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Molecular diagnosis of Lumpy Skin Disease during outbreak in Albania

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Abstract

Lumpy skin disease (LSD) is a viral disease of livestock that cause cutaneous and internal lesions, affect milk production, hide quality and in some cases cause the death of the infected animal. After an outbreak in Greece in 2015, the disease crossed borders and passed to Bulgaria, Macedonia, Serbia, Kosovo, Albania and Montenegro. In Albania the first case is registered on July 6, 2016, in the village Vlashaj, Bulqizë and after this case the disease expanded rapidly in all the country. This study was carried out to determine the situation of the LSDV outbreak in Albania. Samples analyzed with RT-PCR method consisted on swabs, skin nodules, nasal discharge and lymph nodules. The method used was adapted from *Bowden et al., 2008* for the detection of all CaPV. The DNA was extracted with DNeasy Blood& Tissue Mini Kits (Qiagen) and InvitrogenPureLink® Genomic DNA according to the manufacturer's instructions. From 3419 samples collected in Albania during the outbreak of 2016 -2017, 2053 resulted positive with the most outbreaks occurring in spring and summer. Dibra was the districts with the high number of cases of Lumpy Skin Disease. From all the samples collected the nodular skin lesions resulted with higher concentrations of the virus. Warm temperatures and related vector abundance are among the main risk factors for LSD spread. This paper describes the situation of the LSD outbreak in Albania and the confirmation of the disease in dead animals or with clinical signs by the RT- PCR method. The confirmation of the cases during the outbreak was very important for the beginning of the vaccination in cattle with the live homologous vaccine because when the vaccine is applied across the infected population the coverage is most effective in reducing LSD virus spread. Ongoing surveillance should continue as environmental persistence of the virus may lead to further outbreaks of the disease, also it is important to take long-term studies about the seasonality of potential LSD vectors.

Keywords: Lumpy skin, outbreak, livestock, RT-PCR, vaccination

1. Introduction

Lumpy skin disease (LSD) is an economically important acute disease of cattle of all ages. LSD is caused by Capripox virus with a narrow vertebrate host range, infecting only sheep, goats and cattle. The first clinical syndrome of lumpy skin disease was described in Zambia in 1929. The clinical signs of the disease include pyrexia of 40 to 41.5 °C, lachrymation, anorexia and depression. Afterwards, the characteristic skin lumps develop; they may cover the whole body or be restricted to the head, neck, perineum, udder, genitalia or limbs. The regional superficial lymph nodes are enlarged and edematous. There is an increase in nasal and oropharyngeal secretions, which may be associated with the development of lesions on the muzzle and in the mouth. There is no specific antiviral treatment available for this disease. It is recommended that all infected and contact cattle be slaughtered immediately and the carcasses destroyed.

A vaccination cover with a 25 to 50 km radius may then be established around the focus and all cattle movements stopped within that zone. Vaccination will greatly reduce the morbidity and economic effects of an epizootic occurrence. The rapid identification of LSD is essential for the beginning of the vaccination. Also it is important to differentiate the LSD with many other conditions as: bovine herpes virus 2, insect bites, demodicosis etc, this is why the laboratory confirmation is necessary. There is little information about the vectors of LSD. The transmission of the virus is thought to be the result of insect transmission of the virus [1,2,3,4]. Pox viruses are highly resistant and may remain viable in infected tissue for at least four months, and probably longer. Virus is also present in blood, nasal and lachrymal secretions, semen and saliva, which may be sources for transmission. Virus has been isolated from *Stomoxys spp*.,[5] commonly associated with cattle, and from the *Biomyia fasciata* mosquito species.

2. Material and Methods

A total of 3419 samples are analyzed during 2016-2017 in the Laboratory of Molecular Biology of Animal Health Department in Food Safety and Veterinary Institute. The samples were collected from dead cows or with clinical signs of the disease. Samples submitted for RT-PCR diagnosis were nasal swabs, skin nodules, discharge and lymph nodules. Swabs were resuspended in 1 mL of Phosphate Buffered Saline (PBS). Tissues and skin nodules were grinded, approximately 25-50 mg of tissue in 4 mL of Phosphate Buffered Saline (PBS) and centrifuged at 2500 rpm for 10 min and the supernatant was collected. Capripoxvirus DNA extraction from the pathological samples was performed according the instructions provided by the DNA extraction Kits. For this purpose are used Qiagen AllPrep DNA/RNA extraction kit/DNAeasy Mini Kit and InvitrogenPureLink® Genomic DNA. The procedure used for the amplification and detection of Capripoxvirus (CaPV) was one step TaqMan Real-Time PCR assay, (TaqMan Universal PCR Master Mix; Applied Biosystems). Positive controls used consisted on DNA from known positive SPPV, GTPV and LSDV samples and nuclease-free water was used as negative PCR amplification control. Primers and Probes used for Capripox detection were: CaPV074F1: (Forward primer)- 20 µM AAAACGGTATATGGAATAGAGTTGGAA, CaPV074R1: (Reverse primer)- 20µM AAATGAAACCAATGGATGGGATA,CaPV074P1: (Probe)- 10 µM FAM-TGGCTCATAGATTTCCT-MGB/NFQ. Real time PCR amplification and detection was performed using real time PCR machine (Applied Biosystems 7500 Real Time PCR System). All the methods used are according Bowden et al.2008 [6].

