



Comprehensive study of *Capripoxviruses* in Moroccan small ruminants: A RT-PCR analysis

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ABSTRACT

This study aimed to investigate and identify circulating *Capripoxviruses* in different regions of Morocco, using RT-PCR technique on various sample types. The results from the analysis of 980 samples lend considerable perceptions highlighting the diversity in outcomes across the tested samples. The analysis of Ct values revealed distinct patterns dependent on the nature of the sampled materials. Notably, scab samples exhibited lower Ct values indicating a higher concentration of viral load. Swab samples, in contrast, presented average Ct values signifying a moderate presence of viral DNA. Blood samples demonstrated Ct values within the range suggesting a lower viral load in the bloodstream. Furthermore, the study also revealed endemic *Capripoxvirus* outbreaks in Morocco, particularly in regions with a strong sheep breeding industry.

Keywords: *Capripoxvirus*, Goatpox virus, Morocco, Real time PCR, Sheepox virus

Sheepox virus (SPPV) belongs to the genus *Capripoxvirus* (CaPv), subfamily *Chordopoxvirinae*, and family *Poxviridae* along with Goatpox virus (GTPV) and Lumpy skin disease virus (LSDV) (Kumar *et al.* 2022). It causes a highly contagious disease affecting various livestock such as sheep, goats, and cattle (Hamdi *et al.* 2021). They are classified as causative agents of notable, transboundary viral diseases affecting small ruminants, posing a significant threat to the productivity and efficiency of sheep and goat farming in regions where they are endemic. Along the southern Mediterranean shore, where the farming system is often pastoral, the disease is prevalent in Algeria, Tunisia, Morocco, and Egypt.

This disease is manifested by fever, nodules on the body, reduced milk production, infertility and abortion and occasionally resulting in 100% mortality (WOAH 2019, Gupta *et al.* 2020, Mishra and Roy 2020). These diseases are notifiable, due to their significant impact on the economy of livestock farming and the risk of rapid cross-border spread (WOAH 2019). The economic consequences include reduced milk yields, deterioration of skin quality, and other setbacks in production. Furthermore, the presence

of this disease greatly disrupts animal trade and product exports, leading to significant financial losses.

Between 2019 and 2021, LSDV spread rapidly in mainland Asia, invading China, India, Vietnam, Thailand, Malaysia and Mongolia, prompting a focus on prevention and control measures (Tuppurainen *et al.* 2017, Sudhakar *et al.* 2020, Hamdi *et al.* 2021). In addition, SPPV and GTPV are important contagious diseases endemic in India, North and Central Africa, Middle East, Central Asia, East Asia, and South-East Asia (Mishra *et al.* 2018, Hamdi *et al.* 2020).

SPPV, GTPV and LSDV are also being investigated by virus isolation and culture, electron microscopy, animal experimentation and laboratory detection technology (Zewdie 2019, Ochwo 2020, Zewdie *et al.* 2021). However, the presence of mixed infections requires a combination of molecular or serological methods to determine whether the pathogen isolated is CaPv (Jiang *et al.* 2022, Suwankitwat *et al.* 2022). Serological differentiation between SPPV and GTPV is not possible due to the similarity of their antigens (Kumar and Tripathi 2022). Currently, conventional PCR and SYBR Green PCR are the only molecular detection methods recommended by WOAH (Wen *et al.* 2023).

In Morocco, Sheepox has been recognized as an enzootic disease for many decades. The authorities responsible for managing the disease (ONSSA 2017) have constantly implemented control programs since its discovery. However, there is a lack of data on local

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and global virus challenges in Morocco or worldwide, despite significant economic losses (Hajjou *et al.* 2019). So, this study was carried out to identify circulating *Capripoxviruses* in different Moroccan regions using RT-PCR and comparing yields from various sample types.

MATERIALS AND METHODS

Study: Morocco is a country located in North Africa. It is characterized by diverse geographical features, including coastal areas along the Atlantic Ocean and the Mediterranean Sea, mountainous regions such as the Atlas Mountains, and expansive plains and deserts. The country shares borders with the Atlantic Ocean and the Mediterranean Sea to the west and north, respectively. The geographic coordinates of are approximately between 32.4279° N latitude and 9.1218° W longitude.

