported that the lambs in the present flock were immune-suppressed due to orf infection and therefore exposed secondary bronchopneumonia.

**Keywords:** orf, lambs, mortality, bronchopneumonia, vaccines.

**ΠΕΡΙΛΗΨΗ.** Σε μια εκτροφή προβάτων παρατηρήθηκε υψηλή θνησιμότητα των νεογέννητων αμνών λόγω βρογχοπνευμονίας, ενώ τα διάφορα θεραπευτικά σχήματα που χρησιμοποιήθηκαν δεν ήταν αποτελεσματικά. Τέσσερεις ασθενείς αμνοί προσκομίσθηκαν στην Κλινική Παραγωγικών Ζώων και διαπιστώθηκε η ύπαρξη λοιμώδους εκθύματος και βρογχοπνευμονίας. Τα αιματολογικά ευρήματα ήταν εντός των φυσιολογικών ορίων. Οι αλλοιώσεις των πνευμόνων ήταν συμβατές με βρογχοπνευμονία και στις καλλιέργειες απομονώθηκαν *Pasteurella multocida* και *Mycoplasma* spp. Οι ιολογικές εξετάσεις των εφελκίδων ήταν θετικές για τον ιό του λοιμώδους εκθύματος, ενώ ο ιός δεν βρέθηκε στους πνεύμονες. Οι υπόλοιπες έγκυες προβατίνες της εκτροφής εμβολιάσθηκαν κατά του λοιμώδους εκθύματος και το πρόβλημα στη συνέχεια ελέγχθηκε. Συμπερασματικά, κάποια περιστατικά λοιμώδους εκθύματος επιπλέκονται με βρογχοπνευμονία, πιθανόν λόγω ανοσοκαταστολής. Τα κρούσματα αυτά ελέγχονται με τον εμβολιασμό των προβατινών 1 μήνα προ του τοκετού.

**Λέξεις ευρετηριασμού:** λοιμώδης έκθυμα, αμνοί, θνησιμότητα, βρογχοπνευμονία, εμβόλια.

## INTRODUCTION

Orf (contagious ecthyma) is an economically important infectious disease of the small ruminants that can also be transmitted to humans. It is caused by a *Parapoxvirus* that belongs to the family *Poxviridae* (Robinson and Balassu, 1981; Haig, 2006). It usually affects young animals 3-6 months old, although recently the disease has been diagnosed in lambs aged 10-21 days old (Giadinis et al., 2007; McElroy and Bassett, 2007; Stampoulis et al., 2010). Adults are rarely affected, usually when a flock is infected for the first time (Stampoulis et al., 2010).

Orf in young lambs has two main clinical forms, the mild and the severe. The mild form is manifested with skin lesions, especially around the lips and nostrils. These lesions are usually self-limiting and treat-

ment is not attempted most of the times. The severe form appears when orf is complicated with secondary bacterial infections of the internal organs. In this case treatment is recommended especially when high neonatal mortality occurs. Up to date the reason for the mortality has not been elucidated. Orf can be complicated by different infectious agents among them some that cause bronchopneumonia (McKeever and Reid, 1987; McKeever et al., 1987; Giadinis et al., 2007; Stampoulis et al., 2010; Nandi et al., 2011).

Different protocols have been followed for the treatment of orf complicated with bronchopneumonia. These have been proved ineffective at the majority of times. On the contrary, vaccination against orf of pregnant ewes one month before parturition has given the best results in effect to the neonatal mortality (Mayr et al., 1981, Giadinis et al., 2007; Stampoulis

et al., 2010). However, all the aforementioned studies were focused on the results of the vaccination and not in the pathology of the affected animals and the possible pathogenesis of this condition.

The present study presents a case of orf in a sheep flock complicated with bronchopneumonia resulting in high neonatal mortality and its control after vaccination with a commercial vaccine. Also, it focuses on the pathological findings of the affected lambs.

## CASE DESCRIPTION

The flock consisted of 300 adult dairy sheep of East Friesian, Chios and other local breeds that were regularly de-wormed and vaccinated against clostridiosis and mannheimiosis (Dialuene P-MSD®). The animals consumed alfalfa hay *ad libitum* and commercial concentrates according to their nutrient requirements and they were estrus synchronized in groups. For 3 years the animals of the flock manifested orf lesions and high mortality in neonatal lambs 10-15 days old. At the time that the incidence was introduced to Farm Animal Clinic of the Faculty of Veterinary Medicine in Thessaloniki, 200 out of 300 lambs in total had died. They had signs compatible with orf and cough. Any attempt for treatment with antibiotics and anti-inflammatory drugs was unsuccessful.

