

An investigation of the seroprevalence of Crimean-Congo Hemorrhagic Fever and Lumpy Skin Disease in domesticated water buffaloes in northern Turkey

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Abstract. This study was conducted in Samsun Province of Turkey to investigate the serological status of domesticated water buffaloes for both Crimean-Congo Hemorrhagic Fever (CCHF) and Lumpy Skin Disease (LSD). Serum was collected from a total of 272 water buffaloes from different age groups and both genders; of the total, 48.1% had been vaccinated against LSD with heterologous sheep-goat pox vaccine. The serum samples were individually assessed by using a commercial ID screen enzyme-linked immune-sorbent assay (ELISA) to detect neutralizing antibodies against both CCHF virus and LSD virus. All 272 buffaloes were negative for antibodies against the CCHF virus. All the unvaccinated buffaloes (141) were seronegative for LSD virus but of the 131 vaccinated buffaloes, 10 (7.6%) were seropositive for the LSD virus. In addition, 8.6% of vaccinated animals age >1 year old were seropositive for LSD, whereas the seropositivity was 5.1% for the animals age ≤ 1 year old. There was no significant difference for seropositivity between male and female animals in the >1 year old or ≤ 1 year old age groups. When seroprevalances for LSD in the tested water buffaloes are evaluated by gender, there was a significant difference between females (8.6%) and males (0%) in the >1 year old water buffaloes ($X^2=20.24$; $P<0.001$). Separately, the results of this study indicate that Bafra district water buffaloes are not infected by CCHFV and LSDV and some of the buffaloes that vaccinated with LSDV did not develop sufficient antibodies to protect them after they were vaccinated for the LSD virus. Furthermore, the authors of this study conclude that both the commercially produced vaccine that is currently administered and the vaccination strategy have to be urgently evaluated by the veterinary authorities in Turkey. This is essential in order to combat the spread of LSD virus infection with an effective vaccine and a comprehensive management strategy across Turkey.

INTRODUCTION

Infectious diseases are still the most noteworthy agents of morbidity and mortality worldwide and are therefore of considerable interest and concern to both the public and healthcare communities (Mardani and Jahromi, 2007). Crimean-Congo hemorrhagic fever (CCHF) and lumpy skin disease (LSD) are transcontinental viral

diseases that are still endemic in Africa, and in some parts of the Middle East and Europe, and have increasing potential for global spread (Mardani and Jahromi, 2007; Babiuk *et al.*, 2008). Wild ruminants, such as buffaloes, are thought to play a role in the epidemiology of these diseases and other viral hemorrhagic fevers (VHFs) (Davies, 1982).

CCHF is a major, zoonotic, tick-borne VHF that has been reported from more than 30 countries in Africa, Asia, South-East Europe and the Middle East. In Europe, Bulgaria is the only country where CCHF is endemic but outbreaks with higher numbers of cases have been recorded in other countries that include Albania, Kosovo, the south-west of the Russian Federation, Turkey and The Ukraine (Maltezou *et al.*, 2010). The disease is caused by a single-stranded RNA virus belonging to the *Nairovirus* genus of the *Bunyaviridae* family. It is spreading internationally in parallel with the expansion in the distribution of its common tick vectors (*Hyalomma* spp.) and is therefore of increasing public health concern (Ergonul, 2006). CCHFV can infect a wide range of domestic and wild animals, including cattle, sheep, goats, hares, hedgehogs, mice (*Mastomys* spp.) and domestic dogs which do not show clinical signs but may act as reservoirs of infection for humans (Ergonul, 2013) who are infected by the bite of infected ticks. The seroprevalence of CCHF in mammals is estimated at about 13–36% (Gonzalez *et al.*, 1990, Morrill *et al.*, 1990). This situation implies a major threat to persons such as farmers, animal handlers, abattoir workers and veterinarians who are in close contact with animals due to its potential transmission to humans via the blood or tissue of an infected animal, a tick bite or the crushing of a tick on the skin or mucous membranes (The Center for Food Security and Public Health, 2007). Furthermore, human to human transmission has also been reported in various countries (Ergonul, 2006). In Turkey, a higher number of CCHF cases were revealed by serological and molecular evidence between 2002 and 2009 (Ozdarendeli *et al.*, 2012).

