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Seroprevalence and participatory epidemiology of camelpox in Afar region of Ethiopia

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ABSTRACT

Camelpox is endemic in most camel rearing regions of the world, causing significant economic

losses. However, its epidemiology is not extensively investigated. We conducted a cross

sectional seroprevalence study of camelpox in Amibara and Awash Fentale districts in Afar

region of Ethiopia from November 2014 to May 2015. In addition, participatory epidemiology

(PE) was conducted to identify seasonal occurrence of the disease in the study districts. Blood

samples were collected from 384 dromedary camels from 31 herds distributed in five pastoral

associations (PAs) in the two districts. Serum samples were separated from the blood samples

and tested for the presence of viral antibodies using virus neutralization test. Seroprevalence data

were analyzed using multilevel mixed effects logistic regression models accounting for the 4-

level hierarchical data structure (camels nested in herds-herds in PA, and PA in district). For the

participatory data, Kendall's coefficient of concordance was used to assess agreements between

the informants in identifying seasonal occurrences of the top five camel diseases. Camelpox

antibodies were detected in 19.3% of camels (n=384), 81% of herds (n=31), and in all five PAs

from the two districts in the Gabi Rasu zone of Afar region, Ethiopia. The seroprevalence did not

significantly vary between herds, PAs or districts suggesting the widespread occurrence of the

disease. Estimated age stratified basic reproduction number (R_0) was 1.25 (95% CI: 0.62 – 2.19).

Camelpox was identified as one of the top five common camel diseases in the area. The

widespread occurrence of the disease can be attributed mainly to the commingling of camels

from many herds during seasonal migration in search of feed and water, a practice very common

under pastoral production systems. Although the PE informants indicated the clinical disease to

be more common in young animals, seropositivity was higher in older animals. Camelpox

commonly occurs during the minor and major rainy seasons. In conclusion, camelpox is found to

45   be endemic in Afar pastoral region with sporadic outbreaks occurring during rainy seasons.

46   Vaccination and improved camel management practices particularly during the high-risk period

47   can be viable strategies to reduce the burden of the disease.

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49   **Key words:** Camelpox; Camel; Basic reproduction number; Participatory epidemiology;

50   Seroprevalence; Virus neutralization test.

51

52   **Abbreviations:** PE = participatory epidemiology; PA = pastoral association; SSI = semi

53   structured interviews

54

1. Introduction

Camelpox is a contagious infectious disease of dromedary camels (*Camelus dromedarius*) caused by camelpox virus that belongs to the genus *Orthopoxvirus* (Azwai et al., 1996). The disease occurs in all camel rearing countries except in Australia, causing severe economic losses (Fenner et al., 1989; Azwai et al., 1996). The considerable economic loss of the disease is through its high morbidity, a relatively high mortality in young animals, loss of condition in all ages, reduced milk production in lactating camels (Azwai et al., 1996), and abortion in pregnant camels (Al-Zi'abi et al., 2007; Mahmoud et al., 2012). In addition, the appearance of camelpox in herds favors secondary infections from other circulating diseases from which camels can die (Duraffour et al., 2011). The mortality rate in adult animals ranges between 5% and 28%, and in young animals between 25% and 100% (Mayer and Czerny, 1990). The presence of the disease in a country causes restriction in the trade of live camels and their products (Bhanuprakash et al., 2010).

Camelpox is also important because of its potential zoonotic transmission affecting humans (Azwai et al., 1996). Several cases have been reported in humans (Kriz, 1982) most recently in India (Bera et al., 2011) with clinical manifestations such as papules, vesicles, ulceration and finally scabs on fingers and hands. The World Organization for Animal Health (OIE) lists camelpox as a reportable disease (OIE, 2014a). Camelpox is also classified as risk group 2 pathogen for human infection, and recommended to be handled with appropriate measures (OIE, 2014b).

Camelpox virus is host-specific and dependent on a single host, which makes it a potential target for control and eradication. Unfortunately, effective control programs such as

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77 quarantine measures and restriction of camel movement appear to be of limited success owing to

78 the migratory pattern of camels and the difficulty of reaching the animals due to poor

79 infrastructures under most camel rearing pastoral regions. Therefore, vaccination is a viable

80 alternative to control the disease in endemic countries (Khalafalla and El-Dirdiri, 2003).

81 Although vaccination is not widely applied, camelpox vaccine production is being reported from

82 many countries including Saudi Arabia (Hafez et al., 1992), United Arab Emirates (Wernery and

83 Zachariah, 1999), Morocco (El Harrak and Loutfi, 2000) and Mauritania (Nguyen et al., 1996).

84 Recently, camelpox vaccine was developed in Ethiopia by the National Veterinary Institute,

85 which is the first type of vaccine for dromedary camels in the country.

86   To carry out an effective control or eradication programs of camelpox through strategic

87 vaccination, a thorough understanding of the epidemiology of the disease in a specific

88 geographic area has a paramount importance. Although the disease was confirmed to occur in

89 Ethiopia (Ayelet et al., 2013) and recognized for its economic losses, to the best of our

90 knowledge, no study has been reported on the seroepidemiology of the disease in the country.

