1 **¹CROSS SECTIONAL STUDY OF MIDDLE EAST RESPIRATORY**
2 **SYNDROME (Mers-Cov INFECTION) IN CAMELS AT SELECTED SITES OF**
3 **AMIBARA DISTRICT, AFAR REGION, ETHIOPIA**

4
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31 **Abstract**

32 **Background**

33 *A Cross sectional study of Middle East Respiratory Syndrome Corona virus (MERS-CoV)*
 34 *in Camel was conducted between February 2018 to April 2019 in three selected sites of*
 35 *Amibara district of Afar region, Northeast Ethiopia. The study was aimed to observe the*
 36 *current sero-prevalence status of MERS-CoV, assess the presence of active cases through*
 37 *detection RNA Viral particle and investigate possible risk factors of MERS-CoV in camels.*
 38 *A total of 589 sera were collected and tested with indirect Enzyme linked ImmunoSorbent*
 39 *Assay (iELISA).*

40 **Result**

41 *The overall seroprevalance of MERS-CoV was 87.3% (n=514/589, 95% CI: 84.5-*
 42 *89.9). Association of different risk factors with seroprevalance revealed that origin*
 43 *($X^2=13.39, P=0.001$), sex ($X^2=4.5$ $P=0.034$), age ($X^2=185.7$, $P=0.001$) season*
 44 *($X^2=41.7$, $P=0.000$) and reproduction status ($X^2=96.1$, $P=0.001$) displayed a*
 45 *statistical significant difference among the groups ($P<0.05$) while herd size did not*
 46 *show a Significant difference among groups ($p>0.05$). In multivariable logistic*
 47 *regression analysis, age (OR=7.39, 95% CI:3.43-15.91), season (OR=4.83, 95% CI:-*
 48 *2.14-10.90), and in adult female camel reproduction status (OR=7.39,95% C*
 49 *I:3.43-15.91) showed statistically significant difference among the groups for MERS*
 50 *CoV antibody detection while risk factors of origin, animal sex and herd size*
 51 *difference were statistically insignificant. A total of 857 nasal swab samples were*
 52 *collected for the detection of MERS-CoV RNA particle. However, all swab samples*
 53 *tested by Real-time reverse transcription polymerase chain reaction (RT-PCR)*
 54 *technique were Negative for the virus.*

55 **Conclusion**

56 *In conclusion, the present study revealed a high seroprevalance of MERS CoV in*
 57 *adult camels. However, in spite of high seroprevalance the lack of any RNA viral*
 58 *particle in the study suggests the need for further in depth longitudinal study to*
 59 *detect the circulating virus focusing on juveniles and young camels whereby*
 60 *seroprevalance of antibody is low when compared with adult camel in order to get*
 61 *the active virus before the camel develop antibody. Moreover, the zoonotic significance*
 62 *and potential transmission routes of MERS CoV to pastoral communities should also*
 63 *be investigated and design strategy for the preparedness in control of the diseases*
 64 *in Ethiopia.*

65 **Key words:** *Afar, Amibara, Camel, Cross sectional, Ethiopia, MERS-CoV, Sero*
 66 *prevalence*

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69 **INTRODUCTION**

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71 The one-humped camel (*Camelus dromedaries*) is an important livestock species
 72 exceptionally adapted to hot, dry and harsh environment due to heat and
 73 water deprivation tolerance. These tolerances in camels appear to be due to behavioral
 74 response that reduces heat absorption, a relatively efficient sweating mechanism
 75 for heat dissipation, an ability to reduce fecal and urine water loss and the ability
 76 to vary body temperature substantially. It is used for milk and meat production,
 77 transportation, and draught power [1]. Camels are widely distributed in Ethiopian
 78 lowlands especially in Afar, Somali and Oromia region where by pastoralism is
 79 the dominant mode of life and mobility is an inherent strategy to efficiently utilize

the spatially and temporally distributed pasture and water resources. Usually, large numbers of camels and other domestic animals from many different herds/flocks congregate at watering sites, and this may create a perfect condition for disease transmission and spread among animals. The same water sources are also shared by multitudes of wild animals [2]. According to CSA 2016/17 report, the camel population of dromedaries in Ethiopia is estimated to be about 1,209,321. Afar region has 474,146 camels [3] .

