

SPREADING OF CONTAGIOUS ECTHYMA IN SMALL RUMINANTS IN BULGARIA

Lyudmil Ivanov^{1✉}, Raiko Peshev²

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Abstract

Serological and virological investigations were performed to establish the prevalence and impact of the virus of contagious ecthyma (CE) in small ruminants in Bulgaria. Serological surveys were conducted by micro virus neutralization test of 219 serum samples obtained from animals inhabiting 14 sheep and goat farms. Virologically 63 samples (crusts and internal organs) derived from animals were tested by virus isolation with primary and permanent cell cultures. Molecular biological research to confirm the virus of CE was performed on 14 samples by conventional polymerase chain reaction to demonstrate the gene 045 of ORF virus.

From sixty-three surveyed samples grown on primary and permanent cell cultures, ORF viruses were confirmed by PCR in 14 samples (22.2%). After serological testing in all sheep and goat farms 160 positive samples were found for antibodies against CE (73.06%).

From the obtained results it can be concluded that CE is widespread in animals from sheep and goat farms.

Key words: contagious ecthyma, ORF virus, MVNT, PCR

Introduction. Contagious ecthyma (CE) is a generalized viral disease in sheep, goats and wild antelopes, caused by ORF virus (ORFV). It can also affect humans, but the manifestations are local. The disease is known by more than 80 names, which shows the numerous clinical signs. It is most commonly reported as

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infectious pustular stomatitis in France [1], contagious pustular dermatitis in England [2], ORF in Scotland, ulcerative stomatitis and sore mouth in the USA [3,4], rough mouth in Australia and New Zealand, infectious labial dermatitis, boquera in Argentina and Uruguay [5].

In Europe, the incidence of CE reaches 100%, and the mortality is about 10–15%, increasing after complications from bacterial infections [6]. Mortality in our country is about 8–10%. Infected animals cannot eat, are retarded in their development, poorly fattened, and a large part of them die, which is why the losses for the owners are great. In newborns to wild ruminants, CE is severe, and clinical symptoms are similar to those of domestic sheep and goats. Regardless of vaccination or previous infection of the animals with the CE virus, reinfection and its reappearance are possible. The economic losses that CE inflicts on animal husbandry make it necessary to study the spread of this disease among domestic and wild animals in our country [7,8]. CE must be distinguished from sheep and goat pox because the clinical signs of both diseases are similar, but sheep and goat pox is an exotic infectious disease, with high mortality rate up to 58% and animal losses are very high. It is necessary to apply serological, virological and molecular-biological methods to prove CE for quick and accurate diagnosis and taking adequate measures.

The aim of the current study is to establish the spread of the disease in different regions of the country and to prove the circulation of the virus in some farms.

Materials and methods. *Epizootiological studies.* The studies were carried out in 14 settlements from ten administrative regions with data on the presence and manifestations of the disease.

The manifestations of the disease were analyzed in 20 flocks – ten flocks of sheep, six flocks of goats, and four mixed flocks from settlements in the districts of Varna, Veliko Tarnovo, Stara Zagora, Sliven, Plovdiv, Haskovo, Blagoevgrad, Sofia, Pernik and Burgas. The total number of animals included in the study was 2706, of which 1333 sheep and 715 goats from homologous herds and 658 sheep and goats from mixed herds. All farms were subject to an epizootiological survey either through a site visit (within an epizootic outbreak, for a certain time when its clinical manifestation was ascertained) or by obtaining data from the veterinary specialists of the farms.

Serological studies. Serological investigations were performed by microvirus neutralization test (MVNT) on 219 samples originating from animals of 14 sheep and goat farms. MVNT according to the methods described by DILOVSKI et al. [9] were performed. The results were read microscopically according to the presence or absence of cytopathic effect (CPE) in the cells. Uninfected cell culture was used as negative control, and as positive – virus suspension of 100 TCID₅₀/mL. The highest dilution of the sera in which CPE was present was used as the neutralization titer of the sera.