91 The objective of this study was to investigate the seroprevalence and determine the seasonal

92 occurrence of camelpox in Afar region of Ethiopia. This study will enhance our knowledge in the

93 epidemiology of camelpox in Ethiopia, and to improve camelpox mitigation strategies such as

94 the timing of vaccination or other preventive measures to be taken.

95

96 **2. Materials and methods**

97

98 **2.1. Description of the study area**

5

99   The study was carried out from November 2014 to May 2015 using a cross sectional

100   seroprevalence study (Thrusfield, 2005; Dohoo et al., 2009), and a participatory epidemiology

101   (Catley et al., 2012). The study was conducted in Amibara and Awash Fentale districts of Gabi

102   Rasu zone in Afar region of Ethiopia. Afar region is located in North Eastern part of Ethiopia,

103   and is characterized by a pastoral life style. The region has a great potential of livestock

104   resources comprising of 2.3 million cattle, 4.3 million goats, 2.5 million sheep, 0.8 million

105   camels and 0.19 million equines that support the region and contributes to the national economy

106   (CSA, 2010). The Afar region is administratively divided into five zones, 32 districts and 401

107   pastoral associations (PAs; the lowest administrative unit in Ethiopia). Gabi Rasu (formerly

108   known as Zone Three) is one of the administrative zones located in the southern part of the

109   region (Fig. 1). Gabi Rasu zone is characterized by arid and semi-arid agro-climatic conditions

110   with annual rainfall that ranges from 200 to 700 mm. According to the data obtained

111   from the meteorological station of Werer Agricultural Research Center, the mean annual

112   minimum and maximum temperatures are 19.1 and 34.3°C, respectively. Gabi Rasu zone was

113   selected based on its convenience for access by ground transportation, and due its high camel

114   production potential. The zone consists of six districts, predominantly occupied by pastoral and

115   agro-pastoral communities. The two selected districts, Amibara and Awash Fentale, are situated

116   in the dry lowlands of the rift valley, at about 230 and 280 km northeast, respectively from the

117   capital Addis Ababa.

118

119   **2.2. Seroprevalence study**

120

121   *2.2.1. Sampling design*

122   A stratified, 3-stage sampling design in which samples were stratified by two study districts from

123   the Gabi Rasu zone was employed. Of five zones in the Afar region, Gabi Rasu zone was

124   purposively selected based on their camel production potential and accessibility to road

125   transportation. Of six districts in Gabi Rasu zone, Amibara and Awash Fentale districts were

126   further selected due to higher reports of camel health problems by the zone in both districts. Five

127   PAs (three from Amibara district and two from Awash Fentale district) were randomly selected

128   by a lottery system from the list of PAs obtained from the Districts' Pastoral Agriculture and

129   Rural Development Offices. A total of 31 herds (15 from Amibara and 16 from Awash Fentale

130   districts) were selected by systematic random sampling in which the first herd was randomly

131   selected by a lottery system, and then every other herd was sampled until the desired sample size

132   was achieved. If herd owners did not consent, the next herd was sampled. Within the selected

133   herds, camels were selected by simple random sampling method. Camels over six months of age

134   were sampled from each herd to avoid nonspecific serologic reactions due to circulating maternal

135   antibodies (Nothelfer et al., 1995; Mahmoud et al., 2012).

136   The total number of camels for this study was calculated using a formula for the

137   estimation of proportion (Dohoo et al., 2009) by considering 95% confidence level, 5% precision

138   and 50% seroprevalence, due to lack of previous seroprevalence report in the country. This

139   resulted in a total of 384 camels to be sampled for the determination of antibody prevalence

140   against camelpox. However, since random sampling of camels was not cost effective nor

141   practically feasible, we tried to distribute the number of camels sampled over many herds, and

142   PAs to potentially increase the variability and precision of the seroprevalence estimate. The

143   study animals included camels of different age and of both sexes reared under extensive

144   husbandry management system with no previous vaccination against camelpox.

145

2.2.2. Serum sample collection and antibody detection

After animals were physically restrained, blood was collected from jugular vein in to plain

vacutainer tubes. The blood tubes were left to stand overnight at room temperature, after which

the blood was centrifuged at 3000 rpm for 15 min to separate the serum. Separated serum

samples were transferred to a new tube and stored at 4°C until submitted to virology laboratory

where they were stored at -20°C until processed for the detection of camelpox antibodies.

Serum samples were analyzed by virus neutralization test to detect the presence of serum

specific antibodies against camelpox virus by *in vitro* neutralization of the viral cytopathic effect

on cell culture. The test was conducted at the National Animal Health Diagnostic and

Investigation Center (Sebeta, Oromia, Ethiopia) following procedures recommended by the

World Organization for Animal Health (OIE, 2014b). The test method was based on a reaction

between the virus and specific antibody in the test serum. Virus and products containing a

neutralizing antibody were mixed under appropriate conditions and then inoculated into cell

culture. The presence of un-neutralized virus was detected by plaque formation (cytopathic

effect) on the cell culture. A loss of infectivity is caused by interference by the bound antibody

with any of the steps leading to the release of the viral genome from the host cells including

attachment, infection, or viral release.