Middle East Respiratory Syndrome (MERS) is a viral respiratory diseases within the largest group of Corona viruses (CoVs) belonging to Nidovirale order which includes Coronaviridae, Arteriviridae and Ronaviridae families. The coronavirinae are further divide into four groups the alpha, beta, gamma and delta coronaviruses. MERS CoV is within beta corona virus group [4]. Dromedary camels are sturdily suspected of acting as a zoonotic source for human cases of MERS-CoV, by either direct contact through droplet infection via mucous membranes or indirect contact through milk, meat or urine. According to, Miguel *et al.*, (2016) five major points reason out accounts that suggest dromedary camels can play an important role in the epidemiology of MERS-CoV, possibly as a reservoir host:

- Corona-viruses are widespread in the animal kingdom (in bats and livestock), but MERS-CoV does not infect many of the hosts (e.g. sheep, goats, cattle, chickens, water buffaloes, birds, horses and) whereas high levels of sero positivity have been observed in dromedary camelids, ranging from 0% in

103 Asia to as much as 100% in Africa and the Arabian Peninsula (with mean of
104 79%);

- 105 • The Mers-Cov isolated from dromedaries are genetically and phenotypically
106 very similar to those infecting humans;
- 107 • Retrospective serological studies in Africa going back more than 30 years
108 indicate long-term circulation of the virus in dromedary camels;
- 109 • Infection in dromedaries causes no or only mild respiratory symptoms,
110 making it difficult to detect;
- 111 • Mers-Cov genome has likely undergone numerous recent recombination',
112 which suggests frequent co-infection, probably in camels, with distinct
113 lineages of Mers-Cov [5].

114 Studies have demonstrated that dromedary camels can act as a source of human
115 MERS-CoV infection. Indeed, the current state of knowledge indicates that
116 dromedary camels are the only animal species for which there is convincing evidence
117 that they act as host species for Mers-Cov and hence a potential source of human
118 infections [6]. Nonetheless, the route of infection of MERS CoV and types of
119 exposures remain largely unknown, and only a small proportion of the primary cases
120 have reported contact with camels. Other possible sources and vehicles of infection
121 include food-borne transmission such as unpasteurized camel milk and raw meat, and
122 medicinal use of camel urine [7]. Clearly, transmission from camels to humans
123 does take place, and camel exposure is a risk factor for human infection, but such
124 transmission is not efficient and infection is not directly proportional to exposure while
125 in the other hand, many patients with clinically diagnosed MERS did not have an

126 obvious history of direct exposure to camels or their products [8].

127

128 Researchers found high percentages of animals sampled from Nigeria and Ethiopia
129 being seropositive for Mers-Cov with an overall seropositivity of 94% in adult
130 dromedaries in Nigeria and 93% and 97% for juvenile and adult animals, respectively,
131 in Ethiopia [9]. More recently, [10] other researchers displayed a high seropositivity
132 of 99.4% in camel of Ethiopia and also relatively higher Mers-Cov RNA detection
133 in Ethiopia (15.7%) than in Burkina Faso (12.2%) and Morocco (7.6%). Also 10.6%
134 virus detection rate observed by a study in Ethiopia as described by journals [11].

135

136 Other authors also described 93% seropositivity and 7% (n =7/100) MERS CoV RNA
137 detection in Ethiopia, Afar region camels [12]. However, data from experimental
138 camel infections conducted in the Middle East suggest that Mers-Cov causes only
139 mild respiratory infection in camels [13]. Also study in Ethiopia between 2010-
140 2011 reported 93-97% seropositivity [9].