3. Results and Discussion

Lumpy skin disease is a serious disease of cattle caused by a strain of Capripoxvirus. The genome of LSDV is 151 kbp long with a central coding region bounded by identical 2.4 kbp-inverted terminal repeats and coded for 156 putative genes [7]. This virus is one of the largest viruses (170 - 260 nm by 300 - 450 nm). Lumpy skin disease was first recorded in Zambia in 1929, and then spread throughout southern Africa and north to Sudan. It was first diagnosed outside Africa in Israel in 1989 and in subsequent years and during 2015 was spread in different European countries. The disease is characterized by fever and nodular lesions on the skin, mucous membranes, and internal organs and reduction in milk production [8, 9]. It is important to differentiate the disease from the the skin lesions of pseudo LSD (bovine herpesvirus-2, BHV-2), insect bites, Demodex infection, onchocerciasis, besnoitiosis and dermatophilosis in some cases, diseases causing mucosal lesions, can be confused with LSD, such as bovine viral diarrhoea/mucosal disease and bovine malignant catarrhal fever [10]. The transmission of LSDV between animals by contact is inefficient and the inoculation of the virus by intravenously feeding arthropods is required to establish the infection. Lumpy skin disease is listed in Office International des Epizooties in List A which identifies diseases with the potential for rapid and severe economic losses. There is a real threat of capripoxviruses spreading into new geographic regions specifically LSDV into the Europe countries. Spread can occur through trade of infected animals as well as through the insect vectors. Outbreaks of LSD were reported to the OIE in Turkey and Iraq in late 2013 and in Iran in early 2014 [11, 12]. In August 2015, LSD outbreaks were notified in EU from eastern Greece and further spread over the country. In the following season, in spring and summer 2016, LSD spread further over the Balkans to Bulgaria, Macedonia, Kosovo, Serbia, Montenegro, Albania [13]. During 2016 the disease is confirmed in Albania and a rapid spread throughout the country followed. First outbreak in Albania was registered in the villages of Vlashaj, Shupenzë, Dibra country in July 2016. A rapid spread throughout the country followed. In Albania compared with other infected countries of the region was observed the high variation of the intraherd morbidity. This is most likely linked to the small size of the affected farms in the region; the majority of farms in Albania 75% have less than five animals this increase the chance that the whole farm is affected. This paper describes the situation of the LSD outbreak in Albania and the confirmation of the disease in dead animals or with clinical signs by the RT- PCR method. A total of 3419 samples are collected in Albania during the outbreak of 2016 -2017, the samples are submitted in Animal Health Department of FSV-Institute from the field veterinarians, they have collected samples from suspected cows or with clear clinical signs of the disease over the country.

Figure 1. Lumpy skin disease dynamics of outbreaks in the Balkan region

From the largest to the smallest number of suspected samples are collected: in Dibra 617, Elbasan 458, Tirana 357, Shkodra 293, Fieri 231, Kuksi 134, Berat 104, Durresi 77, Korca 62, Lezha 47, Vlora 21 and in Gjirokastra 7 samples. The largest number of confirmed LSD cases during the outbreak in Albania was registered in Dibra, Elbasan and Tirana.