163

2.3. Participatory epidemiology

Initially questionnaire survey using semi structured interviews (SSI) was conducted on 30

respondents from both districts to get a baseline information about the common camel diseases in

the area. At this phase the respondents listed major camel diseases by their local names with their

168   clinical symptoms and ranked them based on their frequency of occurrences using open-ended

169   questions. The top 5 ranked diseases were then selected for subsequent participatory

170   epidemiologic studies. The participatory epidemiology was performed using 12 independent

171   informant groups where each group was composed of 5 - 10 participants. Six groups were

172   interviewed from each district, and the participants included camel owners whose camels were

173   being sampled. Other pastoralists present nearby with good camel herding experiences, and rich

174   indigenous knowledge related to camel diseases and health care were also invited to join the

175   discussion. Seasonal calendars and SSI were used to identify the seasonal occurrences of the five

176   camel diseases identified through simple ranking method (FAO, 2000; Catley et al., 2012). All

177   PAs selected for serum sample collection were included in this study. The method was pre-tested

178   on animal health workers and camel owners before it was used for the actual field work to ensure

179   that the method was understood and the questions were clear. The survey team was composed of

180   three interviewers: a team leader (researcher), a community mobiliser and a translator. The

181   community mobiliser made prior arrangements and preparations with the pastoralists in each PA

182   and ensured time and place for the interview. The local language of the region *"Afar"* was used

183   during the interview in both phases.

184

185   ### *2.3.1. Seasonal calendar*

186   Seasonal calendars- a time-related data source- were used to describe the seasonal occurrences of

187   the five most common camel diseases selected using simple ranking method (Ameri et al., 2009).

188   To construct a seasonal calendar, the four Afar seasons in their local names were represented by

189   objects: a stick with green leaves for *'Kerma'* (the major rainy season between July and August),

190   green stick without leaf for *'Gilal'* (the dry and cold season between September and February),

191   stick with few green leaves for *'Sugum'* (the short rainy season between March and April) and a

192   dry stick without leaves for *'Hagay'* (the hot and dry season between May and June) on the X-

193   axis. Pieces of papers with pictures and local names of the diseases printed on them were placed

194   along the Y-axis. These were then placed on the flip chart and explained to the participants after

195   they were sited in convenient places. The participants were then requested to explain the

196   meaning of each symbol to make sure they understood the symbols. The participants were then

197   given 30 stones and asked to show the relative occurrence of each disease in each season. The

198   seasons, diseases and the number of stones were kept constant across all the participant groups to

199   ensure the reproducibility of the technique.

200
201   *2.3.2. Semi-structured interviews*

202   Following scoring of the seasonal calendars, the results were discussed with the participants

203   using open and probing questions through SSIs. The informant groups were specifically probed

204   more on the disease of interest (camelpox) regarding its seasonal occurrence, impact, age group

205   affected and predisposing factors.

206   **2.4. Data analysis**

207   *2.4.1. Analysis of seroprevalence data*

208   Field and laboratory data were entered in to Excel worksheets (Microsoft Corp., Redmond, WA,

209   USA) double checked, and further verified by a second person. Data were analyzed using

210   Stata/SE 15.1 (Stata Corp. LLC, 2017). For descriptive statistics the proportion of sex, number of

211   herds, and number of camels sampled in each district were calculated and compared by two-

212   sample test for proportions. Median and range were used to describe the frequency distributions

213   of age and herd size, and a two-sample Wilcoxon rank-sum (Mann-Whitney) test was used to

214 compare the median values between the two study districts. Proportion of animals sampled per

215 herd was calculated as the number of camels sampled divided by the herd size.

216   Multilevel mixed effects logistic regression was conducted to account for a 4-hierarchical

217 data structure in which camels are nested in herds, herds nested in PAs, and PAs nested in

218 districts. Herd and PA were modeled as random effects, while the highest level, district, was

219 modeled as a fixed effect variable. In addition, the model consisted of the fixed effects of herd

220 size (continuous variable) and age (as a categorical variable), and sex (as a categorical variable).

221 Age was grouped into four categories: 0-5 years, 6-10 years, 11-15 years, and >15 years old. We

222 first built a full model with all variables included. Then variables were excluded step by step by

223 removing the non-significant terms starting with variables with the highest P-value. The full and

224 the reduced models were compared by maximum likelihood ratio test for significance. Marginal

225 mean prevalence was obtained from the final model. Maximum Likelihood Estimator method

226 was used to calculate the basic reproduction number (R_0) from age stratified seroprevalence data

227 (Ferguson et al., 1999; Boelle and Obadia, 2015).

228

229 *2.4.2. Analysis of participatory epidemiological data*

230 Data collected by seasonal calendar were summarized using median scores and their minimum

231 and maximum scores. The Kendall's coefficient of concordance (W) was used to quantify

232 agreements between the informant groups. Kendall's W values were categorized as weak (<

233 0.26; $P > 0.05$), moderate (0.26 - 0.38; $P < 0.05$), and good (>0.38; $P < 0.01$ to <0.001).

234 Kendall's W was calculated by assuming the rankings of four objects (seasons) by 12 judges

235 (groups of informants) (Seigel and Castellan, 1994).