141

142 In Ethiopia, in spite of the high prevalence of Mers-Cov antibodies in camel as
143 indicated in different studies, no human case has been reported to date, and only
144 few ongoing studies have been carried out to investigate public health significance of
145 MERS in highly exposed pastoralist community of Ethiopia who have close contact
146 with camels requires serious attentions for further surveillance both for camel and
147 exposed human population. So based on the mentioned points the objectives of the
148 study were:

- 149 ▪ To determine the current seroprevalance of MERS-CoV in camels with in
- 150 selected sites of Amibara district, Afar Region.
- 151 ▪ To identify the potential risk factors for MERS CoV in camels in order to
- 152 control the disease.
- 153 ▪ To detect and characterize MERS CoV from nasal swab of camels in the study
- 154 sites.

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156 **RESULT**

157 **Sero-prevalence of MERS CoV antibody**

158 Based on Indirect ELISA test results the overall prevalence of MERS CoV antibody in
 159 camels at study sites was 87.3% (n=514/589) (95% CI:84.5-89.9%). Association of different
 160 risk factors to seropositivity status of camels using X^2 analysis revealed that there was a
 161 statstically significant difference in proportion of MERS Cov antibody positivity among the
 162 three study sites ($X^2=13.7$, $p=0.001$); Age categories ($X^2=185.69$, $p=0.000$); sex categories
 163 ($X^2=4.5$, $p=0.034$) and season ($X^2=41.69$, $p=0.000$); and in reproduction status of adult
 164 female ($X^2=96.13$, $p=0.000$); while no statistical significant difference were observed
 165 between herd sizes ($X^2=5.88$, $p=0.053$) as illustrated in table 1.\

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173 1 : Association of different risk factors to seropositivity of camels MERS-CoV

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Risk factors	No of tested camel	No of Positive	Prevalence (%positive)	X² value	p-value
Origin				13.39	0.001
Andido	289	266	92		
Melka sedi	149	127	85.2		
Angelele	151	121	80.1		
Sex				4.50	0.034
Male	55	43	78.2		
Female	534	471	88.2		
Herd size				5.88	0.053
Small	100	83	83.		
Medium	168	155	92.3		
Large	321	276	86		
Age				185.69	0.001
Juvenile	89	39	43.8		
Young	123	108	87.8		
Adult	377	367	97.3		
Season				41.69	0.001
Winter (December –February)	88	79	89.8		
Autumn (September-November)	272	260	95.6		
Summer (June –August)	229	175	76.4		
Dry	162	157	96.9		
Pregnant	68	64	94.1		
Lactating	146	145	99.3		

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177 In multivariable logistic regression analysis young age (OR=7.39, 95% CI: 3.43-15.91),
 178 season from September -November (OR=4.83, 95% CI: 2.145-10.90), and in adult female
 179 camel lactation status ((OR=10.75, 95% CI: 1.15-100.08)) showed a statistically significant
 180 difference among the groups for MERS CoV antibody detection while risk factors of
 181 origin, animal sex and herd size did not show a statistical significant difference as indicated
 182 in table 2.

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Table 2: Multivariable Logistic regression analysis of MERS CoV prevalence

Risk factor	No of tested	No of positive and prevalence (%)	Crude OR (95% CI)	Adjusted OR (95% CI)
Origin				
Angelele	151	121 (80.1)	1	1
Melka sedi	149	127(85.2)	1.43(0.78-2.62)	1.03(0.44-2.41)
Andido	289	266 (92)	2.87(1.60-5.14)	1.00(0.42-2.41)
Sex				
Male	55	43 (78.2)	1	1
Female	534	471 (88.2)	2.09(1.04-4.16)	0.86(0.35-2.14)
Herd size				
Small	100	83 (83)	1	1
Medium	168	155(92.3)	2.44(1.13-5.27)	2.35(0.89-5.15)
Large	321	276 (86)	1.26(0.68-2.31)	1.51(0.71-3.55)
Age				
Juvenile	89	39 (43.8)	1	1
Young	123	108 (87.8)	9.23(4.55-18.28)	7.39(3.43-15.91)*
Adult	377	367 (97.3)	47.05(22.11-100.10)	21.91(0.27-1743.85)
Season				
Summer (June – August)	229	175 (76.4)	1	1
Autumn (September –November)	272	260 (95.6)	5.59(3.47-12.85)	4.83(2.14-10.90)*
Winter (December – February)	88	79 (89.7)	2.71(1.27-5.76)	4.10(1.30-12.86)*
Production Status(Females)				
Pregnant	68	64 (94.1)	1	1
Lactating	146	145 (99.3)	9.06(0.99-82.59)	10.75(1.15-100.08)
Dry	162	157 (96.9)	1.95(0.51-7.54)	2.49(0.53-9.92)