Four lambs 10-15 days old were admitted to the Farm Animal Clinic in November 2007, the 3 were

alive (1, 2, 3) and 1 dead (4). All the animals had severe orf lesions with stomatitis (Fig. 1). The 3 alive ones had normal rectal temperature, cough, nasal discharge (Fig. 2) and severe dyspnea, while during the auscultation of thorax ronchi were observed. Blood samples with and without EDTA were taken by jugular venipuncture from the 3 alive lambs and after they were euthanized. The 4 lambs were necropsied and samples (skin and lung lesions) were submitted for further laboratory examinations.

The blood samples were analyzed immediately after the collection for the haematological parameters: red blood cells, white blood cells and platelets counts, as well as haemoglobin, haematocrit, MCV, MCH and MCHC values by using an automated analyzer (Scil VET abc™ Hematology Analyzer, Scil Animal Care Co, Viernheim, Germany), while blood smears were prepared and stained with Giemsa for manual differential white blood cell count evaluation (an absolute neutrophil, lymphocyte, monocyte and eosinophil count). Total solids were calculated using a desktop refractometer (ATAGO T 2-NE CLINICAL, Atago Ltd, Tokyo, Japan).

Bacteriological cultures were performed using blood agar plates and mycoplasma broth and plates inoculated with lung tissue samples. Bacteria were isolated and identified using routine methods (Quinn et al., 2002). Susceptibility test was applied according to Kirby-Bauer disc diffusion method (Quinn et al., 2002; VET01S, 2015). The samples were examined for



**Figure 1.** Lamb with stomatiis.



**Figure 2.** Orf lesions and nasal discharge due to bronchopneumonia.



penicillin G, ampicillin, ceftiofur, neomycin, tilmicosin, doxycycline, trimethoprim-sulfamethoxazole and enrofloxacin.

Blood sera were tested by agar gel immunodiffusion for contagious ecthyma and sheep pox antibodies. Conventional polymerase chain reaction (PCR) for contagious ecthyma and for sheep pox was performed based on the previously published studies (Mangana-Vougiouka et al., 1999; Kottaridi et al., 2006) for viral DNA detection from the scab, tongue and lung lesions, as well as from the supernatant of the second and third cell culture passages of the samples.

Virus isolation was performed in primary lamb testis cells (LT), which were grown in plastic flasks of 25 cm<sup>3</sup>. The above mentioned samples were used for inoculation after processing by grinding, followed by freezing at -70°C and thawing three times, according to classical virological methods for virus isolation (Mangana-Vougiouka et al., 2000).

Taking into account the previous experience about orf mortality in lambs (Mayr et al., 1981; Giadinis et al., 2007; Stampoulis et al., 2010), the remaining 80 pregnant ewes that were at the last month of their gestation were vaccinated against orf virus. The commercial vaccine Ecthybel® (Merial-France), which contains live attenuated ecthyma virus cultured in cells at least  $2.5 \times 10^4$  PFU was used. It was administered by subcutaneous injection.

Haematocrit, haemoglobin and red blood cell count values for the 3 examined lambs were within the normal values cited in the literature (Panousis et al., 2007). White blood count was increased for lamb 1 (22.100/ $\mu$ l) and was normal for the other 2 lambs, while neutrophilia and monocytosis were observed only in lamb 1 (Panousis et al., 2007). Platelet counts were normal for the 3 examined lambs (Panousis et al., 2007).

Upon macroscopic examination papules, occasionally coalescing, and crusty scabs were seen on the skin of lips and muzzle. Buccal lesions comprised of raised papules and elevated yellowish crusts that formed layers 5-12mm on the tongue, gums and palate (Fig. 3). The gross lesions of the lungs included a cranio-ventral lobular distribution of consolidation. The affected lobules were firm and red dark to tan in color and collapsed. On cut surface purulent exudate



**Figure 3.** Lesions of the tongue due to orf infection.

expressed from bronchi. On the left cranial lobe of a single lamb there were multiple, round 0.5-1.5 cm in diameter areas of necrosis.