Lumpy skin disease (LSD) is an infectious viral disease of bovine species that is characterized by fever; mastitis; orchitis; swelling of the peripheral lymph nodes; multiple, firm, circumscribed skin nodules; and necrotic plaques in the mucous membranes (Coetzer, 2004) The causative agent is a double-stranded DNA virus belonging to the genus *Capripoxvirus* which is closely related to the goat pox and sheep

pox viruses in the *Chordopoxvirinae* subfamily of the *Poxviridae* family (Buller, *et al.*, 2005, OIE, 2014). This disease, which is endemic and non-zoonotic, causes substantial harm to the livestock industries of many countries (Barnard, 1997; Davies, 1991; Hamblin *et al.*, 1990). The LSD virus (LSDV) can be transmitted in saliva and milk and from infected skin lesions (Tuppurainen *et al.*, 2015). Direct transmission between animals is believed to be inefficient and mechanical transmission by blood-feeding arthropods has been suggested (Chihota *et al.*, 2001). LSD was originally confined to African and Middle Eastern countries but since 2000 it has spread to Europe and Asian countries and has increasingly affected animal production enterprises there (Tuppurainen *et al.*, 2015). In Turkey, the presence of LSD was confirmed for the first time in 2013 and its presence was reported to the World Organization for Animal Health (OIE) (Saraç *et al.*, 2014). LSD is considered an enzootic disease in Turkey because it has borders with other enzootic regions that allow the entrance of LSD infection (Sevik and Dogan, 2016). Enzyme-Linked Immunosorbent Assay (ELISA) is the most suitable serological, diagnostic test for screening large numbers of samples for the presence of LSDV antibodies (Tuppurainen *et al.*, 2005). Vaccination is the most efficient and realistic approach for the control of the disease in countries where it is endemic and/or in resource-poor countries (Tuppurainen *et al.*, 2014). Live, attenuated vaccines based on sheep pox virus have been used to fight LSD outbreaks; they include the Kenyan sheep and goat pox (KSGP “O-180” strain) vaccine, Romanian and Yugoslavian RM 65 sheep pox vaccines in Israel and other Middle-Eastern countries, Gorgan goat pox vaccine (GTP), LSDV (Neethling strain) vaccine in South Africa, and O-240 capripox vaccine. All of these are attenuated vaccines that have been used for the control of LSD in many parts of Africa but their failures have been reported in several countries (Tuppurainen *et al.*, 2014; Gari *et al.*, 2015).

The water buffalo (*Bubalus bubalis* Linnaeus, 1758) is a large, bovid, multi-purpose animal able to convert even low-

quality roughage into meat and milk. It originated in South Asia, South-east Asia and China; today, it is also found in Europe, Australia, North America, South America and some African countries (Moioli and Borghese, 2005).

The water buffaloes in Turkey originated from Mediterranean water buffaloes, a subgroup of river water buffaloes that are commonly known as Anatolian water buffaloes (Ermetin, 2017). Water buffalo husbandry in Turkey is undertaken in limited parts of Turkey that include the Marmara region, Central Anatolia and the Black Sea region (Ermetin, 2017). Water buffaloes may be susceptible to the development of infections and becoming reservoirs for them, which suggests that they might represent a public health risk to humans and other animals via the transmission of many viruses (Fagbo *et al.*, 2014).

The objectives of this study were to investigate the levels of Lumpy Skin Disease (LSD) and Crimean Congo Hemorrhagic Fever (CCHF) infection serologically in domesticated water buffaloes in the Bafra district of Samsun Province in the north of Turkey and to simultaneously investigate the efficacy of a commercial sheep-goat pox vaccine against LSD virus (LSDV).

