Epidemiology of camel contagious ecthyma and molecular detection of the pathogen in Arero district, Ethiopia

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Abstract

Even though camels (*Camelus dromedarius*) 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 *Parapoxvirus* (PPV), is thought to be one of the most common viral diseases of camels in Ethiopia. A cross-sectional study was conducted from November 2013 to April 2014 in the Arero district of Borena Zone, Oromia Regional State of Ethiopia to investigate the epidemiological aspect of CCE and molecularly identify the causative agent. A polymerase chain reaction (PCR) based on B2L gene-specific primers of PPV was used for the confirmatory diagnosis of the CCE virus from the skin lesion of camels showing suspected clinical signs of CCE infection. Eighty-seven percent (87.0%) of camel owners reported the occurrence of CCE outbreaks in their herds in the past year (a year preceding the start of the study). The overall morbidity and mortality rates attributed to CCE were 20% (95% CI: 11– 36%) and 6.3% (95 % CI: 5.2 –7.6%), respectively. Younger camels had higher odds of becoming affected by CCE than adults [OR=3.44 (95 % CI: 2.29 –4.09)] and the difference was statistically significant. Confirmatory diagnosis of the suspected cases using conventional PCR generated the expected amplification product size of 1200bp for one of the samples. Therefore, the study confirms the presence and importance of CCE in Ethiopia and establishes the basis for further investigation.

Keywords: *Camelus dromedaries; Camel; Contagious Ecthyma; Epidemiology; PCR; Ethiopia.*

Introduction

The one-humped camel *(Camelus dromedarius)* is a crucial livestock species uniquely adapted to harsh environments (Mirkena *et al.*, 2018). Dromedaries provide a reliable source of livelihood, especially for some of the most food-insecure pastoral communities. In addition to providing milk, meat, and local transportation to households that keep them, camels are the source of cash income through the sale of live camels and their products (Asiimwe *et al.,* 2020; Salamula *et al.*, 2017).

The world's camel population has been estimated at almost 23 million, and more than 95% of camels are found in developing countries (Faye, 2015). Ethiopia possesses over 7 million dromedaries (CSA, 2020). Major camel-keeping societies in Ethiopia include Afar, Somali, Oromo (Karayu, Gabra, Boran, and Guji groups), Kunama, and Irob peoples, among others. Camel in these areas is becoming a leading animal because of the multipurpose role it has in the provision of milk, meat, social and cultural importance in addition to unpaid transport service (Mirkena *et al*., 2018). Despite all the benefits associated with camel production in the pastoral areas of Ethiopia, camels still face several challenges in their natural environment, the most important of which are camel diseases (Seifu, 2009).

Camel contagious ecthyma (CCE), also known as Orf, is a contagious skin disease of camelids caused by a poxvirus of the genus *Parapoxvirus* (PPV), subfamily *Chordopoxvirinae* of the family *Poxviridae* (Khalafalla *et al*., 2020; Oryan *et al*., 2017; Khalafalla *et al*., 2015). The disease has a worldwide distribution (Khalafalla *et al*., 2015). 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 (Khalafalla *et al.*, 2020; Oryan *et al.*, 2017; Gelaye *et al.*, 2016b; Khalafalla *et al*., 2015). Infected animals are weak, fail to thrive, and are more susceptible to other bacterial infections (Zhu *et al*., 2019). The morbidity rate of CCE was reported as 100% while mortality reached up to 9% in young camels in Arabian Peninsula (Abubakr *et al*., 2007). Molecular technique, namely, PCR based on B2L gene-specific primers of PPV was exten-

sively used for the confirmatory diagnosis of contagious ecthyma in infected animals (Khalafalla *et al.*, 2020; Oryan *et al.*, 2017).

Although the increased occurrence of pox-like diseases in camels has been reported from major camel-keeping areas of Ethiopia, there is only one report on the identification of camelpox virus (CMLV) in the Chifra district of Afar and Jigjiga Zone of Somali Regional States of Ethiopia (Ayelet *et al.*, 2013), with no data available on the existence of camel contagious ecthyma or the identification of the causative agent. Therefore, this study aimed to determine the epidemiology of CCE infection and molecularly identify the causative agent in Arero district, Ethiopia.

