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Serological Evidence of Hepatitis E Virus in Dromedary Camels in the Sahelian Zone of West Africa

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NB, JBO, KAT, PR and AO conceived and designed the review/project/study. Authors JBO, AO, SM, BLO, LHZ and HR executed the experiment and analyzed the sera samples. Authors BLO, JBO, KAT, AT, PR and NB analyzed the data. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

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Short Communication

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ABSTRACT

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.

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

1. INTRODUCTION

Hepatitis E virus (HEV), the one of the most common cause of acute viral hepatitis worldwide [1], is mainly transmitted via the fecal-oral route and blood transfusion, and is well recognized as a zoonotic pathogen. The causative agent of Hepatitis E (HE) is a small, quasi-enveloped, single-stranded positive-sense RNA virus, causing more than 90% of HEV infection cases which is asymptomatic and self-limiting [2]; however, in specific vulnerable groups, the outcome of HEV infection can be much more severe, especially in pregnant women in the third trimester and can become chronic in immunocompromised individuals, such as those receiving organ transplants or chemotherapy and individuals with HIV infection [3,4].

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

Hepeviridae contains several viral species divided into two genera: *Orthohepevirus* with four species (A–D) and *Piscihepevirus* with one species (A) [8]. Eight genotypes exist within

Orthohepevirus A and these HEV strains infects humans and multiple mammals' species. HEV1 and HEV2 are restricted to humans; HEV3 is found among humans, swine, rabbits, deer and mongooses; HEV4, which circulates between humans and swine; HEV5 and HEV6, which are found in wild boars; and HEV7 and HEV8, which were recently identified in dromedary and Bactrian camels, respectively [2,3], HEV-7 has also been isolated from a hepatitis E patient suggesting that Hepatitis E Virus from dromedary camels (DcHEV) could cause zoonotic infection in humans [6]. Moreover, detection of partial DcHEV genome in camel serum or fecal samples in the UAE, Somalia, Kenya, and Pakistan during the period 1983-2015, suggests that DcHEV in dromedary camels is long established, diversified and geographically widespread [9].

In the Sahelian zone of West Africa, dromedary camels are very important livestock animals. Indeed, they have an important socio-economic and cultural role in improving survival of the desert dwellers. According to Food and Agriculture Organization (FAO), the dromedary population in West Africa is estimated at 2, 140, 000 animals. They provide food (milk, meat), fibre (wool, leather) and draft power (for transportation, cultivation and tourism). However, camels being reservoirs of many bacterial and viral diseases transmissible to humans, as Middle East Respiratory Syndrome (MERS) the most frightening [10,11], either by food byproducts or by promiscuity, it is important to provide data on the sero-epidemiology of DcHEV

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in this area. The present work is part of a vast campaign of detection of animal reservoirs of HEV. The aims of this study was to establish the circulation of HEV in dromedary living in West Africa and analysis behaviors that could promote the spread of the virus in human population.

2. MATERIALS AND METHODS

2.1 Study Design

Cross sectional epidemiological survey.

2.2 Place and Duration

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

2.3 Ethics Statement

The protocols for specimen collection and use were submitted and approved by the Ministry of

Environment and fish Resources of Burkina Faso by the ministerial arrest n°2014 2015 - 001 / MERH/CAB.

2.4 Data Collection

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

2.5 Sample Collection

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

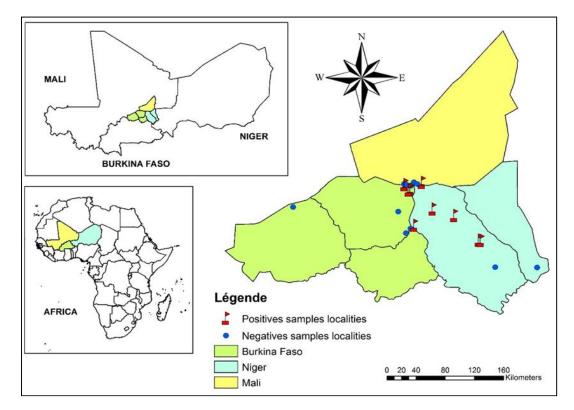


Fig. 1. Geographical location of the sampling site and positive cases of anti-HEV antibodies circulating in camels in West Africa The positives cases are represented by a red color in the figure

