

1 Short communication
2 **Serological evidence of Hepatitis E Virus in**
3 ***Dromedary camels* in the Sahelian zone of West**
4 **Africa**

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8 **ABSTRACT**

9 **Aim:** This study was undertaken to determine the sero-epidemiological information and associated risk factors, of Hepatitis E virus (HEV) among dromedaries in Sub-sahelian region.

Study design: Cross sectional epidemiological survey.

Place and Duration: The study was carried out in three countries of West Africa (Burkina Faso, Mali and Niger), over a two month period from February to March 2015.

Methodology: A total of 133 serum samples collected from dromedaries (*Camelus dromedarius*) were examined for the detection of anti-DcHEV total antibody by a mammals targeted ELISA kit (ID vet, France), using a multispecies conjugate.

Results: Eleven of the 133 (8.33%; 95%CI [3.6 – 13.0]) dromedaries were positive for anti-DcHEV antibodies. Positive cases had a random geographic distribution ($p>0.05$) and all seropositive dromedaries were in close contact with other domestic animals.

Conclusion: This study highlight the circulation of HEV in West African mammals and specifically in dromedaries. This is the first study reporting HEV circulation in dromedaries in West Africa. Further studies is needed to identify the HEV genotype involved in the dromedary camel infection, and evaluation of potential transmission to dromedary's care keepers.

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12 *Keywords: Zoonosis; dromedary camels HEV; anti-DcHEV total antibodies; risk factors; West Africa.*

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16 **1. INTRODUCTION**

17 Hepatitis E virus (HEV), the one of the most common cause of acute viral hepatitis worldwide [1], is
18 mainly transmitted via the fecal-oral route and blood transfusion, and is well recognized as a zoonotic
19 pathogen. The causative agent of Hepatitis E (HE) is a small, quasi-enveloped, single-stranded
20 positive-sense RNA virus, causing more than 90% of HEV infection cases which is asymptomatic and
21 self-limiting [2]; however, in specific vulnerable groups, the outcome of HEV infection can be much
22 more severe, especially in pregnant women in the third trimester and can become chronic in

23 immunocompromised individuals, such as those receiving organ transplants or chemotherapy and
24 individuals with HIV infection [3, 4].

25 Considerable advances have been made towards an understanding of the epidemiology of HEV
26 variants. Usually, HEV infection is mainly linked to contamination water supplies with human fecal
27 material and represents person-to-person transmission [5]. However, hepatitis E may also be
28 predominantly a foodborne zoonosis, which is most commonly transmitted to humans consuming
29 meat or milk of animals including pork, wild boar, deer, rabbits and camels [3, 6, 7].

30 *Hepeviridae* contains several viral species divided into two genera: *Orthohepevirus* with four species
31 (A–D) and *Piscihepevirus* with one species (A) [8]. Eight genotypes exist within *Orthohepevirus A* and
32 these HEV strains infects humans and multiple mammals' species. HEV1 and HEV2 are restricted to
33 humans; HEV3 is found among humans, swine, rabbits, deer and mongooses; HEV4, which circulates
34 between humans and swine; HEV5 and HEV6, which are found in wild boars; and HEV7 and HEV8,
35 which were recently identified in dromedary and Bactrian camels, respectively [2, 3]. HEV-7 has also
36 been isolated from a hepatitis E patient suggesting that *Hepatitis E Virus from dromedary camels*
37 (*DcHEV*) could cause zoonotic infection in humans [6]. Moreover, detection of partial DcHEV genome
38 in camel serum or fecal samples in the UAE, Somalia, Kenya, and Pakistan during the period 1983–
39 2015, suggests that DcHEV in dromedary camels is long established, diversified and geographically
40 widespread [9].

41 In the Sahelian zone of West Africa, dromedary camels are very important livestock animals. Indeed,
42 they have an important socio-economic and cultural role in improving survival of the desert dwellers.
43 According to *Food and Agriculture Organization (FAO)*, the dromedary population in West Africa is
44 estimated at 2, 140, 000 animals. They provide food (milk, meat), fibre (wool, leather) and draft
45 power (for transportation, cultivation and tourism). However, camels being reservoirs of many
46 bacterial and viral diseases transmissible to humans, as *Middle East Respiratory Syndrome (MERS)*
47 the most frightening [10, 11], either by food byproducts or by promiscuity, it is important to provide
48 data on the sero-epidemiology of DcHEV in this area. The present work is part of a vast campaign of
49 detection of animal reservoirs of HEV. The aims of this study was to establish the circulation of HEV
50 in dromedary living in West Africa and analysis behaviors that could promote the spread of the virus in
51 human population.