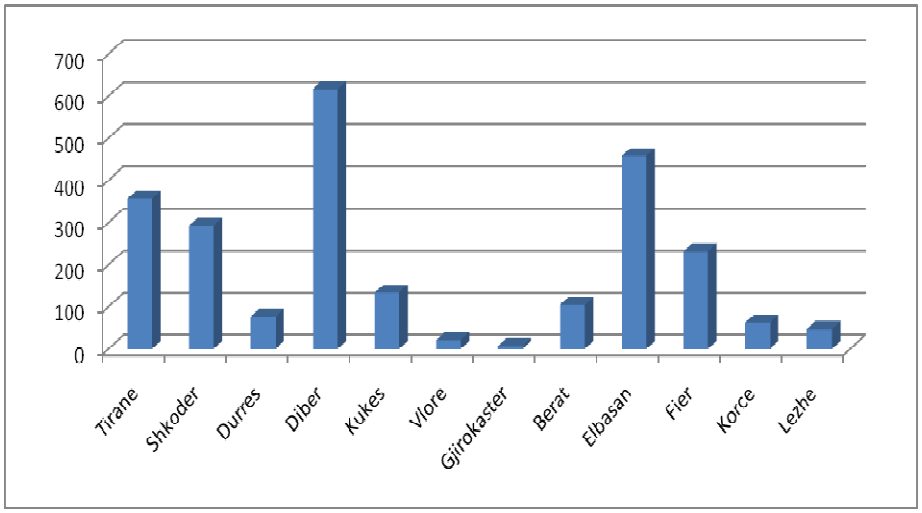

Figure 2. Graphical presentation of district coverage and number of suspected animals.

Rapid diagnostic confirmation of the tentative field diagnosis is fundamental for the successful control and eradication of LSD in endemic and particularly in non-endemic countries. Different molecular tests are the preferred diagnostic tools and are currently replacing other less sensitive and slower diagnostic methods. The real-time PCR method for CaPV displays greater sensitivity. For the diagnosis of the samples submitted in FSVI it was used the PCR assay for Capripox detection. The method used was adapted from *Bowden et al., 2008* for detection of all CaPV including Lumpy skin disease virus [14]. The assay uses a dual labelled fluorogenic (TaqMan®) probe and primers specific to capripoxvirus. For the observation and interpretation of the PCR results few points should be considered: The amplification of SPPV, GTPV and LSDV, no amplification peak must be observed in the negative controls, the presence of sigmoidal curve peak with a Cq value <35 indicates a positive amplification results, Cq values >35 are considered as inconclusive. This assay is rapid, sensitive and specific. It is critical to perform PCR-based assay to enable rapid capripoxvirus diagnostics and surveillance. These tools will increase the capacity to respond to outbreaks, monitor capripoxviruses in endemic regions and study the epidemiology of the diseases.

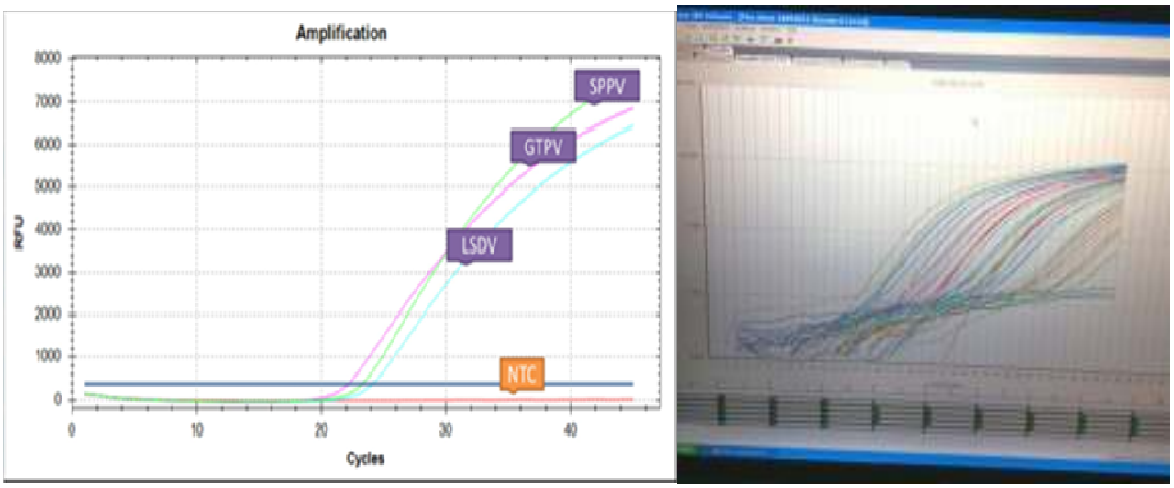

Figure 3. Amplification of positive controls and nonamplification of NTC, important criteria for samples amplification evaluation.

From 3419 samples analyzed 2053 samples resulted positive. From 2053 positive cases confirmed by RT-PCR methods, the most outbreaks occurred in spring and summer. The warm temperatures and related vector abundance are among the main risk factors for LSD spread. From all the samples analyzed the nodular skin lesions resulted with higher concentrations of the virus.