MATERIALS AND METHODS

Ethical considerations

All livestock owners provided verbal consent for their water buffaloes to be included in the study. All procedures were conducted according to the ethical standards protocol

(No. 16/2018 date 31/05/2018) approved by the Scientific Research Assessment and Ethics Committee of the Samsun Veterinary Control Institute.

Collection of samples

Blood sampling was conducted during a routine vaccination of animals in the Bafra district of Samsun Province, which is located on the central coast of the Black Sea Region of Turkey, during the period January 2017 to March 2018 (Fig. 1).

Processing of samples

Blood samples were collected from 272 randomly selected water buffaloes (239 females and 33 males) (Fig. 1) on 10 separate farms. The demographic data concerning the sampled water buffaloes are provided in Table 1. Of those screened for both CCHFV and LSDV, 87.8% were female and 12.1% were male. Forty eight percent (131) of the 272 water buffaloes were vaccinated against LSDV with the commercial, heterologous sheep-goat pox vaccine (SPV-Bk: 107.5 TCID₅₀/dose) which is produced by the Pendik Veterinary Control Institute in Istanbul, Turkey, and the other 51.8% (141) were not vaccinated. Furthermore, 78.6% of the vaccinated animals were female (103) and the other 21.3% were male (28/131). The age distribution of the water buffaloes was determined from their birth records; 16.9% (46/272) of the animals were ≤ 1 year old and 83.09% (226/272) were >1 year old.

All blood samples were taken from the *vena jugularis*, stored in serum collection tubes and transported to the Virology Laboratory of the Faculty of Veterinary

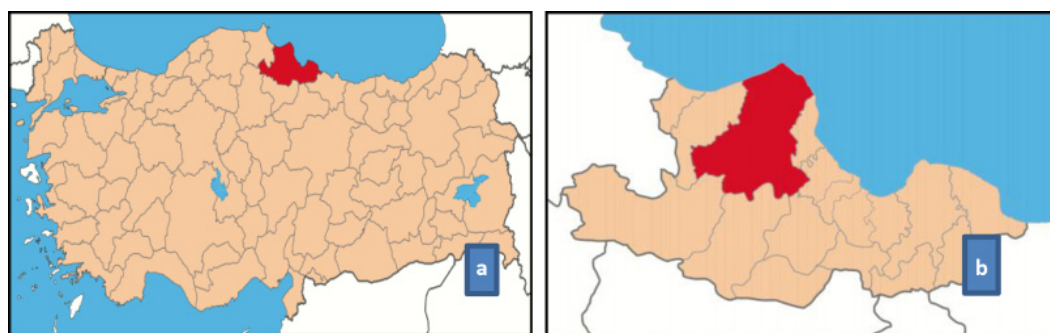


Figure 1. Sampling Region at Turkey (a) in Bafra district (b).

Table 1. Demographic characteristics of tested water buffaloes tested for Female and Male in the Bafra district of Turkey

Parameters	Gender		Total
	Female (%)	Male (%)	
No of sera samples	239 (87.8)	33 (12.1)	272 (100)
Vaccinated *	103 (78.6)	28 (21.3)	131 (48.1)
Unvaccinated	136 (96.4)	5 (3.5)	141 (51.8)
Age ≤ 1 year	22 (47.8)	24 (52.1)	46 (16.9)
Age > 1 year	217 (96.01)	9 (3.9)	226 (83.09)

* Vaccinated against LSDV.

Medicine at Ondokuz Mayıs University under cold chain. The blood samples were centrifuged at 2,500 rpm for 10 min and the sera was then transferred to sterile tubes (Eppendorf, Germany), inactivated at 56°C for 30 min and stored at -20°C until used.

Serological assays

This study determined both the efficacy of a commercially available sheep-pox vaccine that is used against Lumpy skin disease virus (LSDV) and whether there was an presence of antibodies to CCHF and LSD. The level of LSD seroprevalence was assessed on the basis of the antibody response produced in a commercial ID Screen Capripox Double Antigen Multi-species enzyme-linked immunosorbent assay (ELISA). Separately, the ID screen CCHFV Indirect ELISA kit was used to detect the presence of antibodies against CCHFV. Both tests were carried out in accordance with the manufacturers' instructions (IDEXX Laboratories; USA). Plates were read with an ELISA plate reader at 450 nm absorbance and the results were then calculated.