Histopathological examination revealed multifocal thickening of the epithelium due to marked epidermal proliferation. Swollen and vacuolated keratinocytes, showing nuclear pyknosis, were prominent. Intracytoplasmic eosinophilic inclusion bodies were occasionally seen in vacuolated necrotic cells. Ulcers with neutrophilic infiltration, proteinaceous fluid, degenerating neutrophils and cellular debris were frequently seen. In non-ulcerated affected areas, the inflammation was predominantly lymphoplasmacytic.

In the lumen of bronchi there were many neutrophils. Hyperplasia and mucous metaplasia of bronchial epithelium was a common feature. Moderate numbers of macrophages and lymphocytes were seen in the peribronchial tissue. Mild to moderate type-2 pneumocyte hyperplasia and thickening of the interlobular septa was multifocally observed.

Two blood sera and two scab lesions, three tongue lesions, as well as four lung samples from respective number of the animals admitted to the Farm Animal Clinic, were sent to the Department of Virology of the Institute of Infectious and Parasitic Diseases. Both sera were found positive for orf antibodies and negative for sheep pox antibodies, by agar gel immuno-diffusion test.

PCR from both scab lesions and tongue from lamb number 3 was also positive for orf and negative for sheep pox. Contagious ecthyma virus was isolated in lamb testis (LT) cell culture from one scab lesion (lamb 1) and from one tongue lesion (lamb 3). The lungs from all 4 animals were found negative in all tests for orf viral diagnosis.

Tissue samples from the lungs of the 4 lambs were sent to the laboratory of Microbiology of the Ministry of Rural Development and Food in Thessaloniki. From the lung samples of the 3 lambs (1, 3, 4) *Pasteurella multocida* and *Mycoplasma* spp. were isolated, while from the lung of the second lamb (2) *Mannheimia haemolytica* and *Mycoplasma* spp. were isolated. Results on the antibiogram for *P. multocida* and *M. haemolytica* showed sensitivity against doxycycline, enrofloxacin and trimethoprim-sulfamethoxazole.

The 80 vaccinated ewes gave birth to 118 lambs. Twenty of them presented mild orf lesions at their lips at the age of 10-15 days old that disappeared after about 20 days. The rest 98 lambs remained healthy up to the age of 1 month old, when the last report from the supervising veterinarian of the flock was received.

## DISCUSSION

Orf is a common small ruminant disease in many countries, including Greece. Many Greek flocks are infected every year from orf, a disease that has an economic impact that can range from very low to very high, especially when it results in high neonatal mortality (Darbyshire, 1961; Spais, 2005; Stampoulis et al., 2010). Morbidity is usually high (up to 100%), while mortality is increased in small aged animals, when the disease is complicated with secondary infections and especially bronchopneumonia (Brugere-Picoux, 1994; Giadinis et al., 2007). However, once in the past, high lamb mortality has been observed due to orf in animals aged 5 months old in UK that was attributed to heavy stomatitis lesions (Gumbrell and McGregor, 1997).

The mechanism of bronchopneumonia development in cases of orf is not clear. It is known that orf

virus has a local pathogenic effect and therefore is not found in internal organs. This was confirmed in this report by examinations that were conducted in samples from the affected lungs and neither viral DNA was detected, nor the virus was isolated in sensitive cell cultures. So, the most possible explanation for the bronchopneumonia development could be immunosuppression due to severe stomatitis, which leads to reduced food consumption. This is in agreement with the aspect of Reid and Rodger (2007), that the clinical appearance of orf is possibly related to environmental factors. However, further studies seem to be necessary in order to confirm these findings.

It is interesting that although *P. multocida* and *M. haemolytica* isolated in the aforementioned cases of bronchopneumonia were found susceptible *in vitro* to common antibacterial drugs, the conducted treatments were ineffective. This confirms furthermore that the reason for the mortality in orf cases complicated with bronchopneumonia seems to be immuno-suppression.

Taking into account the results of clinical and laboratory examinations, the vaccination against orf was proposed. Vaccination is the most significant measure for the protection of a flock against contagious ecthyma and seems to be necessary when high mortality is observed (Mayr et al., 1981; Giadinis et al., 2007). In the literature there is a controversy for the efficacy of vaccination against orf (Mayr et al., 1981; Buddle and Pulford, 1984; Pye, 1990; Giadinis et al., 2007). Different commercial vaccines and field strains don't seem to be the reason for the inefficiency of the vaccines (Nettleton et al., 1996; Reid and Rodger, 2007).

In conclusion, according to the results of the present study, the vaccination was protective and it is proposed for the control of a severe orf outbreak.

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