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The first isolation and molecular characterization of camelpox virus in Ethiopia

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ABSTRACT

A cross-sectional study was conducted from November 2011 to April 2012 in Chifra district of Afar and in Jigjiga Zone of Somali regional states of Ethiopia with the aims of assessing the epidemiology of camelpox and isolate and molecularly characterize the virus. The study includes questionnaire, active disease search and virus isolation and sequencing. A total of 24 (4.50%) and 12 (3.0%) camels in Afar and Jigjiga respectively were found clinically sick of camelpox during the study period. The questionnaire survey indicated that camelpox is the most common disease in the areas in which 125 (96%) of the respondents reported the frequent occurrence of camelpox in their herds especially during rainy season. The PCR result revealed 12 out of 17 tested samples were positive, of which seven of them collected from Jigjiga zone showed the characteristic PCR positive bands of 881 bp size fragments while five of the Afar samples gave two faint bands. Ethiopian isolates, specially isolated from Somali have very high identity with comparable sequences of CMLV M-96 from Kazakhstan and CMLV CMS from Iran. Out of the total of 780 bp analogous sequences, Ethiopian isolates differ only in two positions, while CMLV-Teheran differed at four nucleotide positions. This is the first study that report isolation and molecularly characterized camelpox virus, which could help for early diagnosis and control of the disease in the country.

1. Introduction

Camelpox is one of the most important infectious and contagious diseases of camels in almost every region where the camel is reared with the exception of Australia. The disease is caused by the camelpox virus (CMLV), which belongs to the Orthopoxvirus (OPV) genus of the *Poxviridae* family. It is closely related to the Variola virus, the etiological agent for smallpox (Duraffour et al., 2011; Gubser and Smith, 2002; OIE, 2008). Camelpox has a considerable economic importance due to high morbidity, relatively high mortality in younger animals, loss of condition and reduced milk production in lactating ones (Azwai et al., 1996).

Transmission of camelpox occurs by direct contacts with sick animals through skin abrasions or via aerosols (Khalafalla and Mohamed, 1996; Wernery and Kaaden, 2002). Scab materials, saliva and secretions of affected camels may shed virus to the environment, such as water which becomes then the source of infection (Khalafalla, 2007). Various studies have demonstrated that the incidence of camelpox outbreaks increases during rainy seasons with the appearance of more severe forms of the disease (Khalafalla, 2007; Wernery et al., 1997a). This may be due to the fact that moisture may enhance virus stability in the environment and increase subsequent transmission to susceptible animal. It could be also associated with the involvement of arthropods which are abundant during rainy seasons which may serve as a mechanical vector of the virus. The latter idea is evidenced by the isolation of CMLV from *Hyalomma dromedarii* ticks (Wernery et al., 1997a, 2000; Wernery and Zachariah, 1999).

The clinical manifestations range from unapparent infection to mild, moderate, and less commonly severe systemic infection and death (Abu Elzein et al., 1999). Young calves and pregnant females are more susceptible (Al-Zi'abi et al., 2007; Wernery and Kaaden, 2002). The disease is characterized by fever, enlarged lymph nodes and skin lesions. Skin lesions appear 1–3 days after the onset of fever, starting as erythematous macules, developing into papules and vesicles and later turning into pustules. These lesions first appear on the head, eyelids, nostrils and the margins of the ears. Later, skin lesions may extend to the neck, limbs, genitalia, mammary glands and perineum. In the generalized form, pox lesions may cover the entire body. Skin lesions may take up to

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4–6 weeks to heal. In the systemic form, pox lesions are found associated with the mucous membranes of the mouth, respiratory and digestive tracts (Wernery and Kaaden, 2002). Tentative diagnosis of camelpox can be made based on clinical signs and pox lesion, but will confuse with other viral diseases, such as contagious ecthyma (parapoxvirus) and papillomatosis (papillomavirus), therefore differential diagnosis is needed (Wernery and Kaaden, 2002). Five complementary techniques might be advised for camelpox diagnosis: transmission electron microscopy (TEM), cell culture isolation, standard PCR assays, immunohistochemistry and demonstration of neutralizing antibodies (Balamurugan et al., 2009; Duraffour et al., 2011; OIE, 2008; Wernery et al., 1997a).

Camelpox can be controlled or prevented by vaccination. Currently there are two types of vaccines, live attenuated and inactivated camelpox vaccines. A live attenuated vaccine gives long-term protection against camelpox. However, a booster vaccination is recommended for young animals vaccinated before the age of 6–9 months. When inactivated vaccine is used, the animals must be vaccinated annually (OIE, 2008; Wernery, 2000; Wernery and Zachariah, 1999).

Camelpox outbreaks have been reported in the Middle East (Bahrain, Iran, Iraq, Oman, Saudi Arabia, United Arab Emirates and Yemen), in Asia (Afghanistan and Pakistan), in Africa (Algeria, Egypt, Ethiopia, Kenya, Mauritania, Morocco, Niger, Somalia and Sudan) and in the southern parts of Russia and India (OIE, 2008; Wernery and Kaaden, 2002; Wernery et al., 1997b). Even though the occurrence of camelpox in Ethiopia is frequently reported by field veterinarians and camel herders; the epidemiology of the disease is not well studied. The only study in Ethiopia was reported from the Borena area (Megerssa, 2010). His study was mainly based on observation of cases and the study reported the occurrence of seasonal variations; 14.2%, 0.3% and 0% prevalence during minor wet, major wet and dry seasons respectively.