Virological and molecular biological studies. Sixty-three samples (crusts and viscera) were examined from animals with signs of CE originating from 14 farms. For the virus isolation primary and permanent cell cultures were used. Sterile 10% homogenates from the crusts and internal organs were prepared and were centrifuged at 4000 rpm/15 min, and the clear supernatant was used to infect the cell cultures. As a growth medium the Eagle MEM with Hanks' salts and 10% fetal calf serum were used, and the same medium with 2% fetal calf serum – as a maintenance medium. The results of the virological test were read microscopically according to the four-cross system, depending on the strength of the cytopathic effect (CPE).

Molecular biological studies were performed on 14 samples by conventional polymerase chain reaction (PCR) to demonstrate 045 gene of the CE virus.

Viral DNA was isolated from the original samples or from adapted to cell cultures samples using a set of columns from the company “Bioline” Germany. The DNA was amplified by the methods described by KOTTARIDI et al. [10]. To perform PCR, were used primers amplifying a region of the highly conserved 045 gene of CE virus, encoding the late transcription factor VLTF-1. The PCR products were visualized by electrophoresis in a 1.5% agarose gel in TBE buffer for 40 min at 120 V.

Results. In most of the farms, the disease appeared after the importation of sheep and goats from other areas of the country, with the exception of a few farms where no animals were imported until the monitoring. The disease occurs most often in newborn and adolescent animals. In the monitored farms, CE occurs with different severity and affects lambs, adult sheep and goats more or less depending on the way the animals are raised and fed and the climatic conditions of the respective areas. CE is much more severe in goats than in sheep, because with bacterial complications and untimely treatment, mortality in some farms has reached 8–10%. In order to analyze the susceptibility of different age categories of animals to CE, the studied sheep were divided into five age groups (suckling lambs, lambs from 3 to 6 months, grown lambs, young barren ewes and adult sheep), and goats – into four (suckling kids, kids from 3 to 6 months, young goats from 6 to 12 months, and adult goats). The highest percentage of sick sheep were found in the group of suckling lambs (19.2%) with a relatively large variability (3.4–36.2%) for individual farms, while in goats the highest percentage was in adult goats (21.5%) with low variability (13.3–30.2%) (Fig. 1).

In the isolation of the virus from 63 samples to primary lamb testicle (LT) and permanent cell cultures sheep fetal timus (SFT) 14 isolates showed characteristic CPE of ORF virus. In LT cell culture CPE was expressed in cell rounding on the second day, after which the cytopathic changes increased on the sixth (Fig. 2b) and eighth days (Fig. 2c). After 9–10 days the CPE was amplified and the cells monolayers fell into the support medium (Fig. 2d). The CPE on cell culture SFT appeared on day 3–4 and was expressed in cell rounding and disruption of the

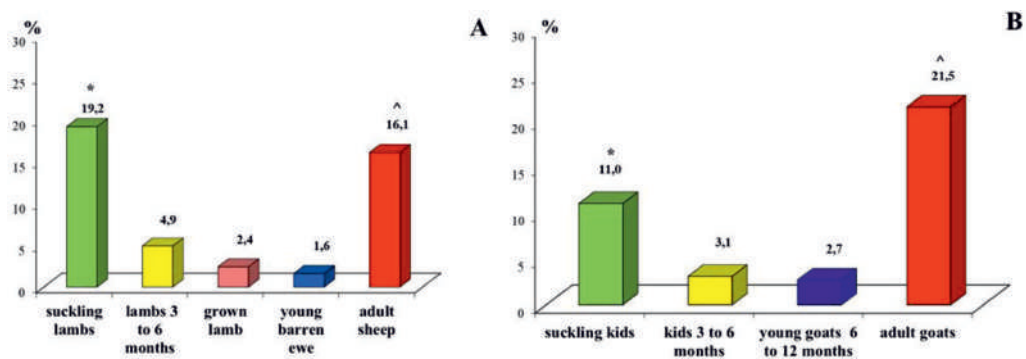


Fig. 1. Percentage distribution of the prevalence of CE in individual age groups of sheep (A) and goats (B) in 12 flocks. * and ^ mark significant differences between groups