2.6 Detection of Total Antibodies against HEV in Serum

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

2.7 Statistical Analyzes

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

3. RESULTS

3.1 Seroprevalence of Anti-HEV Antibodies in dromedaries in West Africa

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

3.2 Analysis of Associated Risk Factors

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

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

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

4. DISCUSSION

4.1 Seroprevalence of HEV

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

4.2 Associated Risk Factors

The circulation of HEV genotype 7 in dromedaries, experimental demonstration of possible infection by DcHEV and a confirmed case of chronic hepatitis E infection (DcHEV) in one patient in 2016 [6] suggest that this genotype can lead to a public health problem. Thus, meat,

| Risk factors | Prevalence of anti-HEV antibodies | | | |
|---------------------|-----------------------------------|--------------------------|---------------------------|--------------|
| | Positive / Total | Positive | Odds ratio OR | P- value |
| Herd size | | | | |
| > 26 | 3 / 59 | 5.1% Cl95% [0.0 – 10.7] | ref | 0.34 (> 0.05 |
| [0-26] | 8 / 74 | 10.8% CI95% [3.7 – 17.9] | 2.26 Cl95% [0.62 – 10.71] | |
| Age | | | | |
| > 4 | 6 / 66 | 9.1% CI95% [2.2 – 16.0] | 1.24 Cl95% [0.35 – 4.51] | 0.73 (> 0.05 |
| [0-4] | 5 / 67 | 7.5% Cl95% [1.2 – 13.8] | ref | |
| Gender | | | | |
| Female | 4 / 65 | 6.2% Cl95% [0.3 – 12.0] | ref | 0.38 (> 0.05 |
| Male | 7 / 68 | 10.3% Cl95% [3.1 – 17.5] | 1.75 Cl95% [0.50 – 6.96] | |
| Use of the a | nimal for tourisn | n | | |
| No | 10 / 129 | 7.8% CI95% [3.1 – 12.4] | ref | 0.29 (> 0.05 |
| Yes | 1/4 | 25.0% Cl95% [0.0 - 67.4] | 3.97 Cl95% [0.19 – 34.45] | |
| Use of the a | nimal for traction | n | | |
| No | 9 / 105 | 8.6% Cl95% [3.2 – 13.9] | 1.21 Cl95% [0.29– 8.31] | 1 (> 0.05) |
| Yes | 2 / 28 | 7.1% Cl95% [0.0 – 16.7] | ref | |
| Sale of anim | al products | | | |
| No | 6 / 64 | 9.4% Cl95% [2.2 – 16.5] | 1.32 Cl95% [0.38– 4.81] | 0.65 (> 0.0 |
| Yes | 5 / 69 | 7.2% Cl95% [1.1 – 13.4] | ref | |
| Live animals | sale | | | |
| No | 8 / 91 | 8.8% Cl95% [3.0 – 14.6] | 1.25 Cl95% [0.34– 5.95] | 1 (> 0.05) |
| Yes | 3 / 42 | 7.1% Cl95% [0.0 – 14.9] | ref | |
| Contact with | pets | | | |
| No | 0/6 | 0.0% Cl95% [0.0 – 0.0] | - | 1 (> 0.05) |
| Yes | 11 / 127 | 8.7% Cl95% [3.8 – 13.6] | | |
| Contact with | wild animals | | | |
| No | 3 / 27 | 11.1% Cl95% [0.0 – 23.0] | 1.53 Cl95% [0.32– 5.75] | 0.69 (> 0.0 |
| Yes | 8 / 106 | 7.5% Cl95% [2.5 – 12.6] | ref | |
| Way of life o | f the camel | | | |
| Nomadic | 1/7 | 14.3% Cl95% [0.0 – 40.2] | 1.93 Cl95% [0.10– 12.98] | 0.46 (> 0.05 |
| Sedentary | 10 / 126 | 7.9% Cl95% [3.2 – 12.7] | ref | |
| Total | 11 / 133 | 8.3% CI95% [3.6 – 13.0] | | |

| Table 1. Factors associated with antibodies anti-HE | V positivity among dromedaries |
|---|--------------------------------|
|---|--------------------------------|

milk and feces from camels might pose a risk of HEV transmission to humans. Nomadic populations should be cautious when handling these mammals (for tourism, traction), processing food products (milk and meat) and feces residues derived from them [14].

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

The observation of the distribution of positive cases is random compared to the sample sites and flock (p>0.05) (Fig.1). This could imply absence of epidemics or a low reinfection of animals in the herd. Investigations of dromedaries could help to further elucidate the

geographic and evolutionary origin of HEV-7. Furthermore, other wild or domestic animals with close contact to dromedaries should be investigated to assess the host range of HEV-7. Considering HEV was detected in fecal samples from camels [14], animal could contribute to disperse virus particles, causing an environmental problem.

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

5. CONCLUSION

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

CONSENT

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

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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