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Prevalence of hepatitis E virus antibodies in cattle in Burkina Faso associated with swine mixed farming

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ABSTRACT

Background: Endemic circulation of human-specific hepatitis E virus (HEV) genotypes 1 and 2 may occult the importance of sporadic zoonotic HEV transmissions in Africa. Increasing numbers of studies reporting anti-HEV antibodies in cattle and the discovery of infectious HEV in cow milk has raised public health concern, but cattle exposure has seldom been investigated in Africa.
Objectives: This study aimed at investigating the role of cows in the epidemiology of HEV in Burkina Faso and farmers habits in terms of dairy product consumption as a prerequisite to estimate the risk of transmission to humans.
Methods: Sera from 475 cattle and 192 pigs were screened for the presence of anti-HEV antibodies while HEV RNA in swine stools was detected by reverse transcription polymerase chain reaction. Data on mixed farming, dairy product consumption and selling habits were gathered through questionnaires.
Results: The overall seroprevalence in cattle was 5.1% and herd seroprevalence reached 32.4% (11/34). Herd seropositivity was not associated with husbandry practice or presence of rabbits on the farms. However, herd seropositivity was associated with on-site presence of pigs, 80.7% of which had anti-HEV antibodies. The majority of farmers reported to preferentially consume raw milk based dairy products.
Conclusions: Concomitant presence of pigs on cattle farms constitutes a risk factor for HEV exposure of cattle. However, the risk of HEV infections associated with raw cow dairy product consumption is currently considered as low.

Keywords: Seroprevalence; Hepatitis E; Cattle; Zoonosis; Public Health; Burkina Faso

INTRODUCTION

The existence of another hepatic virus—different from hepatitis A and B viruses—was suggested in the late 1970s after the occurrence of a very large jaundice epidemic in India

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Conflict of Interest
The authors declare no conflicts of interest.

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with a high fatality rate in pregnant women [1]. Hepatitis E virus (HEV), the now identified causative agent of hepatitis E, has since then been detected in almost every country [1] and its large genetic diversity, extended host range and various transmission routes continue to unravel [2].

HEV circulation is considered widespread in Africa: outbreaks due to genotypes 1 and 2 are frequently reported in the human population [3]. The endemic circulation of those human-specific genotypes may however occult zoonotic transmissions of other HEV genotypes, such as genotype 3, responsible for the vast majority of sporadic cases in Europe or North America [4,5], where genotypes 1 and 2 are virtually absent. Pigs are considered as the main reservoir of zoonotic genotype 3 and 4 strains and occupational exposure to swine as well as consumption of undercooked swine products are known risk factors for zoonotic HEV infections [6]. In Burkina Faso, HEV is likely endemic in humans and in swine [7-9]. Although HEV genotyping in acute jaundice human patients between 2013 and 2016 exclusively identified genotype 2 [10], zoonotic genotype 3 is circulating in swine in Burkina Faso [7], as in other African countries [11-13]. The higher seroprevalence in people with occupational exposure to pigs (76.0% pork butchers in Ouagadougou [7]) compared to the general population in Burkina Faso (39.0% in blood donors [9]) and in Nigeria [14] suggests the potential importance of zoonotic infections on the continent, at least in certain risk groups.

Besides swine, multiple other species such as wild boar, deer, sheep, rabbit or camel are HEV hosts [2]. Direct evidence of animal-to-human HEV transmission has been provided at least for pigs, wild boars and deer [6,15,16]. The first report of anti-HEV antibodies in cattle dates back two decades and came from India [17]. Since then, presence of anti-HEV antibodies in cattle has been reported in the Americas [18,19], in Africa [20-22] and Asia [23-25]. Although no case of cattle-to-human HEV transmission has been documented so far, the recent discovery of infectious HEV particles in cattle milk [26] and HEV RNA in bovine livers [27] suggest the risk of zoonotic transmission from cattle and the necessity of improving HEV surveillance in this host.

In Burkina Faso, a preliminary study in Kadiogo Province suggested past HEV exposure in a small cattle cohort [28]. Given that cattle are frequently reared in close contact with people and swine all over the country, this study therefore aimed at further assessing the potential role of cows in the epidemiology of HEV in Burkina Faso as well as farmers habits in terms of milk consumption as a prerequisite to assess the risk of transmission to humans.

MATERIALS AND METHODS

Ethical considerations

This study received approval from Centre Muraz ethical committee (number 2016-15/MS/SG/CM/IEC). The aim of this study was explained to animal owners and their informed consent was obtained before questionnaire administration and sample collection.