Materials and methods

Study area

The study was conducted in the Arero district of Borana zone, Oromia Regional State of Ethiopia. Arero district is geographically located at 4°45'0'N and 38°49'0'E at a distance of 650 km south of Addis Ababa. The area is bordered on the southwest by Dire, on the West by Yabello, on the North by Bule Hora, on the northeast by the Guji Zone, on the east by the Somali Region, and on the South by Moyale (Olani *et al.*, 2016). The annual average temperature and rainfall are 19 °C and 716 mm, respectively. Animal husbandry in the area is characterized by an extensive pastoral production system with seasonal migration. Camels and cattle are the key livestock species in the area (Mirkena *et al.*, 2018; Faye, 2015). As aridity gradually increases and drought is a recurrent phenomenon in the area, the principal stock is shifting from cattle to camels (Dawo, 2010).

Study methods and sample size determination

The study employed a cross-sectional study design (November 2013 to April 2014). Arero district was selected because of its camel production potential and easy access to a major road. Three pastoral associations (PAs) were randomly included from the sampled district (i.e., Haro-Dimtu, Kaarra-Gumaata, and Silala PAs). A total of 129 volunteer households participated in this study. The sample size for respondents in the house-to-house interview was determined

using the formula ($n = 0.25/SE^2$) proposed by Arsham (Arsham *et al*., 2007), at the standard error (SE) of 0.044 and 95% confidence level.

A standardized, structured questionnaire was used to collect information relevant to the study objectives, such as age structure of the respondents, herd size and herding experience, CCE incidences in the past year (a year preceding the start of the study), age-wise morbidity and mortality rates attributed to CCE, seasonality of the disease, and opinion of camel owners on plant browse that are potentially associated with CCE occurrence. 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. For those who mentioned clinical signs shared by the diseases easily confused with CCE such as Warts, the interviewers reviewed the clinical signs of CCE with camel owners to verify that the respondent had understood the disease correctly. One animal health assistant and a traditional healer were interviewed regarding the local name of CCE in each of the selected PAs. Some of the information collected during interviews was supported by field observation.

Sample collection and sampling procedures

The protocol for field studies and collection of animal samples was carried out following the ethical guideline of Jimma University, College of Agriculture and Veterinary Medicine (JUCAVM).

Fourteen (14) skin scrapings were collected from camels showing suspected clinical signs of PPV infection. Samples were immediately transferred into a cold box and transported to the National Veterinary Institute (NVI) of Ethiopia under the cold-chain system. The samples were then kept at -20 ^{0}C until laboratory analysis.

Viral isolation on cell culture

Skin scraping samples were washed three times with sterile phosphate buffer saline (PBS) containing antibiotics and antifungals, and ground using a sterile pestle and mortar. The supernatant (0.5 mL) was inoculated onto a confluent monolayer of Vero cells grown in a 25 cm^2 tissue culture flask containing 10 mL

Glasgow Minimum Essential Medium (Sigma-Aldrich) supplemented with 2% fetal calf serum (Gibco). The inoculated cultures were incubated at 37 ^{0}C, 5% CO_2, and observed daily for the appearance of virus-induced cytopathic effects (CPEs). Samples were considered negative when no CPE was observed following three blind passages (Gelaye *et al.*, 2016a; Gelaye *et al*., 2016b; Khalafalla *et al.,* 2015).

Polymerase chain reaction (PCR)

After isolating genomic DNA from the virus, the B2L gene was amplified using forward primers (5′-TGA GCT GGT TGG CGC TGT CCT-3′) and reverse primers (5'-CGC AGA CGT GGC TCA GTA CGT-3′). The reaction setup was prepared as follows: 5X standard reaction buffer (5 µl), 2 mM dNTPs (0.5 µl), 500 nM forward primer (1.25 µl), 500 nM reverse primer (1.25 µl), template DNA (5 µl), 2.5 U Taq DNA Polymerase (0.25 µl), nuclease-free water (to 25 µl). The thermal profile was set as follows: Initial denaturation (94 °C, 5 min, 1X cycle), Denaturation (94 °C, 1 min), Annealing (55 °C, 60 s, 35X), Extension (68 °C, 70 s), Final extension (68 °C, 5 min, 1X) (Khalafalla *et al*., 2020; Tedla *et al*., 2018).

Data analysis

A database was constructed in a Microsoft Excel to store the data. Analysis was performed using Statistical Package for Social Science (SPSS 2007 version 20) software. Descriptive (proportion) and inferential (logistic regression model) statistics were used to analyze the data. Potential risk factors associated with CCE occurrence were assessed by using a logistic regression model and an odds ratio (OR) estimate was used to determine the strength of association between the risk factors (independent variables) and disease (dependent variable). In all the analyses, confidence levels of 95 % and a $p < 0.05$ were used for the statistical significance test.