The study commenced in early January 2019 and concluded in December 2023, and a diverse array of samples (n=980), including scabs and skin lesions (14.38%), EDTA-blood (27.04%), nasal (24.90%), lachrymal (6.12%), oral (20.40%), nasopharyngeal (4.08%) and oropharyngeal (3.06%) swabs, were collected in a sterile manner from various regions of Morocco. The samples were obtained from sheep exhibiting typical symptoms of *Capripox* disease and displaying corresponding clinical signs. Furthermore, supplementary samples were obtained at various locations during routine disease surveillance. No consideration was given to the subjects' race, age, or gender.

The field sampling activities were structured by the veterinary services, under the auspices of the National Office of Food Safety (ONSSA), during the autumn-spring period, commonly referred to as the cold season. Upon receipt at the laboratory, the samples were stored at a temperature between 2 and 8°C until analysis in the shortest time (less than 24 h). For long-term storage, samples were being kept < -65°C. Samples had the option to either be combined or processed separately during the laboratory analysis step. Pooling samples can improve efficacy, especially if they are predicted to produce similar results. However, processing samples individually allows for a more in-depth study of each specimen, which may be required for specific types of investigations or when sample variability is expected.

The sampling sites, known for their intensive sheep and goat farming, used in the study are as follows:

- **Fez-Meknes Region:** An important center of intensive livestock farming, benefitting from fertile plains and favourable climatic conditions.
- **Rabat-Sale-Kenitra Region:** Ideal for livestock due to its temperate climate and proximity to the Atlantic Ocean.
- **Marrakech-Safi Region:** Characterized by semi-arid conditions and vast plains, making it conducive to sheep farming.
- **Souss-Massa Region:** Well adapted to sheep and goat farming, with a semi-arid climate and good

infrastructure.

- **Casablanca-Settat Region:** Supports intensive sheep farming with its fertile soils and Mediterranean climate.

DNA extraction: The steps for extracting DNA from various types of samples, following suspension in PBS and clarification, were identical. The skin nodule samples were homogenized with 1 mL of phosphate buffered saline (PBS, pH 7.4). This reagent is primarily used to rinse cells when any trace media must be removed before processing. All procedures and reagent manipulations were performed in the Microbiological Safety Station. DNA was extracted directly from sample homogenate, according to the manufacturer's instructions, using the NucleoSpin Virus Mini kit for viral RNA/DNA purification Kit, MACHEREY-NAGEL® (Düren, Germany). In brief, 150 µl of the sample suspension was incubated with 600 µl of RAV1 lysis buffer and carrier DNA at 70°C for 5 min. After this, 600 µl of absolute ethanol was added to the lysate, and the entire volume was transferred to the silica column for centrifugation. The sample was then washed and centrifuged accordingly. At last, the DNA was eluted in 50 µl of nuclease-free water provided in the kit and stored at -80°C.

RT-PCR amplification: The extracted DNAs were analysed by real-time polymerase chain reaction (PCR) to identify the *Capripoxvirus* genome following the protocol described by Balinsky *et al.* 2008. Reagents from Luna® Universal qPCR Master Mix were used to prepare the reaction mixture as specified in the kit insert. The final test mixture contained 3 µl of DNA with 17 µl of the buffer-containing reaction mixture (Table 1). Cycling conditions consisted of *Taq* activation at 95°C for 1 min, followed by 45 amplification cycles (at 95°C for 10 s and 60°C for 60 s). Positive and negative controls were included within each set of reaction mixtures.

Table 1. Volumes of the Master Mix

Luna Universal Probe Master Mix	Volume per 1 reaction
H ₂ O-RNase Free	5 µL
PCR Buffer 2×	10 µL
FW primer (10 µM)	0.4 µL
5'-GGCGATGTCCATTCCCTG-3'	
REV primer (10 µM)	0.4 µL
5'-AGCATTTTCATTTC CGTGAGGA-3'	
Probe (10 µM)	0.2 µL
FAM -CAATGGGTAAAAGATTCTA-NFQ	
Enzyme mixture 20×	1 µL
Volume of Master Mix	17 µL
Volume of DNA	3 µL

RESULTS AND DISCUSSION

The identification of clinical symptoms plays a crucial role in reporting Sheeppox outbreaks in Morocco. The virus has been circulating in various regions of the kingdom for several years with most part impacting sheep, with no cases reported in goats (Zro *et al.* 2014). Usual

symptoms include fever, enlargement of the lymph nodes, anorexia, depression, sharp decline in milk production, abortion, sterility and development of skin lesions that follow distinct stages, such as maculae, papules, vesicles or vesicular-pustules and ultimately dried scabs (Kumar *et al.* 2021). The classical vesicular form is frequently observed in the clinical manifestation of the Sheeppox virus (SPPV) (Zro *et al.* 2014).