Study area and study population

The study was carried out within a radius of 25 kilometres around Bobo-Dioulasso (Houet Province, Hauts-Bassins Region), which is the second largest city and economic capital of Burkina Faso. The two-stage random sampling method was used for farm and individual animal selection, as explained before [29]. Briefly, as no exhaustive lists of farms locations and production orientation were available, a preliminary survey identified 63 cattle farms breading at least 15 cattle heads. All farms were visited twice, first to inform owners about the purpose of the study and obtain written consent and then for filling epidemiological questionnaires. Sample collection was carried out in a randomly selected subset of 34 cattle farms and animals were randomly selected within each farm to assess HEV seroprevalence.

Sample collection and laboratory testing

Blood was drawn from the jugular vein in a dry vacutainer tube after properly restraining the animals. Serum was separated after allowing blood samples to clot, then stored at −80°C prior to testing. The presence of antibodies, indicators of both recent and old infections (IgG, IgA, IgM), against HEV in bovine and swine sera was investigated by a species-independent competitive enzyme-linked immunosorbent assay (ELISA) (HEV ELISA 4.0v; MP Diagnostics, Eschwege, Germany) that uses a recombinant antigen highly conserved between different HEV strains. The kit was used according to the manufacturer's recommendations.

Swine stools were taken directly from individual animals by transrectal palpation and pooled by farm. Viral RNA was extracted from the supernatant of pools diluted 10% weight/volume in PBS using QIAmp viral RNA minikit (Qiagen, Venlo, The Netherlands) according to the manufacturer's instructions. Presence of HEV RNA was tested by a real-time reverse transcription polymerase chain reaction (RT-PCR) as described before [30].

Statistical analyses

Statistical analyses were performed in SigmaPlot version 12.5 (Systat Software Inc., San Jose, CA, USA). Association between age (in mon) and serological status of animals was tested by Mann-Whitney rank sum test. Association between serological status and sex or breed category (local vs. exotic) was assessed using χ^2 tests, while association between farm serological status and farm orientation (dairy vs. mixed), or presence of pigs (presence vs. absence) or rabbits (presence vs. absence) on the farms was tested using Fisher's exact tests. Differences were considered significant with $p < 0.05$.

RESULTS

Cohort description

Between January and March 2017, 475 cattle were sampled in 24 dairy farms (n = 281), 10 farms with both dairy and cattle meat production orientation (n = 113) and one slaughterhouse (n = 81) located in urban or peri-urban areas of Bobo-Dioulasso. In our study area, dairy herds were exclusively located in peri-urban areas (**Table 1**). These farms were also usually larger (mean number of cattle heads: 220.0) with heavier financial investments and a more generalized use of artificial insemination compared to dairy/meat mixed farms. Mixed farms (mean number of cattle heads: 164.0, **Table 1**) reared female cattle for milk production while males were fattened on site for meat. All dairy/meat mixed farms were located in urban areas. Multiple animal species were usually present on the cattle farms (poultry, 34/34, 100%; equids, 34/34, 100%; rabbits, 7/34, 20.6%; cats and/or dogs, 34/34, 100%; pigs, 24/34, 70.6%). Between 6 and 24 animals per farm were sampled, mainly depending on herd size. Most animals sampled were ≥ 3 yr old (357/475, 75.2%). Cattle from local breeds (216/475, 45.5%) and exotic breeds from Europe or South America or coming from crossed breeding (259/475, 54.5%) were sampled (**Table 2**).

Table 1. Characteristics of the farms sampled and HEV seroprevalence

Characteristics	Type of setting		Total
	Dairy farms	Mixed dairy and meat farms	
Average cattle herd size	220.0 (62–929)	164.0 (72–666)	203.6 (62–929)
No. of farms practicing semi-confined rearing/Total No. of farms	13/24 (54.2)	10/10 (100)	23/34 (67.6)
No. of farms located in peri-urban area/Total No. of farms	24/24 (100)	0/10 (0.0)	24/34 (70.6)
No. of farms with pigs present/Total No. of farms	18/24 (75.0)	6/10 (60.0)	24/34 (70.6)
Average pig herd size in farms with pig present	162.0 (11–402)	106.8 (21–504)	147 (11–504)
No. of farms with rabbits present/Total No. of farms	5/24 (20.8)	2/10 (20.0)	7/34 (20.6)
No. of HEV seropositive herds/Total No. of farms	10/24 (41.7)	1/10 (10.0)	11/34 (32.4)
No. of HEV seropositive animals/Total No. of animals	21/281 (7.5)	3/113 (2.7)	24/475 (5.1)*

Values are presented as number (range) or number (%).
HEV, hepatitis E virus.
*Animals sampled at the slaughterhouse (n = 81) were included in the total of animals tested.