*Note: Risk factors displaying significant difference in Multivariable Logistic regression

204 **Viral RNA detection**

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206 All tested nasal swabs samples were negative for MERS CoV RNA particle by Real time
207 polymerase chain reaction (RT-PCR) both at NAHDIC, Ethiopia and Hong Kong
208 University (HKU).

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210 **DISCUSSIONS**

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213 Middle East respiratory syndrome (MERS) is a zoonotic disease of global health concern,
214 and dromedary camels are the source of human infection. In Ethiopia, a high
215 seroprevalance of MERS-CoV in camel have been reported ranging from 93-97% in
216 pastoral camel rearing areas of the country [9]. In the current study, a high prevalence of
217 MERS-CoV with 87.3% (n=514/589), (95% CI: 84.5-89.9) was observed in camels of
218 Amibara district, Afar Region. This high seroprevalance result was in agreement with
219 previous studies in pastoral areas of Ethiopia who reported 85.1- 99.4% in camels of Afar
220 and Oromia [5], 92.3% in Afar [12] 93-97% in Afar, Somali and Oromia regions [9].

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222 In multivariate logistic regression analysis three significant factors were observed in MERS
223 CoV prevalence. Age; OR=7.39 (95%CI 3.43-15.91) with in this factor Adults >3 year are
224 with high prevalence 97.3 %, young camels 1-3 years 87.8 % and Juvenile <1 year age
225 43.8 %. This study agree with previous study done by [10] in which antibodies detection
226 rates were higher in older animals while Viral RNA was higher in young camels whereby
227 they are free from antibody.

228 The reproduction status of female camels showed a considerable variation with OR=10.75(
229 95%CI 1.15-100.08).With this result pregnant camels were being sated with low sero

230 prevalence 94.1% when comparing with, Dry (96.9%) and lactating camels with (99.3
231 %). From this analysis we observe that high seroprevalance antibodies prevail in lactating
232 camels when comparing with pregnant camels [10].

233

234 Seasonal variation observed in this study (OR =4.83) illustrate high sero-prevalence is
235 prevailed (95.6%) in autumn (September, October and November); (76.4%) in summer
236 (June, July and August) and 89.7% winter (December, January and February). The high
237 prevalence in autumn was due to gathering of camels at one place for prolonged period for
238 the reason that camels are getting sufficient vegetation and grass .For this reason there had
239 been high probability of infection and which induces he development of natural infection
240 antibody. In winter the prevalence is low due to camels are dispersing far places in search
241 of feed and water due to scarce of feed at one place. In this season the possibility of close
242 contact and getting the disease through aerosol and developing antibody is limited.

243

244 Regarding seasonal factors, high seroprevalance was recorded in Autumn (September,
245 October and November) in which prevalence was recorded (n=260/272) (95.5%) ,
246 subsequent winter Dry season (December ,January and February) with prevalence of
247 (n=79/88) (89.7%) and then the relatively low prevalence was seen in summer (n=174/229)
248 (75.98%). The result indicates that there is significance difference related to the season of
249 the study $P<0.05$ (0.000). High seroprevalance was observed in medium herd size 92.3%
250 (n=155/168) subsequently large herd size 86% (n=276/321) and in the last part small
251 herd size 83% (n= 83/100). The result indicates that there is no significance
252 difference related to the herd size of the study $P>0.05$ (0.053) as shown in table 1.