The traditional diagnosis of *Capripox* disease relies on clinical signs, virus isolation, serology, and electron microscopy. Among molecular techniques that have been well-established for the diagnosis of infectious diseases, PCR has been successfully used for the diagnosis of *Capripox* disease and considered one of the best alternatives of conventional assays because of its sensitivity, specificity and reproducibility.

This work was provided to study and identify the circulating *Capripoxvirus* strains in different regions of Morocco by analysing various sample types using real time PCR and comparing their respective yields. The results obtained from RT-PCR test amid the 980 samples analysed revealed significant data (Table 2). Out of these, 671 samples tested positive for the genome *Capripox* virus, demonstrating a notable occurrence of the specified characteristic. Meanwhile, 309 samples brought back had negative results, highlighting the diversity in the outcomes of the testing process. Positive samples were designated as those with cycle threshold (Ct) values below 35, while samples with Ct values above 35 were classified as negative. This comprehensive analysis contributes valuable insights into the presence and absence of the *Capripox* virus genome in different types of samples tested, collected from six major regions in Morocco.

Table 2. Distribution of positive and negative results during 2019 to 2023

Year	Positives samples (in %)	Negatives samples (in %)
2019	50.90	49.09
2020	61.11	38.90
2021	72.41	27.59
2022	74.92	25.08
2023	64.39	35.60

The Cycle Threshold (Ct) values indicate a perceptible variation dependent upon the specific nature of the sampled materials. In particular, scab samples showed significantly lower Ct values within the range of 11.3 to 30.97, suggesting a relatively higher concentration of viral load in these samples. In contrast, swab samples yielded average Ct values between 19 to 34.5, indicating a moderate presence of the viral DNA. On the other hand, Ct values of blood samples fell within the range of 29 to 35, standing for a lower viral load.

The difference between Ct values throughout sample species may be assigned to integral variances in viral load. The real time PCR technique, that is usually recruited to define Ct values, count on the amplification of viral

DNA. For this reason, a lower Ct value is revelatory of a higher initial concentration of viral genetic material in the sample. This fact is especially appropriate in the context of *Capripoxviruses*, designated their propensity to cause skin infections in infected animals. The scabs, formed as a result of the infection, serve as reservoirs of viral genetic material.

This study looked at the distribution of *Capripoxvirus* outbreaks in Morocco from 2019 to 2023, producing intriguing results. It was found that *Capripoxvirus* was endemic in large sections of the country during this period. The sampling strategy consistently targeted both central and eastern regions of the kingdom, which are known for their robust sheep farming industry and extensive husbandry procedures. In contrast, the southern and north-western regions displayed distinct patterns, with lower sheep and animal densities indicating a less severe impact from the virus. This sampling approach aimed to capture the diverse dynamics of sheep breeding across the country, ensuring a comprehensive understanding of the variations in intensity and prevalence within different geographical zones. As well as the research conducted by Lafar *et al.* (2019) found that Sheeppox was widespread in Morocco, with varied incidence rates recorded across provinces. The disease was particularly prevalent in locations with intense sheep farming. Their analysis also highlighted a significant association between the endemicity of Sheeppox and rural animal markets, suggesting a significant correlation. Furthermore, RT-PCR analysis confirmed the presence of the virus in skin scabs, lung nodules, and other samples.

In conclusion, *Capripoxvirus* causes infectious and endemic disease in many parts of the world. In Morocco, it's important to highlight that despite the various control measures undertaken by the veterinary services over the years, the disease persists in an enzootic form due to the regular and significant number of sheep pox cases recorded annually. The findings of the study provide crucial insights for disease management and surveillance in the livestock sector.

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