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ABSTRACT

Background: While camels (dromedaries) were traditionally believed to be resistant to most livestock diseases, research has demonstrated that they are susceptible to a large number of infectious agents. Based on the clinical appearance of typical lesions, Camel contagious ecthyma (CCE), caused by a Orf virus, is thought to be one of the most common viral diseases of camelids in Ethiopia. However, the epidemiology of the disease has not been formally described and the causative agent has never been molecularly confirmed.

Methods: a cross-sectional study was conducted from November 2013 to April 2014 in Arero district of Borena Zone, to assess morbidity and mortality rates of the of disease consistent with CCE, isolate and molecularly identify the causative agents and to find out the potential risk factors. Molecular technique, namely, PCR based on B2L gene-specific primers of ORFV was used for the confirmatory diagnosis of CCE virus from the skin lesions.

Results: Majority (86.8%) of the respondents indicated occurrence of CCE outbreaks in their herds in the past one year (a year preceding the start of the study). The overall morbidity and mortality rates attributed to CCE was 43.6% (95 % CI: 41.2%–46%) and 6.3% (95 % CI: 5.2 –7.6%) respectively. Confirmatory diagnosis of the suspected Orf virus isolates using conventional PCR techniques generated the expected amplification product of 1200bp for one of the samples. No product was amplified from the DNA samples of the negative control. This study showed that young camels (calves) had higher odds of becoming affected by CCE than adults [OR=3.44 (95 % CI: 2.29 –4.09); ($p<0.05$)]. The disease had marked seasonality with most of the cases occurring during rainy season. Acacia trees significantly contribute to virus dissemination through damaging the lips of browsing camels.

Conclusions: This study confirms the presence and importance of CCE in Ethiopia and establishes the basis for further research.

Key words: *B2L gene, Camelus dromedaries, CCE, Epidemiology, PCR, Ethiopia*

Background

The one-humped camel (*Camelus dromedarius*) is a key livestock species that is uniquely adapted to harsh environments [1]. Dromedaries provide a reliable source of livelihood especially for some of the most food insecure pastoral and nomadic communities. In addition to providing milk, meat and local transportation, they are the source of cash [1, 2, &3] for households.

East Africa is home to a large number of dromedaries [4]. Ethiopia possesses over 1 million dromedaries [5], mainly found in the Afar, Somali and Borana areas [6]. The Borana pastoralists, who traditionally depend on cattle husbandry for milk production, have increasingly turned their attention to camel milk in recent years. As a result there is an increased demand for camel production in the area [7], however regional disease knowledge is limited [8 & 9].

Camel contagious ecthyma (CCE), also known as Orf in camels is caused by a pox virus of the genus parapoxvirus (PPV) of the family Poxviridae [10]. CCE is clinically recognized by the appearance of papules, vesicles, pustules and rapidly growing scabs confined to the lips and muzzle of the affected animals [11-14]. Infected animals are weak, fail to thrive, and are more disposed to other bacterial infections [13& 14]. In addition to its economic impact (from morbidity and mortality), CCE has important zoonotic implications [15]. Field observations have indicated that CCE as one of the major viral infections causing economic losses and potential public health threat to camel herders in many parts of Ethiopia including Borena pastoral area. Molecular technique, namely, PCR based on B2L gene-specific primers of ORFV was extensively used for the confirmatory diagnosis of contagious ecthyma in

infected animals [16]. Therefore, the aim of this study was to quantify morbid and mortality caused by CCE, isolate and molecularly identify ORF virus and to find out the potential risk factors of CCE in Arero district, Ethiopia.

Methods

Description of the study area

The study was conducted in Arero district of Borana zone, Oromia regional state of Ethiopia. Arero district was selected on the basis of its camel population, and easy access from a major road. The district has an area of 10,890 Km². Its altitude ranges from 750- 1700 meters above sea level. The annual average temperature and rainfall is 19°C and 716 mm respectively. Animal husbandry in the area is characterized by extensive pastoral productions system with seasonal migration. Camels and cattle are the key livestock species in the area. As aridity is gradually increasing and drought is a recurrent phenomenon in the area, the principal stock is shifting from cattle to camels [17]. Our study was specifically carried out in three pastoral associations (Haro-Dimtu, Kaarra-Gumaata and Silala) where there are large numbers of camel populations.