253

254 This analysis also coincides with previous studies Camels in the larger herd size have
 255 slightly higher prevalence (n=324/347) (93.4%) than the small herd sized 92.3%
 256 (n=205/222), [12]. But the difference between the herd's categories was not statistically
 257 significant the current study have little variation in the prevalence. Sero-prevalence
 258 of MERS-CoV in relation to production status was highly significant. With the study high
 259 prevalence was seen in lactating camels (n=145/146) (99.3%) following dry camels
 260 (158/163) (96.9%) consequently Pregnant camels (n=62/66) (93.9%) at the last N/A
 261 (young and Juveniles) Sero positivity indicates (n=149/214) (69.6%). In general, the result
 262 denotes that there is significant difference in sero positivity ratio among different
 263 production status of camels. The result indicates that there is significance difference $P < 0.05$
 264 as indicated in table 1 by which the juvenile with lactating camels may shed the virus and
 265 by transmitting the virus develop Sero positivity for MERS CoV.

266

267 Despite high Sero-positivity of MERS CoV antibody, the virus couldn't be detected in the
 268 current study. This has been due to the development of MERS CoV antibody by large
 269 number of camels [10]. However in previous studies at Afar area (Fekadu *et al.*, 2017) (n=
 270 7/100 (7%) of samples had detected by RT- PCR technique which was an indicative for
 271 the existence of circulating virus where it can be an evident for high sero positivity. Higher
 272 virus RNA detection rate in young animals compared with older animals could be related
 273 to a lack of prior immunity as published in previous studies in Saudi Arabia. Young animals
 274 were naïve and more susceptible to virus infection [10].

275

276 CONCLUSION

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278 From the current study, there was clear evidence for overall high Sero-positivity of MERS-
279 CoV in the study sites of Amibara district which was 87% (n=514/589). Among the study
280 sites (Andido=45.16%, Melka Sedi 21.56 % and Angellele 20.54 %. Within the risk factors
281 Age, Production status and season have significant difference in multivariate analysis for
282 the prevalence of MERS CoV antibody.

283

284 The correlations of different risk factors were assessed in this study. In doing so, almost all
285 risk factors were highly associated and were an important determinant for the disease In
286 this study despite high Sero prevalence of MERS CoV antibody, the viral RNA is not able
287 to be detected by RT- PCR test both at NAHDIC and HKU referral laboratories as previous
288 studies indicated. This result disagree with the past studies high MERS CoV RNA rate
289 detected in Ethiopia up to 15.7% ;(C.I. 95%, 8.2-28.0) [10]. In another study MERS CoV
290 RNA with 7% was detected in Ethiopia between October 2014 and May 2015 [12].

291

292 The possible causes for not getting /detecting the Viral RNA in the study area would be
293 due to the following factors and challenges:-

294

295 Lack of sufficient information in understanding the viral shedding period or incubation
296 time of the disease, lack of observation for apparent form of clinical sign of MERS CoV
297 on camels as to enable taking the swab sample at early time of the disease, difficulty in
298 deep swab sample taking process due to far distance of posterior turbinate of elongated

nasal cavity of camels whereby it is the virus replication site compared to application swab stick length.

301

Based on the above conclusion the following recommendations are forwarded:-

303

Further study on the disease should be conducted in the study area by considering all aspects of the disease including in identifying other risk factors which will have value in the control of the disease.

307

Even- though that, priority is given for swab sampling from nasal cavity of camel due to nature of replication site of virus; milk, urine and feces might be appropriate samples to detect the virus . Hence, these samples should be included at sampling.

311

Camel abattoirs /slaughter houses to be included in taking swab samples from slaughtered camels to get access to the deep of nasal turbinate in getting the virus.

314

A study to be considered by repeated swab sampling or as longitudinal study and focus of sampling to be given to well-marked and known juvenile and young camels as they are considered that, most of them are not developing MERS CoV antibody. This intensifies a chance of getting active virus to understand the virus characteristics.

320

Since MERS CoV is one of the recently recognized zoonotic disease & camels are the sources of the virus to humans’ public awareness about the disease should be created in camel rearing pastoralist area.

