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Molecular epidemiology of camel contagious ecthyma in Arero district, Ethiopia

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Abstract

Background: While dromedary 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, caused by a *Parapoxvirus*, is thought to be one of the most common viral diseases of dromedary camels in Ethiopia.

Methods: A cross-sectional study was conducted from November 2013 to April 2014 in Arero district of Borana Zone, Oromia Regional State of Ethiopia to investigate the epidemiological aspect of camel contagious ecthyma and molecularly identify the causative agent. A polymerase chain reaction based on B2L gene-specific primers of *Parapoxvirus* was used for confirmatory diagnosis of the disease from camels showing suspected clinical signs of *Parapoxvirus* infection.

Results: The majority (87%) of camel owners reported the occurrence of camel contagious ecthyma outbreaks in their herds in the past year (a year preceding the start of the study). The overall morbidity and mortality rates attributed to camel contagious ecthyma were 20% (95% CI 11–36%) and 6.3% (95% CI 5.2–7.6%), respectively. Camel calves had higher odds of becoming affected by the disease than adults [OR = 3.44 (95% CI 2.29–4.09)] and the difference was statistically significant. The disease has a marked seasonality with most of the cases occurring during the rainy season. Acacia trees significantly contribute to virus dissemination by damaging the lips of browsing camels. Confirmatory diagnosis of the suspected cases using conventional polymerase chain reaction generated the expected amplification product of 1200 bp for one of the samples.

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

Keywords: Camel contagious ecthyma, *Dromedaries*, Epidemiology, Ethiopia, PCR

Background

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 on the provision of milk, meat, social and cultural importance in addition to unpaid transport service

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(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. 2015, 2020; Oryan et al. 2017). 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 (Gelaye et al. 2016a, b; Khalafalla et al. 2015, 2020; Oryan et al. 2017). 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, polymerase chain reaction (PCR) based on B2L gene-specific primers of PPV was extensively 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 by major camel-keeping areas of Ethiopia, there is only one report on the identification of camelpox virus in Chifra district of Afar and in Jigjiga Zone of Somali Regional States of Ethiopia (Ayelet et al. 2013), with no data available on the existence of CCE 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.

Methods

Description of the study area

The study was conducted in Arero district of the Borana zone, Oromia Regional State of Ethiopia. Arero district is geographically located 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 Yabelo, 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 (Faye 2015; Mirkena et al. 2018). 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–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 were participated in this study. The sample size for respondents in the house-to-house interviews was determined using the formula ($n = 0.25/\mathrm{SE}^2$), at the standard error (SE) of 0.044 and 95% confidence level (Arsham et al. 2007).

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 outbreak, 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 in accordance with the ethics guideline of Jimma University College of Agriculture and Veterinary Medicine.

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 °C until laboratory analysis.

Viral isolation on cell culture

Skin scraping samples were washed three times with sterile PBS containing antibiotics and antifungal 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 °C, 5% CO2 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, b; Khalafalla et al. 2015).

Polymerase chain reaction (PCR)

After isolating genomic DNA from the virus, 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 set up was prepared as follows: 5× 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, 1× cycle), Denaturation (94 °C, 1 min), Annealing (55 °C, 60 s, 35×), Extension (68 °C, 70 s), Final extension (68 °C, 5 min, 1×) (Khalafalla et al. 2020; Tedla et al. 2018).

Data collection and 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 survey findings. Potential risk factors associated with CCE occurrence were assessed by using a logistic regression model and 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 at 95% and a $p < 0.05$ were used for statistical significance test.

Results

Questionnaire survey results

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

Herders' knowledge on diagnosis of CCE is described in Table 2. The result indicated that over 90% (95%) of respondents were aware about CCE, and 76 (62%) mentioned clinical signs suggestive of CCE infection 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 11–36) and 6.3% (95% CI 5.2–7.6%), respectively.

Table 1 Herd profile of the respondents

No	Characteristic	Categories	*N*	Percentage (%)
		25–41	27	21
1	Age of the respondents	42–61	62	48
		62–81	40	31
		1–7	41	32.3
2	Herd size of the respondents	8–17	50	38.7
		≥ 18	38	29
		0–10	33	25.4
3	Herding experience (years)	11–21	60	46.3
		22–31	36	28.3

N number of respondents

Table 2 Selected variables of herders' knowledge on CCE

Variables	*N*	Percentage
Heard about CCE		
Yes	123	95.4
No	6	4.6
Major clinical signs mentioned		
1. Animals fail to grow	14	11
2. Mortality	11	9
3. Sores and blisters on the lips, nose and ears	76	62
4. Abortion	2	1.6
5. Loss of appetite	9	7.4
6. Mentioned 1, 3, and 5	11	9
7. Diarrhea	0	0
Experienced CCE outbreaks in their herds in the past year		
Yes	107	87
No	16	13

Table 3 describes epidemiological information related to CCE infection. The results indicated that camels calves (age less than two years) had higher odds of becoming affected by CCE than adults (OR = 3.44; 95% CI 1.75–8.8) and the difference was statistically significant ($P < 0.05$). CCE outbreaks was 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 to CCE infection than camels that browsed at low Acacia-dominated trees.