Study Methods and sample size determination

The study employed cross-sectional study design (November 2013 to April 2014). A total of 129 volunteer camel owners were interviewed to explore data relevant to the study objectives such as herd size of the respondents, herding experiences, occurrence and seasonality of CCE outbreak in the past one year (a year preceding the start of the study), number of animals affected and died due to CCE and common plant browse that are potentially associated with CCE occurrences. Herders' ability to identify CCE infection from other diseases with similar

clinical signs and symptoms was cross-checked by enquiring about the clinical signs of the diseases. The questionnaire data were collected only after confirming that the respondent had described the disease correctly.

Sample collection and sampling procedures

The protocol for field studies and collection of animal samples was carried out in accordance with the ethics guideline of the College of Agriculture and Veterinary Medicine, Jimma University. Fourteen (14) skin scrapings were collected from camels showing suspected clinical signs of poxvirus infection for isolation and molecular identification. During sample collection from camels showing suggestive clinical signs, hair around the lesion was shaved; the area was disinfected with 70% alcohol and the scabs were aseptically collected using a scalpel blade. The collected samples were put in a labeled sterile universal tube containing phosphate buffered saline (PBS) at pH 7.2 and supplemented by antifungal and antibiotics to prevent further contamination. Samples with PBS were instantly transferred into a cold box and transported to the National Veterinary Institute (NVI, Ethiopia) for laboratory analysis.

Virus isolation on cell culture

Tissue homogenates (10 % w/v) were prepared using sterile PBS and centrifuged at high speed using a refrigerated centrifuge. Then, 2% Glasgow Minimum Essential Medium (GMEM), calf serum and PBS at 37°C were kept warm in water bath. The laminar flow hood was disinfected with alcohol and the UV light. The media was discarded from the cellular monolayer and 0.5ml of sample suspension was inoculated into each 25cm² tissue clutter flask. Inoculated flasks were then incubated at 37°C for one hour to adsorb on to the cell and the suspension was subsequently discarded for monolayer formation. After washing with PBS suspension, 2% GMEM media was added. Loosely closing the stopper, cells and virus

containing flask was incubated at 37°C. Samples were considered negative when no cytopathic effect (CPE) was observed following three blind passages [18].

Polymerase chain reaction (PCR)

Confirmatory diagnosis of the cell culture positive samples was attempted using conventional PCR techniques with primers that amplify the **B2L** gene. This gene helps to distinguish ORF virus from other orthopoxviruses [19]. DNA was extracted using the DNeasy Tissue Kit (Qiagen, Germany) based on the manufacturer's instructions. Amplification was carried out in a final volume of 50µl containing 5µl of 10 × PCR buffer, 1.5µl of MgCl₂ (50mM), 1µl of dNTP (10mM), 1µl of forward primer, 1µl of reverse primer, 5µl of DNA template, 0.5µl of Taq DNA polymerase and 35µl of nuclease-free water.

Amplification was performed by using primer pair 5'CGCAGACGTGGCTCAGTACGT-3' and 5'TGAGCTGGTTGCCGCTGTCCT-3'. The reaction conditions were first denaturation for five minutes at 94°C followed by 29 cycles of one minute at 94°C, followed by one minute at 45°C, and finally 2.5 minutes at 72°C. A final extension step at 72°C for 10 minutes completed the amplification. Gel electrophoresis was prepared and ethidium bromide (loading dye) was added after forming a comb. The sample was subjected to electrophoresis for 1 hour and 20 minutes, and subsequently observed under UV light.

Data collection and analysis

Data related to the location of the herds/specific PAs, seasonal occurrence of CCE, the age groups of camels diseased and died of CCE, and the types of camel browses potentially predisposing to CCE were collected through the questionnaires. Laboratory results abstained

from each tested sample were also recorded. The collected data were entered and managed in Microsoft excel sheet.