Despite the fact that Camelpox has been recognized clinically and known for its economic importance, to our knowledge no successful attempts have been made to isolate the virus in Ethiopia. Therefore, this study was designed to isolate and conduct molecular characterization on locally circulating Camelpox virus in Ethiopia.

2. Materials and methods

2.1. Study area

The study was conducted from November 2011 to April 2012 in Chifra district of Afar and in four districts (Jigjiga, Fafaan, Hordha and Golajoo) Jigjiga Zone of Somali regional states of Ethiopia. The two regions are known for their large camel population in the country. The population of Afar and Somali Regional States are predominantly pastoralist whose livelihoods largely depend on livestock in the semi-arid and arid environment.

Chifra district of Afar lies in the Northern parts of the Rift Valley at about 450 km East of Addis Ababa. The topography of the area varies from hilly escarpment on the western and southern edges with an altitude of 1,000–1,500 m above sea level. The Jigjiga Zone is located in Somali regional state in Eastern part of Ethiopia about 630 km from Addis Ababa. The altitude of the zone ranges from 500 to 1650 m above sea level. The climate of Jigjiga is semi-arid type which is characterized by high temperature (MOARD-PADS, 2004).

2.2. Study design, study population and sampling strategy

The study populations were one humped camels herd. The sampling procedure followed was purposive sampling because strictly random sampling procedure might not be possible due to mobile, scattered and less accessible nature of pastoral communities. The starting point for sampling was that 10% of the animals would be sick from camel pox (Megerssa, 2010) and we hypothesized that 80% of sick animals would give us an isolate. Further, we wanted to compare between Afar and Somali areas, assuming 10% in one and a lower level at 5% in the other. This would lead to a sample size of 474 from each area (Sergeant, 2012). At the field a total 553 camels from Afar and 400 camels from Jigjiga were examined during study period. Selection of villages from the study districts were performed based on their proximity to the towns and access of transportation service. The household heads were selected based on camel possessions and all camels within the herd were clinically observed for the presence of any camelpox lesion.

2.3. Study methods

2.3.1. Questionnaire survey

Totally 130 respondents (eighty in Chifra and fifty in Jigjiga) were purposively selected and interviewed using a semi-structured questionnaire to assess the presence of camelpox in their herds. Data on age, sex, herd size, animal management, productivity, seasonality and other hypothesized risk factors were recorded.

2.3.2. Sample collection

Tissue samples of skin biopsies were collected from the total of 24 (twelve from each area) camels showing suspected camelpox lesions during the study period. About 2–3 g of tissue samples were collected and placed in a bottle with a 50% phosphate buffer saline (PBS) at a pH of 7.2–7.6 with antibiotics (gentamycin). Species, identification number, sex, age and village were labeled, and samples were immediately placed in a cold box and transported to National Veterinary Institute (NVI), Debre Zeit. Once the samples arrived at NVI, they were placed at −20 °C until processed.

2.3.3. Virus isolation

The biopsy samples were thawed at room temperature and washed three times using sterile PBS at a pH of 7.2 under laminar air flow hood. About 1 g of the samples was grounded using sterile mortar and pestle by adding 10 ml of sterile phosphate buffer saline (PBS) containing gentamycin. The tissue suspension was centrifuged at 1,500 rpm for 15 min. The supernatant was collected and about 1 ml was inoculated on Vero monolayer cells grown on 25 cm^2 tissue culture flasks and then flashed with growth media and incubated at 37 °C and 5% CO_2 in a humidified incubator. Cells were monitored for cytopathic effect (CPE) daily, and frozen when CPE was exhibited.

2.3.4. Polymerase chain reaction and sequencing

The confirmatory diagnosis of the cell culture positive samples were made by conventional PCR using primers that amplify the Acidophilic-type inclusion protein (ATIP) gene, which could enable to differentiate camelpox virus from other othopoxviruses (Meyer et al., 1997). DNA was extracted by using the DNeasy Tissue Kit (Qiagen, Germany) according to the manufacturer's instructions. DNA amplification was carried out in a final volume of 50 µl containing: 5 µl of 10 × PCR buffer, 1.5 µl of $MgCl_2$ (50 mM), 1 µl of dNTP (10 mM), 1 µl of forward primer, 1 µl of reverse primer, 5 µl of DNA template, 0.5 µl of *Taq* DNA polymerase and 35 µl of nuclease-free water. Amplification was performed using primer pair: 5′-AATACAAGGAGGATCT-3′ and 5′-CTTAACTTTTTCTTTCTC-3′ (OIE, 2008). The reaction conditions were: first denaturation for 5 min at 94 °C followed by second cycle: 1 min at 94 °C, 1 min at 45 °C, 2.5 min at 72 °C for 29 cycles and final extension at 72 °C for 10 min.

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