3. Results

3.1. Description of the study animals

238 Thirty-one herds (15 from Amibara, and 16 from Awash Fentale) were selected from the two

239 study districts. The number of camels sampled per herd ranged from 10% to 100% (Table 1).

240 Herd size and the number of camels studied did not significantly ($P > 0.05$) differ between the

241 two districts (Table 2). Female camels represented 87% of the total camels studied (n=384).

242 There was significant (likelihood-ratio χ^2 $P = 0.002$) difference in the sex distribution between

243 the two districts: the proportion of females was higher in the Amibara district while the

244 proportion of males was higher in the Awash Fentale district (Table 2). Similarly, the age

245 distribution of the study camels was significantly (LR χ^2 $P < 0.001$) different between the

246 districts: while higher proportions of older camels were sampled in Amibara, relatively younger

247 camels were sampled from Awash Fentale district (Table 2).

248

3.2. Seroprevalence of camelpox

250 Across the two districts of the Gabi Rasu zone of Afar region, the overall seroprevalence of

251 camelpox was 19.3% (95% CI: 15.4 - 23.1%). When stratified by district the seroprevalence was

252 20% (95% CI: 14.7 – 25.3%) in Amibara, and 18.3% (95% CI: 12.5 – 24.2%) in Awash Fentale

253 district. Seropositive camels were detected in 81% of the herds (n=31). No seropositive camel

254 was detected in two herds from Amibara, and three herds in Awash Fentale. Among the positive

255 herds, herd level prevalence varied from 7% (in Awash Fentale) to 50% (in Amibara) as shown

256   in Fig. 2. All the six PAs had at least one herd with seropositive camel. However, all the levels of

257   potential clustering effects (herd, PA, and district) were not significantly associated with

258   seroprevalence (Table 3). Only the age of the animal was found to be significantly associated

259   with seropositivity after adjusting for the various levels of clustering variables both in the full

260   model (Table 3), and the final model (Table 4). Older camels (>10 years of age) are three times

261   more likely to be seropositive when compared to the younger age group, 0 – 5 years old (Table

262   4). The seroprevalence shows an increase with age (Fig. 3). The age stratified basic reproduction

263   number (R_0) estimate for the camelpox infection using Maximum Likelihood method was 1.25

264   and the 95% CI range was between 0.62 – 2.19. The point estimate indicates that the disease will

265   spread out in the population (will not die out in the long term), but the estimate was not

266   statistically significant.

267

268   **3.3. Questionnaire and participatory epidemiological surveys**

269   During the initial questionnaire survey, various camel diseases with their symptoms were

270   identified by the pastoralists based on their common occurrences. Subsequently top five diseases

271   including camelpox (Fig. 4) were selected by simple ranking method (Ameri et al., 2009) for the

272   seasonal calendar study. In addition, the occurrence of tick infestation was included because of

273   its possible association with the occurrence of the targeted diseases. Results of the 12 seasonal

274   calendars are presented in Fig. 4. Good agreement was observed among the 12 informant groups

275   concerning seasonal patterns of the selected camel diseases except for *galideli* (abscess) which

276   scores weak agreement (W= 0.171). Camelpox (*galiguduf/ambraruk*) incidence peaks during

13

277 minor *(Sugum)* and major *(Kerma)* rainy seasons with median scores of 16 (95% CI: 8-28) and

278 13.5 (95% CI: 2-22) respectively (Fig. 4).

279

280 **4. Discussion**

281 **4.1. Seroprevalence**

282 The epidemiology of most camel diseases including camelpox is not extensively investigated.

283 Most of the studies on camelpox were mainly focused on the isolation and characterization of the

284 causative virus (Renner-Müller et al., 1995; Pfeffer et al., 1998; Salem et al., 2008; Yousif and

285 Al-Naeem, 2012; Ayelet et al., 2013). In this seroepidemiologic investigation, out of 384 camels,

286 31 herds and five PAs sampled from two districts of Gabi Rasu zone in Afar region, camelpox

287 antibodies were detected in 74 (19.3%) camels, 25 herds (81%) and all PAs. These findings show

288 that there is a high seroprevalence of camelpox in the herds investigated regardless of their

289 geographic location. Serological evidence of the present study together with the confirmatory

290 report of the disease (Ayelet et al, 2013) from Afar region suggest that the disease is endemic in

291 the area. According to the information collected from the animal health sector of the Pastoral

292 Agriculture and Rural Development Office of the districts and camel owners, the camel

293 population in the study area has never been vaccinated against any diseases including camelpox.

294 Therefore, our findings indicate a seropositivity in response to natural infection, and we

295 speculate that camelpox infection is active in the area. However, confirmation of this claim

296 requires isolation of the virus from clinical cases or detection of the viral antigen as proxy (OIE,

297 2014b). Unfortunately, however, we did not encounter any active cases of camelpox in this

298 cross-sectional study population or in a cohort of camels, from 15 of the herds included in this

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299   report that were followed. A previous study (Ayelet et al., 2013) that looked for the occurrence

300   of clinical cases of camelpox in another district in Afar region, and four other districts in Somali

301   region isolated the virus and further characterized by PCR.