Statistical analysis

Data was analyzed with GENMOD (Generalized Linear Modelling). As the data was binomially distributed, the probit model was used in the analyses with log link functions used as per Lipsitz *et al.* (1994). In addition, to avoid a type 1 error, Pearson's chi square analysis was carried out with Yate's correction for continuity. All analyses and calculations were executed with the SAS package (2009).

RESULTS

A total of 272 buffaloes were tested for antibodies against CCHFV and LSDV. All samples were seronegative for CCHFV. No antibodies against LSDV were detected among unvaccinated water buffaloes. But, The overall seroprevalence of LSDV was 7.6% (Table 2) in vaccinated animals. Furthermore, there was a significant difference between the proportion of seropositive vaccinated females and males > 1 year old ($X^2=20.24$; $P<0.001$) (Table 2).

The Eight of 92 water buffaloes that had been vaccinated for LSDV age > 1 year old were found seropositive (8.6%) against to LSD and all of them were female. Also, ≤ 1 year old two (5.1%) water buffaloes (one (5.8%) female and one (4.5% male), that had been vaccinated for LSDV were seropositive against to LSD. Statistical analysis revealed no significant difference between the numbers of vaccinated water buffaloes aged >1 year and ≤ 1 year of age in terms of gender ($X^2=0.8334$; $P>0.05$). In addition, there was no significant difference between females and males ≤ 1 year old in terms of the proportion that was seropositive for LSDV ($X^2=0.035$; $P>0.05$) (Table 2).

DISCUSSION

There are many methods for the diagnosis of CCHF, including virus isolation, immunological assays like ELISA and molecular diagnostic methods such as reverse transcription-polymerase chain

Table 2. The distribution of LSDV seropositivity among water buffaloes in the Bafra district of Turkey with respect to age, gender and vaccine status

Age (year)	Sex	Vaccine status	
		n (%)	P (%)
> 1	F	86 (93.4)	8 (9.3)
	M	6 (6.5)	–
	Total	92	8 (8.6)
X ² value			20.24
P value			< 0.001
≤ 1	F	17 (43.5)	1 (5.8)
	M	22 (46.4)	1 (4.5)
	Total	39	2 (5.1)
X ² value			0.0352
P value			> 0.05
Grand Total		131	10 (7.6)
X ² value			0.8334
P value			> 0.05

* p: seropositive, * n: tested, * F: female, * M: male, * NA: not available.

reaction (RT-PCR) (Center for Food Security and Public Health, 2007; Mardani and Jahromi, 2007). Indirect immunofluorescence assay (IFA) and enzyme-linked immune-sorbent assay (ELISA) can detect both IgM and IgG antibodies after 7-9 days after infection (Whitehouse, 2004). The present study detected no antibodies to CCHFV in 282 water buffaloes in the Bafra district in northern Turkey. In contrast, seropositivity has been reported for CCHFV in domesticated cattle, but not including buffaloes, in neighbouring or nearby European countries like Greece (25%) (Papa *et al.*, 2014), Kosovo (18.4%) (Fajs *et al.*, 2014), Republic of Macedonia (14.6%) (Mertens *et al.*, 2015) and Albania (4.7%) (Lugaj and Bërzhhol, 2014).

In the Marmara region of Turkey, 33.1% of domesticated cattle were seropositive for CCHF (Tuncer *et al.*, 2014) and 78 of 105 goat and sheep serum samples were seropositive for CCHFV in the central Black Sea Region (Albayrak *et al.*, 2012). However, no data have been reported on the seroprevalence of CCHF in water buffaloes in Turkey. Therefore, this was the first on this subject although no seropositive animals were detected.