Ethical consideration

Ethical clearance was obtained from the Ethics Committee of the College of Agriculture and Veterinary Medicine, Jimma University (ethical code R/GS /217/2007). Written and oral consent was obtained from camel owners before the questionnaire survey and sample collection.

Results

Questionnaire survey results

Herd profile of the respondents was described in Table 1. Herd size was used to evaluate the relative contribution of camels to pastoralists and herding years were used to estimate their practices in camel husbandry. Out of the total interviewed respondents (n = 129), the majority (48%) were in the age range of 42-61years. The average herd size of the respondents was 13 camels. Over 40% (46.3%) of the respondents had been herding camels for more than ten years.

Table 1. Herd profile of the respondents

No	Characteristic	Categories	N	Percentage
1	Age of the respondents	25-41	27	21.0
		42-61	62	48.0
		62-81	40	31.0
2	Herd size of the respondents	1-7	41	32.3
		8-17	50	38.7
		≥18	38	29.0
3	Herding experience (years)	0-10	33	25.4
		11-21	60	46.3
		22-31	36	28.3

N=Number of respondents

Herders' knowledge of the diagnosis of CCE is described in Table 2. The result indicated that over 90% of the respondents were aware of CCE, and 62.0% (76/129) mentioned clinical signs suggestive of CCE such as sores and blisters on the lips, nose, and ears. The majority (87%) of the participants reported occurrence of CCE outbreaks in their herds in the past year (a year preceding the start of the study) with the overall morbidity and mortality rates of 20% (95% CI: 11.0– 36.0%) and 6.3% (95 % CI: 5.2 –7.6%), respectively.

Table 2. Selected variables of herders' knowledge and attitude on CCE

Variables	N	Percentage
Heard about CCE		
Yes	123	95.4
No	6	4.6
Major clinical signs mentioned		
Animals fail to grow	14	11.0
Mortality	11	9.0
Sores & blisters on the lips, nose, and ears	76	62.0
Abortion	2	1.6
Loss of appetite	9	7.4
Mentioned 1, 3, & 5	11	9.0
Diarrhea	0	0.0
Experienced CCE outbreaks in their herds in the past year?		
Yes	107	87.0
No	16	13.0

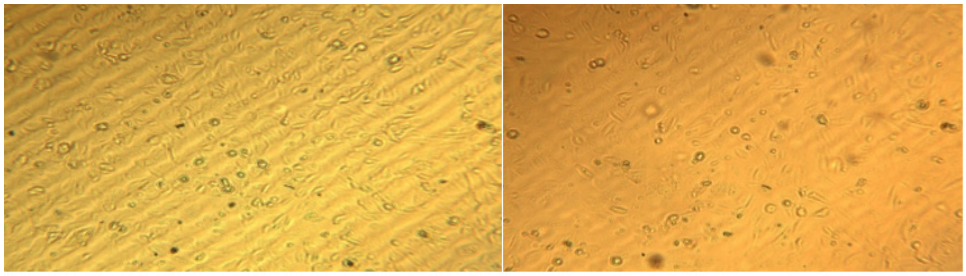
Table 3 describes epidemiological information related to CCE infection. The results indicated that camel calves (age less than two years) had higher odds of becoming affected by CCE than adults (OR=3.44; 95% CI: 1.75-8.80%) and the difference was statistically significant ($p < 0.05$). CCE outbreaks were nearly six times more severe (OR=5.8; 95% CI: 3.34 - 10.52%) in the rainy season than in the dry season. Camels that browsed at Acacia-dominated trees were nearly ten times (OR=9.6; 95% CI: 0.42 - 17.83%) more at risk of CCE infection than camels that browsed at low Acacia-dominated trees.