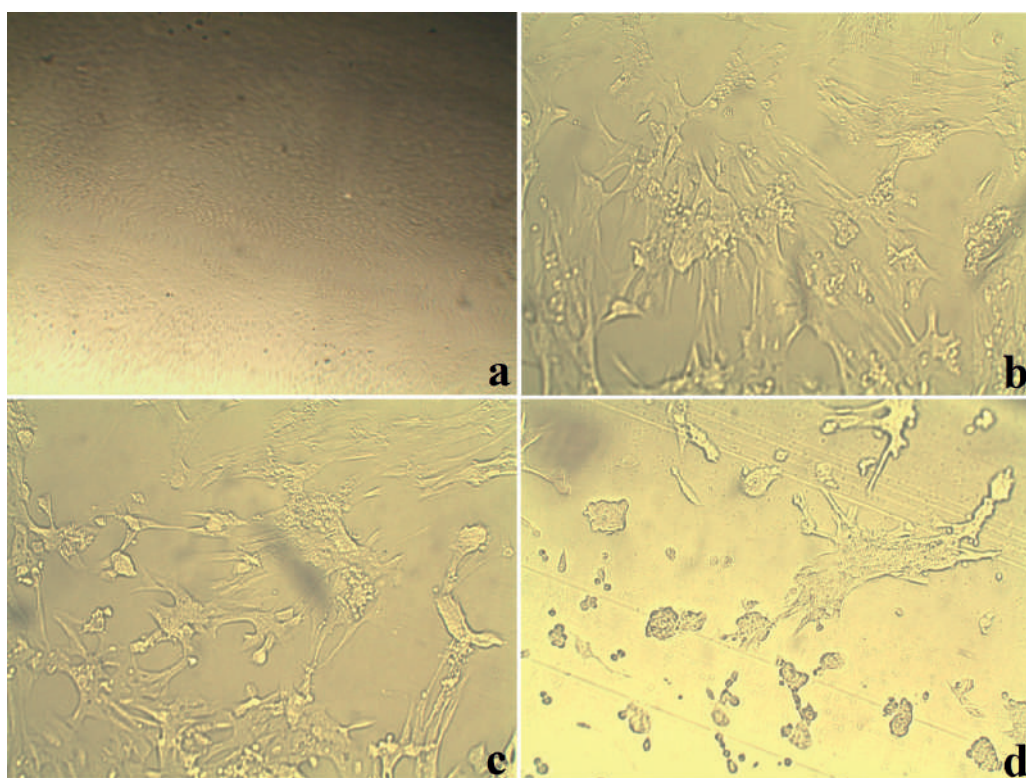


Fig. 2. Cytopathic effect of “Tsarevo” isolate on primary LT cell culture. a) Control uninfected cell culture LT; b) Day 6; c) Day 8; d) Day 10 after infection

cell monolayer. The changes intensified after day 6, and after 9–10 days, a large proportion of cells fell into the support medium. CPE was not observed in the control uninfected cell cultures. The virus titers after the titration ranged between $10^{3.5}$ and $10^{4.7}$ TCID₅₀/mL.

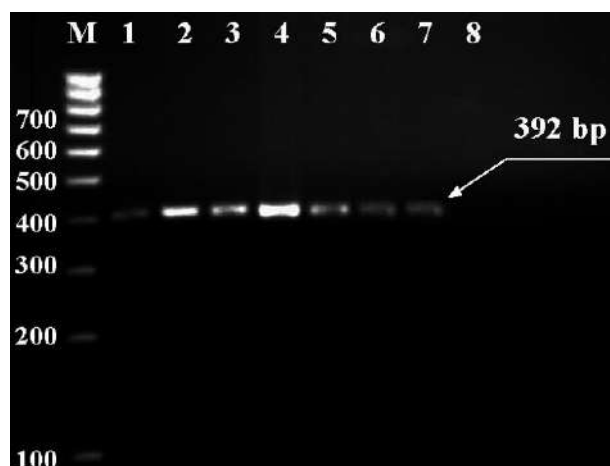


Fig. 3. Amplification of DNA obtained from the field and adapted on cell cultures CE virus isolates. M – Marker of molecular weights 100 bp, 1. Tsarevo (Burgas), 2. Avren, 3. V. Tarnovo, 4. Pernik, 5. Dragovishtitsa, 6. Burgas, 7. Positive control – Phylaxia, 8. Negative control – cell culture