Virus isolation results

From the total of 14 skin scrapings (obtained from 14 different camels), ten samples were found to be positive for PPV infection showing apparent CPE in the form of cell

Table 3 The epidemiological aspect of CCE outbreaks in Arero district

Major risk factors	Number affected	%	OR	95% CI	*P* value
Morbidity					
Calves affected	269	77.5	3.44	1.75–8.80	0.012
Adults affected	78	22.5	Ref.*		
Mortality					
Calves died	89	84	7.2	4.22–11.55	0.002
Adults died	17	16	Ref.*		
Seasonal occurrence of CCE as reported by camel owners					
During rainy season	219	63	5.8	3.34–10.52	0.006
Dry season	128	37	Ref.*		
Plant brows and CCE outbreaks as reported by camel owners					
During acacia trees abundance	293	84.4	9.6	5.42–17.83	0.024
At low acacia trees abundance	54	15.6	Ref.*		

* *Ref* reference category, *CI* confidence interval, *OR* odds ratio

rounding and enlargement, pyknosis, granularity of the cytoplasm and cell detachment four days post-infection (Fig. 1).

PCR result

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

Discussion

Contagious ecthyma infection in camels is generally neglected worldwide. In Ethiopia, despite the frequent outbreaks, there hasn't been any attempt 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 also determined the potential risk factors in Arero district of Borana zone, Oromia Regional State of Ethiopia. Camel owners were interviewed to get some epidemiological information relevant to CCE infection.

Significant numbers of respondents in the present study were middle-aged and engaged mainly in camel rearing. This, therefore, demonstrates that camel husbandry is a substantial livelihood endeavor for pastoralists in Ethiopia which is in line with the study reports in many African countries including Uganda (Salamula et al. 2017), Nigeria (Jaji et al. 2017) and Kenya (Kagunyu and Wanjohi 2014). Also the majority of participants had plentiful experience in camel rearing. Their knowledge in describing CCE outbreaks and ability to recognize its effect indicate their expertise in camel health problems. The long herding experience might have helped them to easily identify camel production constraints in their vicinities which is in agreement with the study report in Kenya (Kagunyu and Wanjohi 2014).

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

During field clinical investigation, authors noticed that majority of the animals showing clinical signs of suspected pox virus infection were camel calves. Also the morbidity and mortality rates attributed to CCE were higher in calves than in adults. Our findings agree with the reports of different researchers (Khalafalla 1998; Khalafalla et al. 2015, 2020; Mombeni et al. 2013). 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 fully developed immune system (Oryan et al. 2017; Hosamani et al. 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 (Buchnev et al. 1987; Khalafalla et al. 1994, 2020; Mombeni et al. 2013). A factor responsible for this epizootiological feature seems 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. The same opinion was reflected by a researcher from the Union of Soviet Socialist Republics (USSR), who argued that thorny plants damaged the lips allowing transmission of parapoxvirus through skin abrasions caused by browsing thorny trees (Buchnev et al. 1987).

Inoculation of scabs supernatants on Vero cell cultures revealed CPE in the form of cell rounding and enlargement, pyknosis, the granularity of the cytoplasm and cell detachment four days post-infection. This can be considered a first step in screening for PPV infections from suspected samples. Furthermore, PPV-specific amplification by PCR revealed the 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);