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56 **2. MATERIAL AND METHODS**

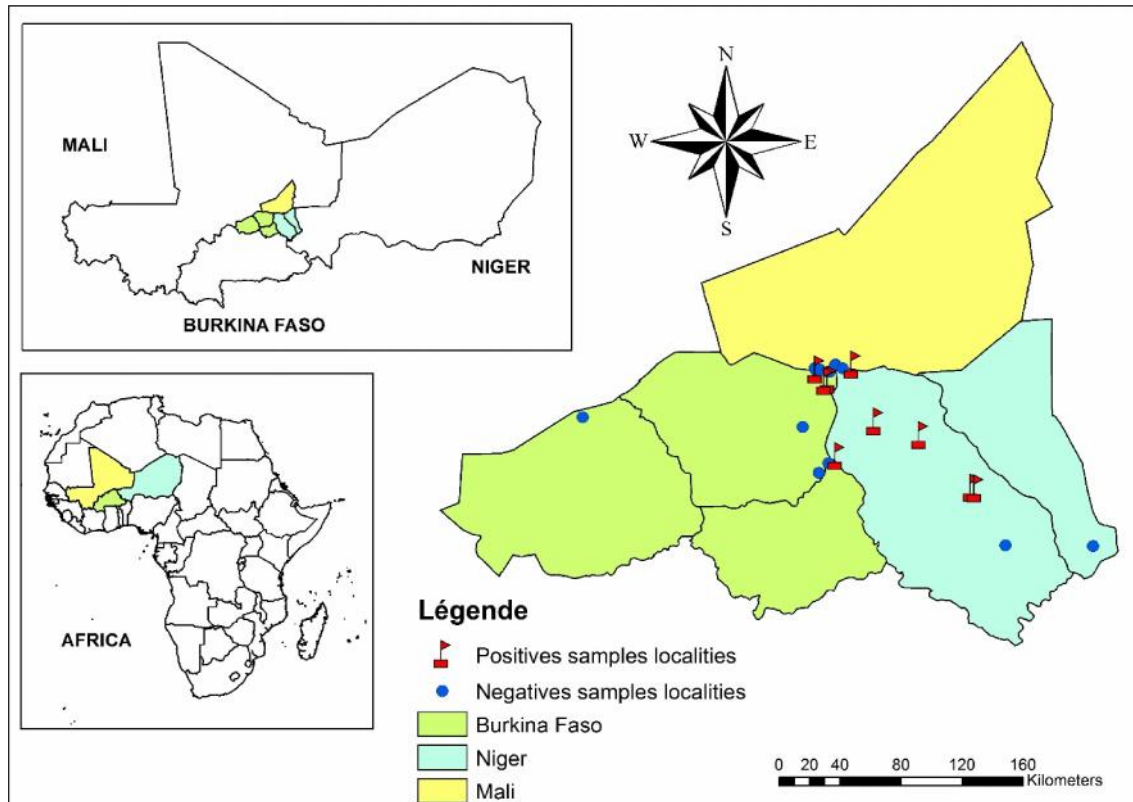
57 **2.1 Study Design**

58 Cross sectional epidemiological survey.

59 **2.2 Place and Duration**

60 The study was carried out from February to March 2015 in the sahelian area of West Africa grouping
61 three countries: Burkina Faso, Mali and Niger (Figure 1).

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64 **Fig. 1. Geographical location of the sampling site and positive cases of anti-HEV antibodies**
65 **circulating in camels in West Africa.** The positives cases are represented by a red color in the
66 figure.

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68 **2.3 Ethics statement**

69 The protocols for specimen collection and use were submitted and approved by the Ministry of
70 Environment and fish Resources of Burkina Faso by the ministerial arrest n°2014 2015 – 001 /
71 MERH/CAB.

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73 **2.4 Data collection**

74 A Questionnaire based on direct and indirect questions to obtain demographic characteristics such
75 as age, sex, as well as possible associated risk factors was administered and filled by consenting
76 animal owners/handlers before sample collection. Those who could neither read nor write were
77 assisted using the local lingua franca.

78 **2.5 Sample collection**

79 Blood samples from 133 (68 males and 65 females) symptomless dromedary camels (*Camelus*
80 *dromedaries*) were collected. Sampling was done randomly and animals were sampled from 48
81 herds. The ages of the dromedary camels ranged from 0.1 to 18 years old. Sera were obtained by
82 centrifugation for 5 minutes at 2,500g. All the sera were separated, aliquoted and stored at -20 °C
83 until testing.