322

323

324 MATERIALS AND METHOD

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326 Study localities

327 A cross sectional study was carried out in Amibara districts of Afar region, Ethiopia Map
328 of study sites for Amibara district Amibara sites as illustrated in figure 1. The district is
329 located at latitude: 9° 39' N. Longitude 40°19'E within Administrative Zone three of Afar
330 region bordered to the south by Awash Fentale district, to the west by Awash River which
331 separates it from Dulecha, on the northwest by the Zone five administrative, to the north
332 by Gewane, to the east by the Somali Region, and to the south east by Oromia Region.
333 Amibara district has an average altitude of 867 m.a.s.l. Within the district, three study sites
334 (Angellele, Melka Sedi, and Andido) were selected based on camel population density and
335 being not previously studied.

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339 Figure 1: Map of study sites for Amibara district

340

341 **Study design and population**

342

343 A cross sectional study design was used to assess the seroprevalance of MERS-CoV in
344 Amibara. The target populations for the study were dromedary camel of all age groups,
345 (juvenile, young and adult) and both sexes (male and female). Camel population in
346 Amibara district was 148,769 [14]. The herd size of study population was composed of,
347 high >30, medium =11-30 and low/small number of camel herds. =1-10 and the age
348 categories is described as Juvenile <1 year, Young 1-3 years, Adult >3years [15].

349 **Sample Size Determination and Sampling**

350 The sample size determined for serological study was calculated by considering previously
351 achieved epidemiological investigation of MERS-CoV with an expected prevalence of
352 (92.3%) in the study area [16]. Thus, the calculated sample size using a 95% confidence
353 interval at 5% absolute precision was 95% using the formula as described by [17]. The
354 total sample size in camels was 110. Increasing the sample size was considered to increase
355 the precision.

356

$$357 \quad n = \frac{1.96^2 (P_{exp}) (1 - P_{exp})}{d^2}$$

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$$361 \quad n = \frac{1.96^2 (92.3) (1 - 0.923)}{0.05^2}$$

362

363

$$364 \quad n = \frac{3.84 (.923) (1 - 0.923)}{0.0025}$$

365

366

$$367 \quad n = 110 \text{ camels}$$

368

369 The increasing of sample size by 5 fold to enhance the precision of sampling and hence
370 total sample was 589.

371 **Sampling techniques**

372 Camels are restrained in all cases before sampling. Adequate health safety measures like
373 wearing hand gloves, overall and mouth masks had been used at sampling site while
374 sampling.

375 ***Blood sample for sera harvesting***

376 Blood Samples were taken in duplicate from camels of each study three site. 10 ml of blood
377 sample was collected from jugular vein using sterile needle and plain Vacutainer tube .The
378 blood was allowed to clot at room temperature. Serum was separated from the clot by
379 centrifugation at 3000rpm for 3 min and transferred to 2 ml cryo vial with a volume of 1.5-
380 2 ml sera. The separated serum was labeled and kept under refrigeration (-20°C) until
381 transported to NAHDIC for laboratory analysis both at NAHDIC and HKU. A total 589
382 sera were collected.

383 ***Nasal Swab sampling for detection of the virus***

384
385 A total of 857 nasal swabs samples were collected in duplicate (for NAHDIC and HKU)
386 by using applicator cotton swab [18]. The swab was taken for deep lateral turbinate. After
387 taking sample, the swabs are immersed into 2 ml cryo vial containing 1.2 ml Viral transport
388 medium (VTM) & preserved in liquid Nitrogen at -196°C until transported to NAHDIC
389 for keeping at -80°C freezer. Finally the swabs samples belonging to NAHDIC were tested
390 in molecular laboratory and the other swab samples were shipped to HKU laboratory for
391 MERS CoV RNA detection.

392

393 **Laboratory analysis**

394 MERS CoV antibody detection through indirect ELISA test

395 The MERS CoV antibody detection was carried out using the indirect ELISA test which is
396 EUROIMMUN Anti –MERS-CoV S1 ELISA Camel (IgG) kit AG product of Lübeck,
397 Germany according to manufacturer’s instructions [19].