Data were analyzed using the Statistical Packages for Social Sciences (SPSS of version 17). Descriptive (proportion) and inferential (logistic regression model) statistics were used to analyze the data. Potential risk factors associated with occurrence of CCE were assessed using logistic regression model and odds ratio (OR) were used to determine the strength of association between the risk factors (independent variables) and disease (dependent variable). In all the analyses, confidence levels at 95 % and a $p < 0.05$ were used for statistical significance test.

Results

Results of the questionnaire survey

Herd profile in relation to camel CCE and herding years of the respondents was described in Table 1. Herd size was used to evaluate the relative contribution of camels to pastoralists in the study area, and herding year was used to estimate their practices in camel husbandry. Average herd size of the respondents was 14 camels (range one to 29) and average herding year was 37 (range one to 75). A total of 129 herds (1,679 camels) were investigated in the present study. Majority (86.8%) of the respondents indicated occurrence of CCE outbreaks in their herds in the past one year. The overall morbidity and mortality rates attributed to CCE was 43.6% (95 % CI: 41.2%–46%) and 6.3% (95 % CI: 5.2 –7.6%) respectively.

Age wise morbidity and mortality rate of the disease was presented in Table 2. The results, therefore, showed that young animals (below two years of age) had higher odds of becoming affected by CCE than adults (OR=3.44; 95%CI: 2.3–6.4) and the difference was statistically

significant ($p < 0.05$). The disease had marked seasonality with most of the cases occurring during rainy season. Acacia trees significantly contributed to virus dissemination in the study area through damaging the lips of browsing camels.

Result for virus isolation

From the total of 14 skin scrapings, 10 samples were found to be positive for CCE virus showing clear cytopathic effects. This was clearly evidenced by cell floating, rounding, aggregation and detachment (sloughing) of the cell on verocell line of African Green Monkey kidney cultures within eight days after inoculation (Figure 1).

PCR result

The sequence of the PPVB2L gene, a homologue of the major enveloped antigen, p37K of the vaccinia virus, has been used as a molecular target for virus recognition, diagnosis of infection and for identification of species within the genus *Parapoxvirus* [20]. In the present study, the extracted DNA was amplified and only 1 out of 10 tested samples were found positive with the primers (5'-CGCAGACGTGGCTCAGTACGT-3' and 5'-TGAGCTGGTTGCCGCTGTCCT-3') showing bands with 1200 bp (Figure 2).

Discussions

Contagious ecthyma infection in camels is generally neglected worldwide. In Ethiopia, in spite of the frequent outbreaks, there hasn't been much attempt to further investigate the disease in the areas where camels are important assets to the local community. This study is the first in its kind to address the epidemiological aspect of camel contagious ecthyma and isolate and molecularly identify the causative agent in Arero district of Borana zone, Oromia Regional state of Ethiopia, where camel husbandry is one of the major sustenance factors for the local community. Herders' knowledge in describing CCE and their ability of recognizing its effect indicate their expertise in distinguishing camel health problems in their vicinities. They considered clinical signs of CCE consistently with the descriptions of the disease signs in the standard veterinary text books [20; 21].

The overall morbidity and mortality rate rates of CCE in the present study were comparable with the reports from Southwest Iran [22] and Mongolia [23]; but lower when compared to the findings of Khalafalla of Sudan [24]. The variation could be related to the age structure of animals included in the studies, difference in the husbandry and health management system in different countries.

During our field clinical investigation, we noticed that majority of the animals showing clinical signs of suspected pox virus infection were camel calves. The morbidity and the mortality rates attributed to CCE are also higher in this age group than in adults. Our findings concur with the reports of different researchers [25, 26]. Severity of the disease in young

animals might be due to lack of prior exposure to infecting pathogens or due to absence of fully developed immune system.