84 **2.6 Detection of total antibodies against HEV in serum**

85 DcHEV has the same serotype as that of G1, G3 and G4 HEV [12, 13], for this purpose, sera of
86 dromedaries were tested for the detection of HEV antibodies by multi-species ELISA kit (ID Screen®
87 Hepatitis E Indirect Multi-species, Paris, France). This commercially available double antigen
88 sandwich ELISA (das-ELISA) test developed strictly for veterinary use, is indirect ELISA for the
89 detection of anti-Hepatitis E total antibodies in serum and plasma from swine and other animal
90 species using a recombinant genotype 3 capsid antigen and a multispecies conjugate. The laboratory
91 analysis was performed following manufacturer's instruction. Interpretation of the results for each
92 sample is based on the calculation of the percentage S / P. S for each sample corresponds to the
93 difference in optical density (OD) of the cups that are sensitized to the antigens and cups that have no
94 antigen. P correspond to the mean of difference in OD of the cups that are sensitized to the antigens
95 and cups that have no antigen for two positive controls. Samples with an S / P greater than 70% are
96 considered positive. Samples with S / P less than or equal to 60% are considered as negatives.
97 Samples with S / P between 60% and 70% are considered as doubtful.

98 **2.7 Statistical analyzes**

99 The data were processed and analyzed using Excel 2013. Logistic regression analyses were carried
100 out to determine which variables (locality, gender, use of the animal, way of life and contact with other
101 animals) were significantly associated with detection of HEV antibodies. Logistic regression was
102 performed using R software version 2.13.0. P <0.05 was considered significant. The lower and upper
103 limits of the 95% confidence interval (CI) for a proportion were also calculated.

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108 **3. RESULTS**

109 **3.1 Seroprevalence of Anti-HEV Antibodies in dromedaries in West Africa.**

110 In our study 68/133 (51.1%) of dromedaries were males; the mean age of animals was 5.6 years
111 (range: 5.6±0.8 years) and the median age was 4 years. The prevalence of anti-DcHEV total antibody
112 was 8.11% (11/133; 95%CI [3.6 – 13.0]).

113 **3.2 Analysis of Associated Risk Factors**

114 There is no statistically significant relationship between HEV positive cases and parameters such as
115 age, sex, or use for traction or tourism (Table 1). However, among the animals studied, HEV
116 seroprevalence appeared to increase with increase in age and decrease with increase in Herd size.

117 The result showed that male had a higher prevalence of 10.3% (7/68) while female had the lowest
118 prevalence of 6.2% (4/65) as shown in Table 1.

119 All seropositive animals were in contact with other domestic animals and the distribution of positive
120 cases is random compared to the sample sites and flock ($p>0.05$) (Figure 1).

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Table 1: Factors associated with antibodies anti-HEV positivity among dromedaries

Risk factors	Prevalence of anti-HEV antibodies			
	Positive / Total	Positive	Odds ratio OR	P- value
Herd size				
> 26	3 / 59	5.1% CI95% [0.0 – 10.7]	ref	0.34 (> 0.05)
[0-26]	8 / 74	10.8% CI95% [3.7 – 17.9]	2.26 CI95% [0.62 – 10.71]	
Age				
> 4	6 / 66	9.1% CI95% [2.2 – 16.0]	1.24 CI95% [0.35 – 4.51]	0.73 (> 0.05)
[0-4]	5 / 67	7.5% CI95% [1.2 – 13.8]	ref	
Gender				
Female	4 / 65	6.2% CI95% [0.3 – 12.0]	ref	0.38 (> 0.05)
Male	7 / 68	10.3% CI95% [3.1 – 17.5]	1.75 CI95% [0.50 – 6.96]	
Use of the animal for tourism				
No	10 / 129	7.8% CI95% [3.1 – 12.4]	ref	0.29 (> 0.05)
Yes	1 / 4	25.0% CI95% [0.0 – 67.4]	3.97 CI95% [0.19 – 34.45]	
Use of the animal for traction				
No	9 / 105	8.6% CI95% [3.2 – 13.9]	1.21 CI95% [0.29– 8.31]	1 (> 0.05)
Yes	2 / 28	7.1% CI95% [0.0 – 16.7]	ref	
Sale of animal products				
No	6 / 64	9.4% CI95% [2.2 – 16.5]	1.32 CI95% [0.38– 4.81]	0.65 (> 0.05)
Yes	5 / 69	7.2% CI95% [1.1 – 13.4]	ref	
Live animals sale				
No	8 / 91	8.8% CI95% [3.0 – 14.6]	1.25 CI95% [0.34– 5.95]	1 (> 0.05)
Yes	3 / 42	7.1% CI95% [0.0 – 14.9]	ref	
Contact with pets				
No	0 / 6	0.0% CI95% [0.0 – 0.0]	-	1 (> 0.05)
Yes	11 / 127	8.7% CI95% [3.8 – 13.6]		
Contact with wild animals				
No	3 / 27	11.1% CI95% [0.0 – 23.0]	1.53 CI95% [0.32– 5.75]	0.69 (> 0.05)
Yes	8 / 106	7.5% CI95% [2.5 – 12.6]	ref	
Way of life of the camel				
Nomadic	1 / 7	14.3% CI95% [0.0 – 40.2]	1.93 CI95% [0.10– 12.98]	0.46 (> 0.05)
Sedentary	10 / 126	7.9% CI95% [3.2 – 12.7]	ref	
Total	11 / 133	8.3% CI95% [3.6 – 13.0]		