302   The antibody prevalence reported here is relatively higher than other seroepidemiologic

303   studies of camelpox that reported the presence of neutralizing antibodies from similarly

304   unvaccinated camels: 9.8% in Libya (Azwai et al., 1996) and 9.14% in Saudi Arabia (Housawi,

305   2007). On the other hand, higher prevalence values of neutralizing antibodies have been reported

306   from similarly unvaccinated camel populations but following disease outbreaks in 72.5% of

307   camels in Sudan (Khalafalla et al., 1998) and in 100% of clinical cases and in-contact apparently

308   healthy camels in Egypt (Mahmoud et al., 2012). Camelpox prevalence based on clinical cases,

309   ranging from 3% to 14.2%, has been reported from various parts of Ethiopia (Tefera and

310   Gebreab, 2001; Megersa, 2010; Ayelet et al, 2013). It was expected to find higher serological

311   prevalence than clinical prevalence as poxvirus antibodies can be detected in animal sera much

312   more frequently than poxviruses can be isolated from them (Marennikova et al., 1975).

313   Among the risk factors screened for an association with seropositivity of camelpox

314   antibody, age group was found to be the only significantly associated factor, where

315   seroprevalence increased positively with age. This can be explained by the fact that adult animals

316   are consistently exposed resulting in a cumulative increase in neutralizing antibody titer than the

317   younger age group. In addition, camelpox is commonly fatal in young camels (Kriz, 1982;

318   Khalafalla and Mohamed, 1996; Mahmoud et al., 2012), where antibody detection is less likely.

319   Khalafalla and Mohamed (1996) reported increasing clinical cases of camelpox in younger

320   (under 1 year of age) camels than in the adult age group. Generally, young animals under 4 years

15

321 of age in a herd experience a more severe and generalized form of the disease resulting in high

322 mortality occasionally due to waning of acquired maternal immunity after 5–8 months (Nothelfer

323 et al., 1995; Mahmoud et al., 2012). Low basic reproduction number either indicates the disease

324 occurs at endemic level, or the age record obtained from the owners was not accurate resulting in

325 the underestimation of the parameter. Further studies may be needed to supplement the estimated

326 parameter.

327   We did not observe any statistically significant difference in the seroprevalence between

328 males and females. There are contradictory reports in the literature (Kriz, 1982; Khalafalla and

329 Mohamed, 1996; Mahmoud et al., 2012) with regards to the role of the sex of camel for

330 susceptibility to camelpox. However, we note that sex was disproportionately distributed in the

331 current study in which over 87% of the camels tested were females. Typically, under the pastoral

332 production systems female camels are kept for breeding stock and milk production whereas

333 males are sold for meat. Seropositivity was not significantly affected by herd size as well as by

334 study districts. These findings can be attributed to the contagious nature of camelpox virus which

335 spreads between animals within the same herd or between herds when camels congregate during

336 grazing and watering. In addition, the pastoral production system itself which is characterized by

337 migratory nature of camel herders coupled with sharing communal grazing and watering points,

338 as well as the close proximity of the two districts (Fig. 1) also contribute for the observed lack of

339 statistically significant variations among the herds, PAs and the districts. Under pastoral

340 production system it is difficult to consider herds as independent units, rather they comprise part

341 of a larger animal production ecology. Furthermore, selection bias (Dohoo, 2014) might have

342 been introduced at each sampling stages: districts-herds-animals as strict random sampling of

343 these units was not possible for logistical and practical reasons during this study. Nevertheless,

16

344   our findings suggest the widespread occurrence of camelpox in the Afar pastoral production

345   system, and that it is not limited to specific herds, PAs or districts.

346   **4.2. Participatory epidemiology**

347   Our participatory epidemiologic investigation identified the seasonal occurrence of camelpox

348   during the short and long rainy seasons. As explained by the informant groups, the high

349   incidence of camelpox during the rainy seasons is associated with the return of camels from

350   grazing places during the migration season in the previous dry season, and congregation of the

351   animals in small areas that favors the transmission of the disease. High humidity (hot

352   temperature and rain) during the minor rain season (*Sugum*) was also pointed as another

353   predisposing factor for the higher occurrence of the disease during this season. As indicated by

354   the informants, the disease clinically affects mainly young camels that are just allowed to graze

355   during the rainy seasons. Increased frequency of contacts with other camels, and grazing on

356   thorny plant species and bushes which are abundant during rainy seasons were also mentioned as

357   predisposing factors for the disease in young camels. Our participatory epidemiology based

358   finding generally agrees with previous reports that demonstrated the frequent outbreaks with a

359   more severe form of the disease during rainy seasons (Wernery et al., 1997a, 1997b; Wernery

360   and Kaaden, 2002; Khalafalla and Ali, 2007; OIE, 2014b). Consistent with our finding, a

361   relatively higher incidence of camelpox during *Sugum* (short rainy season) compared to *Kerma*

362   (major rainy season) has been reported (Megersa, 2010). The author suggested that moisture

363   enhances the mechanism of virus stability in the environment and increases subsequent

364   transmission to susceptible animals during the rainy seasons.