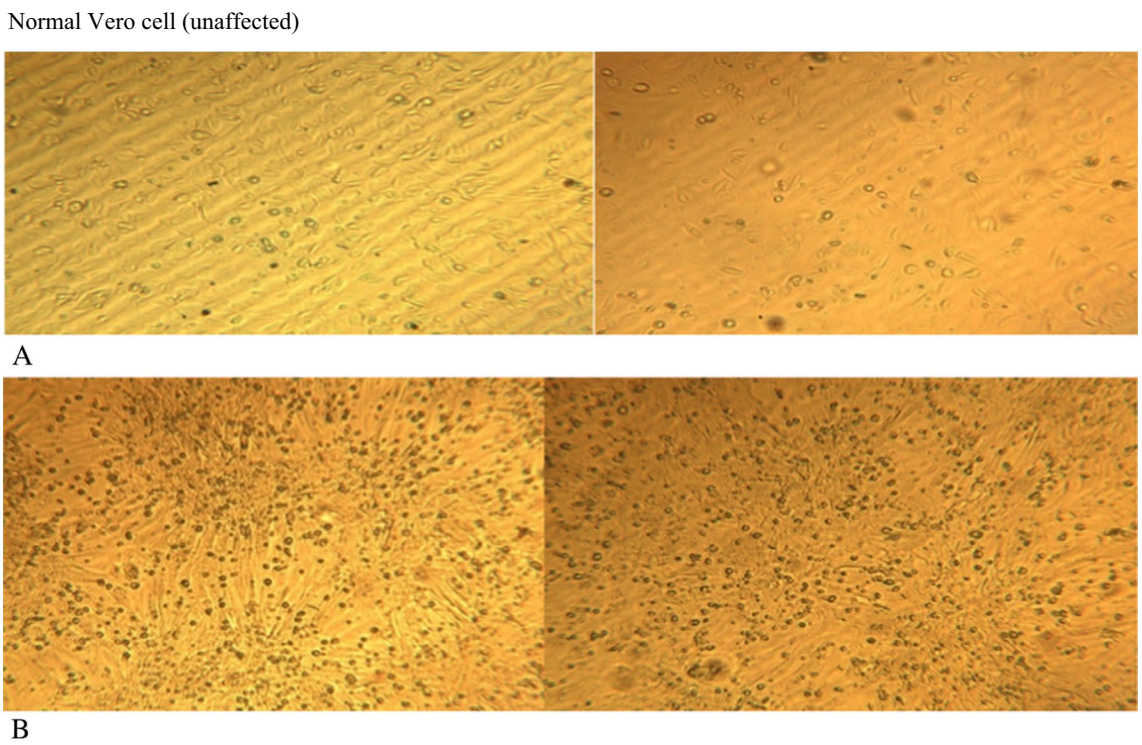

Fig. 1 African green monkey Vero cell cultures before (top left) or non-inoculated normal monolayers (top right) (**A**), and CPE observed four days post-infection (**B**). The figure demonstrates a monolayer of African green monkey Vero cells cultures after infection with camel contagious ecthyma virus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

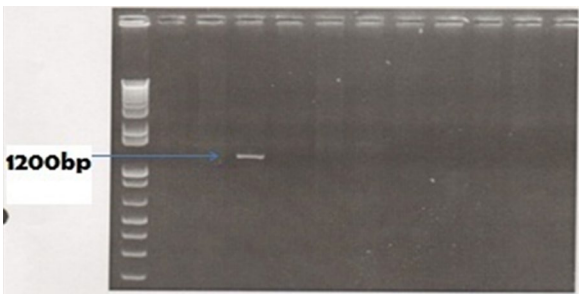

Fig. 2 Molecular detection of CCEV. The figure shows gel electrophoretic separation of PCR products (read from left to right). Lane 1 = 100 bp DNA ladder. Lane 2 is a negative control, whereas all the rest are CCEV suspected tissue samples. Lane 4 is positive for CCEV around 1200 bp; and no amplification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

Iran (Mombeni et al. 2013; Oryan et al. 2017); and Sudan (Khalafalla et al. 2015).

Given the increased incidences and economic importance of CCEV infections in 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 the disease outbreaks.

Conclusions

The present study confirmed the existence of CCE in Arero district of Borana zone, Ethiopia. Camel calves had the highest morbidity and mortality rates attributed to CCE compared with adult camels. Confirmatory diagnosis of the suspected cases using conventional PCR techniques generated the expected amplification product size of 1200 bp, confirming that the disease in camels was associated with CCEV. 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.

Abbreviations
BA: Birhanu Ayele; BD: Bareda Diba; BdA: Bedane Adane; BdG: Benti Deressa Gelalcha; bp: Base pair; CCE: Camel contagious ecthyma; CPE: Cytopathic effect; 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-violet.

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Author contributions
BD conceived the research idea, collected the data and drafted the manuscript. BdA supervised the sample collection and was involved in data analysis. BA 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 editing. All authors read and approved the final manuscript.

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Availability of data and materials
This article contains all data used in the study.

Declarations

Ethics approval and consent to participate
Written approval for skin sample collection and shipping of samples to National Veterinary Institute, Bishoftu, Ethiopia, for laboratory analysis was granted by respective Authorities responsible for livestock in Borana zone, Oromia Regional State of Ethiopia in 2013. Written and oral consent was obtained from camel owners before questionnaire survey and sample collection.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interest.

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