Of the examined 63 samples cultured on primary and permanent cell cultures, the ORF virus was confirmed by PCR in 14 samples (22.2%). The used primers specifically amplified gene 045, encoding the late transcription factor of the ORF virus with a size of 392 bp. In the control uninfected cell culture, an amplification product was not found (Fig. 3).

In the serological tests performed by MVNT on 219 animal samples from 14 sheep and goat farms, 160 were positive for antibodies against CE (73.06%).

Discussion. In the surveyed farms, newborn lambs and kids and adult sheep and goats were most frequently affected. In sheep, the prevalence rate was significantly higher in adolescent lambs, while in goats, the prevalence rate was significantly higher in adult goats. No significant difference was found in the other groups of animals. In juvenile animals a higher lethality was also found. The most probable reason is the complication of the infectious process by secondary bacterial microflora and an immature immune system [11, 12].

CE is known as a moderately ongoing infectious disease, which in adults most often ends with spontaneous self-healing in a period of 3–5 weeks without medical treatment [13]. In the present clinical and epizootiological studies in different farms, affected by CE, all three forms of the disease were observed – labial, podal and genital. In newborn and adolescent lambs and kids the appearance of papules and pustules on the skin of the lips, nose and the oral commissures was observed most often. After 4–5 weeks the crusts healed but in some lambs and kids this period was longer as was observed by GUO et al. [14] and ABU ELZEIN and HOUSAWI [15].

ORF virus is difficult to culture in vitro. Primary cell cultures are most suitable for virus isolation [16]. In the current study, primary cell cultures from

lamb testes and permanent cell lines of ovine origin were used for isolation and multiplication. Probably, these cells have receptors on their surface that allow the adsorption and penetration of the ORF virus, and also provide the most optimal conditions for its multiplication.

For the diagnosis of parapoxvirus infections a conventional PCR based on the detection of B2L or VIR genes was developed [17]. Comparative studies between two different conventional PCR protocols after viral isolation of the 045 gene of the ORF virus were made in Greece [10]. In present molecular biological studies a laboratory verified PCR method amplifying a part of the highly conserved ecthyma 045 gene encoding the late transcription factor VLTF-1 of the virus was used. This method successfully confirmed both the field isolates of the ORF virus and cell culture-adapted isolates.

Serum samples from acutely ill and recovered animals were investigated by using the MVNT for spreading of the infection on the territory of Bulgaria. The increasing of antibody titers by two or more logarithms was the proof for virus circulation in the monitored herds. Serum neutralization test is most commonly used for serologic testing, with MVNT titers ≥ 8 considered positive [18]. In the current study all sera with MVNT titers ≥ 2 were considered positive. Most probably that titer is the low threshold of that test for antibodies detection against the ORF virus.

Conclusions.

1. During the conducted clinical-epizootiological and serological studies, the widespread of the infectious CE virus among the sheep and goat herds in the country was established.

2. Strains cultivated on primary cell cultures from different regions of the country have been proven by molecular biological methods as CE viruses.

3. The applied molecular methods for the study of the isolated ORF virus by PCR specifically amplify the conservative gene of the virus and can be successfully used in the daily and research activities of the laboratory.

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¹*Veterinary Medicine Faculty, Trakia University, 6000 Stara Zagora, Bulgaria*
e-mail: lyudmilivanov@abv.bg

²*National Diagnostic and Research Veterinary Medical Institute, 15 P. Slaveykov Blvd, 1606 Sofia, Bulgaria*
e-mail: p_raiko@hotmail.com