398 ***Virus detection through RT –PCR***

399

400 The Real-time polymerase chain reaction (RT-PCR) was used for detection of RNA of
401 MERS-CoV. RNA extraction was carried out as described by the manufacturer instruction
402 [20]. Screening of the upstream of envelope gene (UpE) was done using UpE- FWD primer
403 (GCAACGCGCGATTTCAGTT) and UpE-Rev primer (GCCTCTACACGGGACCCATA)
404 by reverse transcription quantitative PCR (RT-PCR) hydrolysis probe assay [10].

405 **Data analysis**

406 The Data obtained from the investigations was coded and stored in Excel spread sheets.
407 The data was analyzed using STATA software version 15.0 software. Logistic regressions
408 reporting the odd ratio at 95% confidence interval were used to determine the level of
409 variation between the Sero-prevalence and the independent variable factors. The
410 association of the explanatory and outcome variables was also analyzed by Chi² test where
411 p<0.05 indicates the significance level of the risk factors.

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416 **ABREVIATIONS**

417	DNA	Deoxy ribonucleic acid
418	DPP	Dipeptidyl peptidase
419	E	Envelope protein
420	ELISA	Enzyme Linked ImmunoSorbent Assay
421	FAO	Food and Agriculture organization
422	HKU	Hong Kong University
423	IELISA	Indirect Enzyme Linked Immuno-Sorbent Assay
424	M	Matrix protein
425	MERS-CoV	Middle East Respiratory Syndrome Corona virus
426	N	Nucleocapsid protein
427	NAHDIC	National Animal Health Disease Investigation Centre
428	NSP	Non-structural protein
429	ORF	Open reading frame
430	P	Protein
431	RNA	Ribonucleic acid
432	RT-rtPCR	Reverse transcriptase real-time polymerase chain reaction
433	RT -PCR	Real time polymerase chain reaction
434	S	Spike-(surface glycoprotein)
435	SARS	Severe Acute Respiratory Syndrome
436	SP	Structural protein
437	UpE	Upstream Envelope
438	URT	Upper Respiratory Tract

439 **Declarations**

440

441 **Ethics approval and consent to participate**

442

443 Ethical clearance is approved and got permission from National Animal Health Diagnostic
444 & Investigation Center (NAHDIC) Animal Research Scientific and Ethics Review
445 Committee (ARSERC).

446 **Consent for publication**

447 Not applicable

448 **Availability of data and materials**

449 The data and materials are available.

450

451 **Competing interests**

452 None of the authors of this paper have a financial or personal relationship with other people
453 or organizations that could inappropriately influence or bias the content of this paper by
454 any means.

455 **Funding**

456 The study was sponsored by FAO, Ethiopia.

457

458 **Authors' contributions**

459

460 D.S contributed in sampling, epidemiological data gathering, laboratory tests, data
461 acquisition, statistical analysis and drafting of the manuscript. F.A involved in the
462 designing of the study, D.Z. Analysis of data and write up of the manuscript A.M. for lab
463 test and analysis, G.M. contributed critical data analysis, interpretation and critical revision

464 of the manuscript. E.W contributed participation in field work, data management and
465 provision of logistics apart from these all authors included with in the manuscript
466 contributed in editing and offering comment and approved the final version of the
467 manuscript.

468 **Acknowledgements**

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470 The authors would like to thank National Animal Health and Disease Investigation Centre
471 (NAHDIC) for allowing the laboratory facility in doing the tests, Food and Agricultural
472 Organization (FAO) , Ethiopia for the financial support for running costs of field activity
473 and provision of laboratory materials and covering the costs for shipment and diagnostic
474 costs of samples at H.K.U. Thanks also to H.K.U for generous cooperation of sample
475 testing , analysis and offer a training in PCR and serology diagnostic techniques. Many
476 thanks to Amibara Agricultural and Livestock staffs Dr Mekonnen, Seid, Million and
477 Hummed for their cooperation in mobilizing and convincing camel owners of the pastoral
478 community. In sample collection from camels.

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