365   The occurrences of the other camel diseases including respiratory diseases, mange mites,

366   trypanosomosis, and ticks were associated with the long dry season, *Gilal* (September-February),

367   and short dry season, *Hagay* (May-June), as opposed to that of camelpox (Fig. 4). This can be

368   mainly attributed to feed shortage and subsequent starvation during dry seasons which

369   predispose animals to various infections. Contrary to the present finding of higher density of the

370   tick population during the long and short dry seasons (Fig. 4), the occurrence of camelpox with

371   the increased density of the tick population during the rainy season has been reported (Wernery

372   *et al.*, 1997a). This requires further study on the diversity of biological rhythms, diapauses,

373   biological clocks, daily and seasonal cycles utilized by arthropods (ticks and flies) for

374   adaptations under Afar arid and semi-arid environmental conditions. Further studies are also

375   recommended to ensure the involvement of arthropods in the transmission of camelpox virus

376   (Duraffour *et al.,* 2011).

377   Pastoralists have a clear knowledge of the seasonality of camelpox which agrees with

378   clinical and modern laboratory based scientific reports (Wernery et al., 1997a; Khalafalla and

379   Ali, 2007; Megersa, 2010). However, some camel diseases such as trypanosomosis were reported

380   to occur during the dry seasons by the informant groups, although it was known to occur

381   commonly during the wet season when the biting fly population is abundant (Luckins, 1988). Its

382   increased occurrence during the dry season as suggested by the informant groups can be

383   associated with feed and water shortages that can result in reduced immune status of camels,

384   with subsequent susceptibility to infections. The situation in camelpox was however different

385   which was reported to occur mainly during the rainy season that shows synergic finding with the

386   scientific reports based on standard epidemiologic investigations. Participatory epidemiology

387   played a significant role in identifying the seasonal occurrence of camelpox, and other important

18

388 diseases of camels in the pastoral area which otherwise would have been very difficult using

389 conventional epidemiologic studies.

390

391 **5. Conclusion**

392 Our results indicate the widespread occurrence of camelpox in Gabi Rasu zone of Afar region. In

393 addition, camelpox was identified as one of the top five common camel diseases with its seasonal

394 occurrence during rainy seasons. Targeted vaccination strategies before the start of the rainy

395 seasons in combination with improved camel husbandry management practices can improve herd

396 immunity thus reducing the disease burden. Since camels are the only reservoir host of camelpox

397 virus, camelpox can be targeted for eradication through national and international strategic

398 vaccinations and surveillance systems. The eradication effort requires the development of

399 effective heat stable camelpox vaccine for use in hot, dry and inaccessible camel rearing regions

400 of the world, and community involvement in the vaccination programs.

401

402 **Conflict of interest**

403 The authors declare that there are no conflicts of interest.

404

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522   virus from skin 12 months after onset of clinical signs reveals possible mechanism of virus

523   persistence in herds. Vet. Microbiol. 159, 320–326.

524   **Figure legends**

525   **Fig. 1.** Geographical location of Gabi Rasu zone and the study districts for seroepidemiological

526   and participatory epidemiology of camelpox in Afar region of Ethiopia.

527

528   **Fig. 2.** Seroprevalence of camelpox in camel herds in Afar region of Ethiopia.

529

530   **Fig. 3.** Model adjusted marginal seroprevalence of camelpox by age categories, and stratified by

531   the study districts.

532

533   **Fig. 4.** Seasonal calendar for the seasonal occurrences of the most common camel diseases in

534   Gabi Rasu zone, Afar region of Ethiopia. Number of informant groups = 12; W= Kendall's

535   Coefficient of Concordance ($^{ns}P > 0.05$ [non-significant]; $**P < 0.01$; $***P < 0.001$). W values

536   vary from 0 to 1.0; the higher the value, the higher the level of agreement between the informant

537   groups. The black dots represent the median scores (number of stones) that were used during

538   construction of the seasonal calendars; minimum and maximum limits are shown in parentheses.

539   Afar terms are italicized.

540

541 **Table 1** Number of camels sampled from each herd for seroprevalence study of camelpox in Afar, Ethiopia

		Amibara					Awash Fentale		
PA[*]	Herd id	Herd size	# sampled	% sampled	PA[*]	Herd id	Herd size	# sampled	% sampled
Halidegi	1	45	34	80	Deho	16	60	18	30
	2	20	14	70		17	40	17	40
	3	25	6	20		18	35	15	40
	4	120	26	20		19	60	15	30
	5	80	21	30		20	30	15	50
	6	55	24	40		21	20	10	50
Bedhamo	7	60	20	30		22	15	3	20
	8	45	3	10		23	17	7	40
Uduleisi	9	4	3	80	Sabure	24	200	20	10
	10	11	11	100		25	50	10	20
	11	7	7	100		26	65	15	20
	12	20	16	80		27	60	6	10
	13	7	4	60		28	30	6	20
	14	11	7	60		29	25	7	30
	15	9	8	90	Deho	30	40	6	20
						31	55	10	20