In this study we used ELISA to detect neutralizing antibodies for LSDV. That choice was made because the Serum Neutralization Test (SNT) is time-consuming and lacks the sensitivity to differentiate between antibodies for different capripox viruses but it is still a solid and reliable serological test (Babiuk *et al.*, 2009). Earlier research that used a cloned capripox virus structural protein (P32) antigen demonstrated that the ELISA test was more sensitive than the SNT for the detection of LSDV antibodies in bovine sera (Carn *et al.*, 1994). In previous similar LSDV prevalence studies, sera were collected from African buffalo between 1963 and 1996 (Barnard, 1997; Hamblin *et al.*, 1990), and the SNT was the main test used to detect neutralizing antibodies (Barnard, 1997).

In the present study, neutralizing antibodies for LSDV were detected in 10 of 131 (7.6%) of LSDV-vaccinated buffaloes but LSDV antibodies were not found in any unvaccinated animals. In the study by Barnard (1997), no antibodies to LSDV were detected in African buffaloes. In contrast, neutralizing antibodies were detected with the IFAT and SNT techniques in the sera of 150 of 254 buffaloes (59.1%) that had been collected during an epidemic and during

inter-epidemic periods in Kenya, Tanzania and Uganda (Davies, 1991). Another study, with a much larger and more diverse sample size than the present study, tested for the presence of LSDV in buffalo sera samples collected between 1963 and 1982 in 11 sub-Saharan African countries; all of the samples (1,413) were negative for LSDV neutralizing antibodies (Hedger and Hamblin, 1983).

The current study on water buffalo sera had a reasonable sample size and the Double Antigen-ELISA test detected a low percentage of positives (7.6%) in the vaccinated animals; this indicates that the protection level afforded by the heterologous sheep-goat pox vaccine (SPV-Bk: 107.5 TCID₅₀/dose) produced by the Pendik Veterinary Control Institute, Istanbul, Turkey appears to be not sufficient to produce proper immunogenicity against LSDV in water buffaloes. Many researchers have reported mixed results after comparing different types of LSD vaccines; Gari *et al.* (2015) stated that the Gorgan GTP vaccine, which is manufactured by Jordan Bio-Industries Centre (JOVAC), can effectively protect cattle against LSDV but the homologue Neethling strain of Onderstepoort Biological Products (OBP) and KSGP O-180 strain vaccines did not provide protection. In contrast, Ben-Gera *et al.* (2015) reported that in a field study the Neethling strain vaccine was significantly more effective than the x10RM65 attenuated sheep-pox vaccine in preventing LSD morbidity.

In this study, despite Bafra district water buffalo herds having been vaccinated with the heterologous sheep-goat pox vaccine (SPV-Bk: 107.5 TCID₅₀/dose vaccine) against LSD there was a low level of antibodies to LSDV, which suggests that this vaccine may have been administered in a dose or concentration that was not capable of producing sufficient immunization or its cross immunization capability is lacking in that it doesn't stimulate the production of sufficient protecting antibodies against LSDV in especially water buffaloes. Thus, it is difficult to determine the actual effectiveness of the SPV-Bk: 107.5 TCID₅₀/dose vaccine in the control of the disease in Turkey.

In summary, the present study revealed that the subject water buffaloes in the Bafra district of Turkey were free of CCHFV. This study also determined that the overall seroprevalence of the LSD virus in the LSD vaccinated water buffaloes aged >1 year was 8.6% and that there was a significant difference between females and males ($X^2=20.24$; $P<0.001$). This result was not extensively interpreted because male and female sample were not equal. So, more in-depth studies are needed to investigate the effects of age and gender on the success rate of vaccination against LSDV.

Also, the results of this study showed that some of the water buffaloes did not develop antibodies after being vaccinated for LSDV. Therefore, the authors of the present study emphasize that the currently used commercial vaccine and vaccination strategy need to be re-evaluated urgently by the veterinary authorities to combat both the non-suppression of LSDV infection and the growing threat of its spread. The disease was first reported in Turkey 5 years ago yet it is still severely threatening the health of large ruminants and hence the economic status of their owners.

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