Table 3. The epidemiological aspect of CCE outbreaks in the study district

Major risk factors	Number Affected	Percentage	OR	95 % CI	P-value
Morbidity					
Calves affected	269	77.5	3.44	1.75 - 8.80	0.012
Adults affected	78	22.5			
Mortality					
Calves died	89	84.0	7.2	4.22 - 11.55	0.002
Adults died	17	16.0			
Seasonal occurrence of CCE as reported by camel owners					
Rainy season	219	63.0	5.8	3.34 - 10.52	0.006
Dry season	128	37.0			
Plant brows and CCE outbreaks as reported by camel owners					
During acaria trees abundance	293	84.4	9.6	5.42 – 17.83	0.024
At low acacia trees abundance	54	15.6			

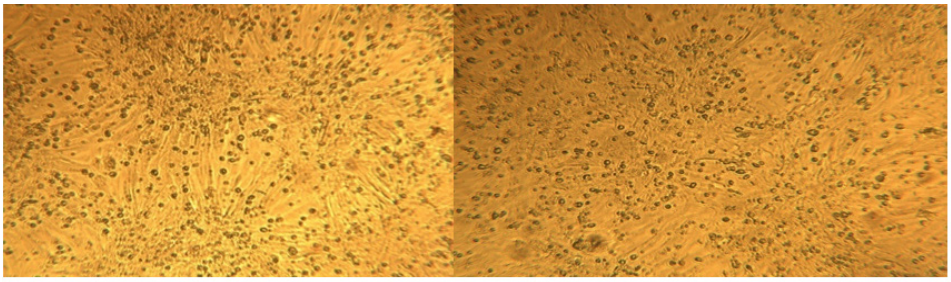
CI= Confidence interval; OR= Odds ratio

Virus isolation results



Normal Vero cell (unaffected)

A.



B.

Fig. 1. African green monkey Vero cell cultures before (top left) or non-inoculated normal monolayers (top right) (A), and CPE observed four days post-infection (B). The figure demonstrates a monolayer of African green monkey Vero cells cultures after infection with CCEV. The inoculated CCEV produces a CPE characterized by cell rounding and enlargement, pyknosis, granularity of the cytoplasm, and cell detachment.

PCR result

PPV-specific amplification by PCR confirmed that the disease was associated with **CCEV** infection showing bands with 1200 bp in one of the culture-positive samples (Figure 2).

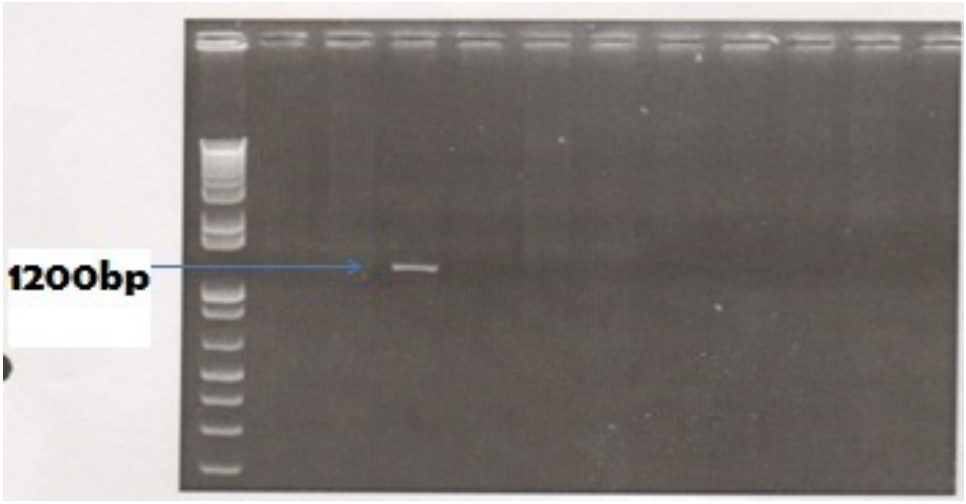


Figure 2. Molecular detection of CCEV. The figure shows gel electrophoretic separation of PCR products (read from left to right). Lane 1=100bp DNA ladder. Lane 2 is a negative control whereas all the rest are CCEV suspected tissue samples. Lane 4 is positive for CCEV around 1200bp, and no amplification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

Discussion

Contagious ecthyma infection in camels is generally neglected worldwide. In Ethiopia, despite frequent outbreaks, there hasn't been any attempts to investigate the disease in the country where camels are important assets to the local community. This study investigated morbidity and mortality rates of the disease consistent with CCE, molecularly identified the causative agent, and determined the potential risk factors in the Arero district of Borana zone, Oromia Regional State of Ethiopia. Camel owners were interviewed to get some epidemiological information on the CCE infection. Their knowledge in describing CCE and ability to recognize its effect indicate their expertise in distinguishing camel health problems in their vicinities, which is in line with the results reported in Sudan (Khalafalla *et al*., 2020). Camel owners considered clinical signs of CCE consistently with the descriptions of the disease signs in the standard veterinary literature (Khalafalla *et al*., 2015; Radostits *et al.,* 2007; Munz *et al*., 1986).