542 PA= pastoral association

28

543 **Table 2** Description of the study animals for the seroprevalence study of camelpox in two
544 districts in Afar region of Ethiopia

| | District | | | |
Variable	Amibara	Awash Fentale	Total	P-value
Number of herds	15	16	31	0.8587
Herd size (median, range)	45 (4-120)	50 (15-200)	45 (4-200)	0.05
Number of camels examined	204	180	384	0.2216
% Female	92.2	81.7	87.2	0.002
Age (in years; median, range)	8 (0.8-34)	3.75 (1-20)	5 (0.8-34)	<0.0001

545

546

547

548　**Table 3** Full model multilevel mixed effects logistic regression for the analysis of seroprevalence of camelpox in Afar region of

549　Ethiopia

Variables[#]		Coefficients	Standard error	P-value	95% confidence interval	
Herd size (number of animals)		0.004	0.0029	0.169	-0.002	0.01
Age*	6-10 years	0.15	0.3695	0.694	-0.58	0.87
	11-15 years	0.93	0.3511	0.008	0.24	1.62
	>15 years	0.96	0.4578	0.036	0.06	1.86
Sex=male		-0.372	0.515	0.470	-1.38	0.64
District=Awash Fentale		-0.132	0.278	0.635	-0.68	0.41
Intercept		-1.85	0.3137	-	-2.47	-1.24

550　[#]only the fixed effects part of the model is presented. The random effects, pastoral association, and herd had a variance of 0.00

551　indicating they are not significant.

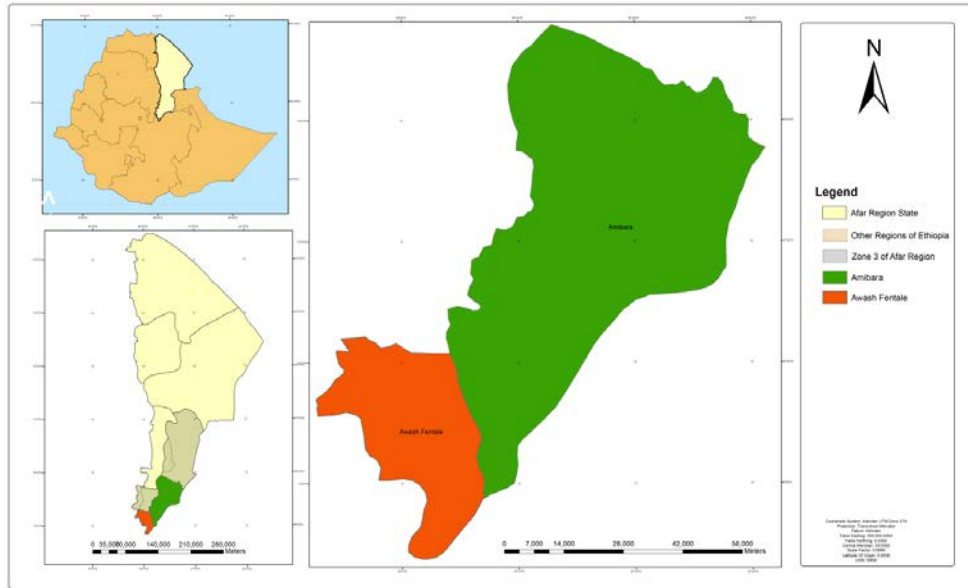552　*0-5 years old is a referent age category

553

554 **Table 4** Final multilevel mixed effects logistic regression model for the association between age

555 of camels and camelpox seropositivity in Afar region of Ethiopia.
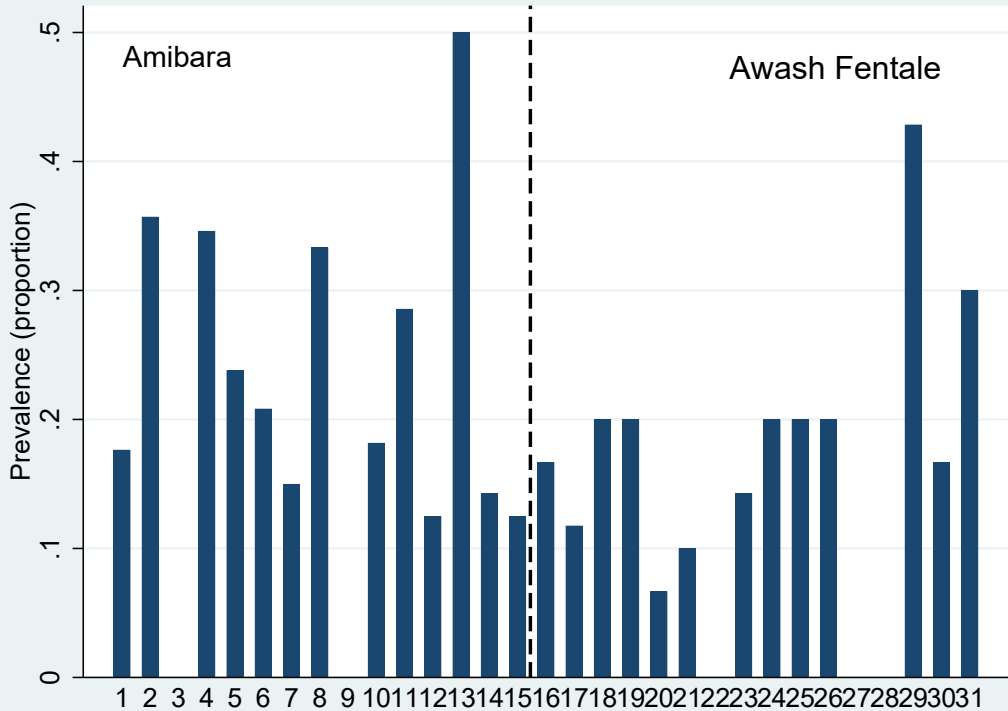
| Variable		Odds Ratio	Standard error	P-value	95% confidence interval	
Age*	6-10 years	1.3	0.4528	0.509	0.63	2.6
	11-15 years	2.7	0.8996	0.003	1.4	5.2
	>15 years	2.9	1.3072	0.016	1.2	7.0
District=Awash Fentale		0.85	0.2331	0.545	0.5	1.5