The present study season was evaluated for potential risk factors for occurrence of CCD; rainy season being associated with the disease. This finding concurs with previous reports [25-28]. The increase in incidence of CCE in the rainy season could be associated with the abrasion of the lips due to browsing thorny Acacia trees locally named '*Ammarressa*' and '*Ogoraa (Saphansa)*') that is abundant in this time. It could also be related to the abundance of insects (in rainy season) which have been incriminated as a mechanical vector [29] and might increase the risk of transmission of ORF from infected animals to the healthy ones.

Infected Vero cells cultures developed characteristic pox-virus induced cytopathic effects (CPE) with floating of dead cells on top of the monolayer, plaque formation, cytoplasmic elongation and multinucleated giant cell formation. These findings were comparable with the report by Khalafalla and Mohamed [30], who reported similar cell presentations of CCE scab samples grown in Vero cells.

Confirmatory diagnosis of the suspected Orf virus isolates by conventional PCR techniques generated the expected amplification product of 1200bp in one sample. These findings are consistent with previous reports [31] of PCR amplification of the B2L gene in two outbreaks from India. In that study they demonstrated a PCR product of 1206 bp in size with an open reading frame of 1137 bp in length. It is unclear why only one of the 10 samples yielded a positive by PCR. Given that these samples were collected from animals with direct contact with each other and during an outbreak, the finding of one positive suggest that the other animals are likely infected with the same virus. Possible explanations for the low positive

rate in this study could include overloading of template DNA, the presence of PCR inhibitors, and poor primer binding due to variation in DNA sequence at the binding sites.

Conclusions

The present study confirmed the existence of CCE in Arero district of Borena zone. Morbidity and mortality attributed to CCE was higher among camel calves. The disease had a marked seasonal pattern of occurrence. Confirmatory diagnosis of the suspected Orf virus isolates by conventional PCR techniques generated the expected amplification product size of 1200bp. Hence, the information obtained would be worthwhile to improve the farmers' livelihood and also may open new research avenues for control and eradication of the disease at local and national level.

Abbreviations

BA: Bedane Adane; BDG: Benti Deressa Gelalcha; bp: Base pair; BAS: Birhanu Ayele Shigute; BD: Bareda Diba; CCE: Camel contagious ecthyma; CPE: Cytopathic effect; NVI: National Veterinary Institute; OR: Odds ratio; PAs: Peasant associations; PBS: Phosphate buffered saline; PCR: Polymerase chain reaction; PP: Paul J. Plummer; PPV: Parapoxvirus; SPSS: Statistical Packages for Social Sciences; UV: Ultra violet.

Declarations

Ethics approval and consent to participate

Ethical clearance was obtained from Jimma University, College of Agriculture and Veterinary Medicine. Written and oral consent was obtained from camel owners before questionnaire survey and sample collection.

Consent for publication

Not applicable

Availability of data and material

The dataset analyzed during the current study is available from the corresponding author on reasonable request.

Competing interest

The authors would like to declare that they have no any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work.

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Authors' contribution

BD conceived the research idea, collected the data and drafted the manuscript. BA supervised the sample collection and involved in data analysis. BAS involved in manuscript formatting, data analysis and final write up. BDG conceived the research idea, supervised field and laboratory works, analyzed the data and drafted and edited the manuscript. PP involved in manuscript writes up. All authors read and approved the final manuscript.

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Figures

A.

Normal vero cell (unaffected)

B.

Figure 1

African green monkey vero cells cultures before (A) and after infection with ORF virus (B). The figure demonstrates mono layer of African green monkey vero cells cultures after infection with ORF virus. The inoculated ORF virus produces cytopathic effect characterized by rounding of the cells, formation of hyperplastic foci and also small plaques

Figure 2

Molecular identification of camel contagious ecthyma virus. The figure shows gel electrophoretic separation of PCR products (read from left to right). Lane 1=100bp DNA ladder. Lane 2 is a negative control whereas all the rest are ORF virus suspected tissue samples. Lane 4 is positive for ORF virus around 1200bp; and no amplification is observed in all of the rest.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Tables2.pdf](#)
- [Questionnaire.pdf](#)