141 Note: OR, odds ratio; CI, confidence interval.

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147 **4. DISCUSSION**

148 **4.1 Seroprevalence of HEV**

149 The seroprevalence of HEV antibodies recorded for dromedaries (8.11%) in our study, suggest that
150 DcHEV infection is common among dromedary camels in the Sahelian zone of West Africa. The
151 prevalence observed in our study is lower than that recorded in East African cost: Ethiopia 22.4% in
152 2013 (using In-house test) [13], in Somalia 40% in 2016 (using HEV- ELISA-EU-ROIMMUN) [9], 15%
153 in Soudan in 2016 (using HEV- ELISA-EU-ROIMMUN) [9], 62.9% in Egypt (using HEV- ELISA-EU-
154 ROIMMUN) and 31.4% in Kenya in 2016 (using HEV- ELISA-EU-ROIMMUN) [9]. In Middle Est area,
155 seroprevalence of 37.1% in United Arab Emirates [9] and 60.0% in Pakistan [9] were recorded. The
156 low prevalence observed in our study could indicates assumed minor importance of West African
157 dromedaries in the epidemiology of HEV. Factors other than farm size, housing and husbandry
158 systems or stocking density, associated to the low sensitivity, as we did not use a specific anti-
159 dromedary Ig sensitivity of the test used might have influenced the low HEV seroprevalence observed
160 in ours study.

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162 **4.2 Associated Risk Factors**

163 The circulation of HEV genotype 7 in dromedaries, experimental demonstration of possible infection
164 by DcHEV and a confirmed case of chronic hepatitis E infection (DcHEV) in one patient in 2016 [6]
165 suggest that this genotype can lead to a public health problem. Thus, meat, milk and feces from
166 camels might pose a risk of HEV transmission to humans. Nomadic populations should be cautious
167 when handling these mammals (for tourism, traction), processing food products (milk and meat) and
168 feces residues derived from them [14].

169 There is no statistically significant relationship between HEV positive cases and parameters such as
170 age, sex, or use for traction or tourism. However, all seropositive animals were in contact with other
171 domestic animals. This could be considered a risk factor for HEV transmission if these animals shared
172 the same genotype of the virus. Molecular studies should therefore be undertaken to identify HEV
173 genotypes common to these species.

174 The observation of the distribution of positive cases is random compared to the sample sites and flock
175 ($p>0.05$) (Figure 1). This could imply absence of epidemics or a low reinfection of animals in the herd.
176 Investigations of dromedaries could help to further elucidate the geographic and evolutionary origin of
177 HEV-7. Furthermore, other wild or domestic animals with close contact to dromedaries should be
178 investigated to assess the host range of HEV-7. Considering HEV was detected in fecal samples from
179 camels [14], animal could contribute to disperse virus particles, causing an environmental problem.

180 Our study has some limitations. We did not perform a molecular study and only one antibody
181 detection methods was used; the antibody prevalence in camels should be confirmed by larger
182 studies including virus neutralization studies to determine potential genotype variability.

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184 5. CONCLUSION

185 The present study reports the description of anti-HEV antibodies found in a survey dromedaries in
186 West Africa. This is the first study reporting HEV circulation in *Camelidae* in West Africa. Nomadic
187 populations should then take precautions regarding the use of food products derived from camels.

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189 Ethical: NA

190 Consent: As per international standard written consent from the camel owners has been collected
191 and preserved by the authors.

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