556 *0-5 years old is a referent age category. Results are adjusted for clustering effects of herds,

557 pastoral associations and districts.

558

Legend
- Afar Region State
- Other Regions of Ethiopia
- Zone 3 of Afar Region
- Amibara
- Awash Fentale
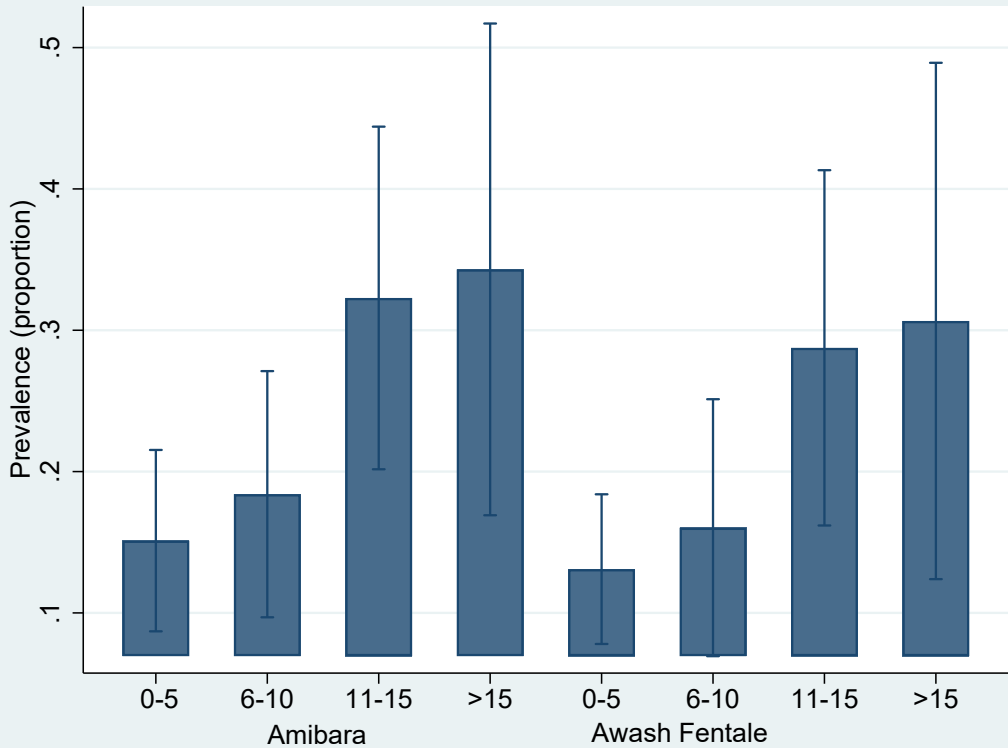
Diseases	Afar seasons			
	Kerma (July-August)	*Gilal* (September-February)	*Sugum* (March-April)	*Hagay* (May-June)
Galideli Abscess (W = 0.17 ns)	• • • • • • • • • • • 10.5 (2-19)	• • • • • • • • • • 10 (5-24)	• • • • 4 (2-9)	• • 2 (0-15)
Galiguduf/Ambraruk Camelpox (W = 0.67***)	• • • • • • • • • • • • • • 13.5 (2-22)	0 (0-2)	• • • • • • • • • • • • • • • 16 (8-28)	0 (0-3)
Kahu/Bahu Respiratory disease (W = 0.49**)	• 0.5 (0-6)	• • • • • • • • • • • • • • • • • • • • • • • • • • • 25 (18-30)	• 0.5 (0-6)	• • 2 (0-15)
Agara Mange (W = 0.59***)	0 (0-4)	• • • • • • • • • • • • • • • • • • • • • • • • • • • • 26 (15-30)	0 (0-4)	• • • 2.5 (0-15)
Dahan Trypanosomosis (W = 0.51**)	0 (0-8)	• • • • • 5 (0-22)	0 (0-4)	• • • • • • • • • • • • • • • • • • • • • • • • • • • 24 (8-30)
Kilim Ticks (W = 0.59***)	• 1 (0-4)	• • • • • • • • • • • • • • 12.5 (5-20)	• • 1.5 (0-4)	• • • • • • • • • • • • • • • 15 (6-23)