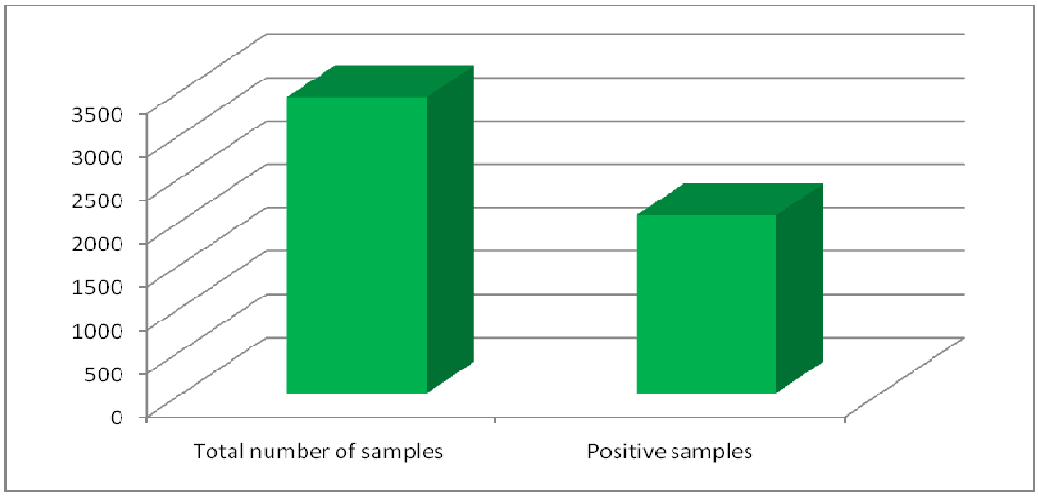

Figure 4. Graphical presentation of total samples/positive samples.

For the diagnosis of LSD in Laboratory is important to know the period of the virus shed in different samples, LSDV can be detected in animal ocular secretions, nasal discharge up to 15 days post infection. If protected from sunlight, the virus can survive in environment for up to 57 months. Oral swabs, conjunctival swabs, nasal swabs, skin lesion were evaluated as clinical samples for early SPPV detection. According the literature oral and conjunctival swabs are not considered good candidates for early detection of SPPV since positive results were not obtained until after the onset of clinical signs. Nasal swabs were found to be positive by real-time PCR starting on day two post infection with all samples positive by day four. The real-time assay was able to detect viral DNA in nasal swabs one to five days prior to the onset of clinical disease as defined by fever and the presence of skin lesions, and up to two days before positive results were seen. Virus was detected in nasal secretions of infected animals until the day of death. Sometimes the presence of inhibitors in blood, or in the samples container or the relatively low viremia associated with SPPV infections affect the analyze result. This can be solved after performing 10 fold dilutions of the samples. The confirmation of positive cases is important in the disease control. Mass vaccination campaigns with live homologous vaccines against LSD were carried out at regional

level. Vaccination of cattle with homologous strain vaccine started on 26 July 2016 and by 22 September around 250,000 animals were vaccinated. Albania was carried out alongside LSD spread (reactive vaccination). The vaccination was conducted first in close proximity to the affected herds, where the actual risk of getting affected was higher. The protection provided by the vaccine was significant. According to EFSA the vaccination effectiveness in Albania was estimated to be around 70% at farm level and 77% at animal level. Adverse effects to live homologous vaccine, including fever, decreased milk production and oedema at injection site mostly within 2 weeks after vaccination are reported in 0.09% of the vaccinated animals [13].

4. Conclusions

LSD is a disease of domestic cattle caused by viruses of the genus Capripoxvirus., characterized by significant losses, reduced milk production and weight, infertility, abortion and death. CaPVs are not considered to be zoonotic agents. Economically, LSD is a significant cattle disease and high mortality rates of 40 percent or more have been encountered. LSDV can be detected in animal secretions (e.g. ocular, nasal discharge) up to at least 15 days post infection but skin biopsies are the best samples to submit in the laboratory for disease confirmation as they contain much more viral particles. Real-time PCR method is the best choice for rapid detection and identification of the LSD outbreak causative agent. The *Bowden et.al 2008* protocol was used during the outbreak of LSD in Albania for the confirmation of the disease in affected cows. From 3419 samples analyzed, 2053 samples resulted positive in the district where the disease was present. Dibra was the district with the largest number of LSD outbreak. The most positive cases occurred in spring and summer. From all the samples analyzed the nodular skin lesions resulted with higher concentrations of the virus. The intraherd morbidity of the disease in Albania was very high, most likely linked to the small size of the affected farms in the region, the majority of farms in Albania. The LSD situation in Albania was a good case study to verify the protective effect of vaccination. The LSD control strategy should include restriction of animal movement, quarantine, import ban of live bovines and their products from zones or countries with reported outbreaks, the improvement of monitoring and clinical surveillance activities in border regions, cleaning and disinfection in outbreak areas, vector control and vaccination in outbreak area (older than 3 months and under 9 months pregnant).

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