The overall morbidity and mortality rates attributed to CCE in the present study were comparable with the reports from Sudan (Khalafalla *et al*., 2020; Khalafalla *et al*., 2015; Khalafalla, 1998) and Somalia (Moallin and Zessin, 1988), but lower when compared to the findings of Iran (Oryan *et al*., 2017; Mombeni *et al*., 2013), and Sudan (Khalafalla, 2000). The variation could be related to the age structure of animals included in the studies, and/or differences in the husbandry and health management systems of the countries.

During field clinical investigation, authors noticed that the majority of the animals showing clinical signs of suspected poxvirus infection were younger camels. In addition, the morbidity and mortality rates attributed to CCE were higher in younger than in adults. Our result is in agreement with the reports of different researchers (Khalafalla *et al*., 2020; Khalafalla *et al*., 2015; Mombeni *et al*., 2013; Khalafalla, 1998). The severity of the disease in young animals

might be due to a lack of prior exposure to infecting pathogens (Radostits *et al.*, 2007), and/or due to the absence of a fully developed immune system (Oryan *et al.*, 2017; Hosamani, 2006).

The present study identified that CCE had a marked seasonality, being associated with the rainy season, and seemed to occur at this particular time every year. These findings substantiate the previous reports on the seasonality of CCE and its association with the rainy season (Khalafalla *et al.*, 2020; Mombeni *et al.*, 2013; Khalafalla *et al.*, 1994; Buchnev *et al.*, 1987). Factors responsible for this epizootiological feature seem to be the abrasion of the skin of the lips, resulting from eating thorny acacia plants at this time of the year when no other source of food was available (Khalafalla, 2000). The same view was reflected by a researcher from the former *Union of Soviet Socialist Republics* (*USSR*), who argued that thorny plants damaged the lips allowing transmission of parapox virus through skin abrasions caused by browsing thorny trees (Buchnev *et al.*, 1987).

Inoculation of scabs supernatants on Vero cell cultures revealed a CPE in the form of cell rounding and enlargement, pyknosis, and granularity of the cytoplasm and cell detachment four days post-infection. This can be considered the first step in the screening of PPV infections from suspected samples. Furthermore, PPV-specific amplification by PCR revealed an amplification product of 1200 bp size, confirming that the disease in camels was associated with CCEV. This finding substantiates reports from different countries including Ethiopia (Gelaye *et al.*, 2016b); Iran (Oryan *et al.*, 2017; Mombeni *et al.*, 2013); and Sudan (Khalafalla *et al.*, 2015).

Given the increased incidences and economic importance of PPV infections in the camel population in Ethiopia, it will be worthwhile to obtain more epidemiological information about this disease for effective surveillance and to carefully monitor and handle disease outbreaks.

Conclusions

The present study confirmed the existence of CCE in the Arero district of Borena zone, Ethiopia. Younger camels accounted for the highest morbidity and

mortality rates compared with adults. Confirmatory diagnosis of the suspected cases using conventional PCR techniques generated the expected amplification product size of 1200bp. However, due to scarcity in the laboratory facility, the amplicon with the compatible size was not sequenced to confirm the specificity. Overall, the information obtained in this study would be worthwhile to improve the farmers' livelihood and may open new research avenues for the control and eradication of the disease at the local and national levels.

Acknowledgments

The authors are very much grateful to the National Veterinary Institute for providing space and laboratory inputs to carry out the laboratory work.

Abbreviations

BA: Birhanu Ayele; BD: Bareda Diba; BdA: Bedane Adane; BdG: Benti Deressa Gelalcha; bp: Base pair; CCE: Camel contagious ecthyma; CCEV: Camel contagious ecthyma virus; CMLV: camelpox virus; CPE: Cytopathic effect; JUCAVM: Jimma University College of Agriculture and Veterinary Medicine; NVI: National Veterinary Institute; OR: Odds ratio; PAs: Pastoral associations; PBS: Phosphate buffered saline; PCR: Polymerase chain reaction; PPV: Parapoxvirus; SPSS: Statistical Packages for Social Sciences; UV: Ultra violate.

Conflicts of Interest

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

Funding

The present study received financial support from Jimma University, the Vice president of the research and publication office.

Author contributions

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

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