Table 2. Characteristics of sampled animals and individual HEV seroprevalence rates

Characteristics	No. of animals in each category/Total No. of animals sampled	No. of seropositive animals/No. of animals in the category
Sex		
Female	253/475 (53.3)	16/253 (6.3)
Male	222/475 (46.7)	8/222 (3.6)
Age class (in mon)		
6–11	43/475 (9.1)	2/43 (4.7)
12–23	35/475 (7.4)	1/35 (2.9)
24–35	40/475 (8.4)	1/40 (2.5)
36–59	122/475 (25.7)	9/122 (7.4)
60–119	209/475 (44.0)	11/209 (5.3)
≥ 120	26/475 (5.5)	0/26 (0.0)
Animal breed		
Local*	216/475 (45.5)	16/216 (6.2)
Exotic†	259/475 (54.5)	8/259 (3.7)

Values are presented as number (%).
HEV, hepatitis E virus.
*The local breeds included the Sudanese Peuhl zebu (n = 109), the Goudali zebu (n = 34), the Azawak zebu (n = 58) and Taurin N'Dama (n = 15).
†The exotic breeds originated mainly from Europe (Holstein, n = 66; Montbélliarde, n = 35; Tarentaise, n = 29; Jersey, n = 17; Brune des Alpes, n = 12) or Brazil (Gyr zebu, n = 28) and included various crossed breeding (n = 72).

In October-November 2016, swine sera (n = 192) and pools of swine stools (1 pool/farm) were collected in 11/24 farms rearing both cattle and pigs. All pigs were of exotic breeds introduced in the country for their production performance. All animals were 6 mon old or more (range: 6–48 mon, mean age: 12 mon). Sera were obtained from 2 to 30 animals per farm (**Fig. 1**).

HEV prevalence and associated factors

In cattle, the overall prevalence of anti-hepatitis E antibodies reached 5.1% (24/475; **Table 1**) and the overall herd seroprevalence was 32.4% (11/34). Within-herd seroprevalence in positive farms ranged from 5.0 (1/20 positive animals) to 50.0% (3/6 positive animals). No seropositive animals were identified at the slaughterhouse. At the individual animal level, no association between cattle age and serological status was observed ($p = 0.612$). The seroprevalence in female cattle (16/253, 6.3%) was not significantly different than in males (8/222, 3.6%; $p = 0.254$). No statistical difference was observed when comparing the seroprevalence in local (8/216, 3.7%) to exotic breeds (16/259, 6.2%; $p = 0.310$).

Most seropositive cattle (21/24, 87.5%) were reared in dairy farms while only few (3/24, 12.5%) originated from farms where both dairy and meat cattle were reared. However, seropositivity status was not significantly different in dairy farms (10/24, 41.7%) compared to dairy/meat mixed farms (1/10, 10%, $p = 0.113$). Herd seropositivity was not associated with rearing practice (5/11, 45.5% confined vs. 6/23, 26.1% semi-confined; $p = 0.434$) or with the

Journal of Veterinary Science

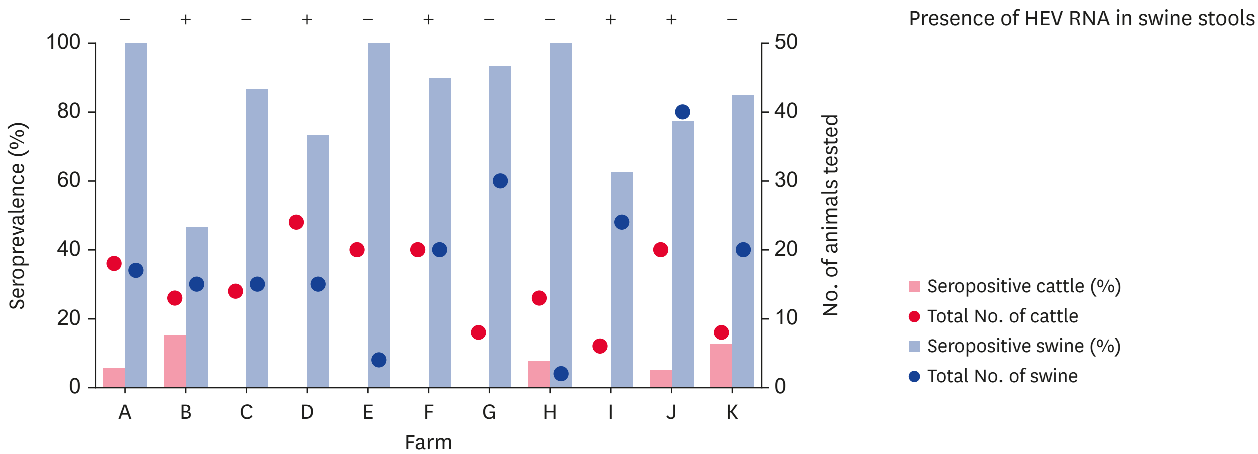

Fig. 1. HEV seroprevalence in mixed cattle/swine farms. The prevalence of anti-hepatitis E antibodies (left axis) in cattle and swine in 11 farms where both species were sampled is shown. The number of cattle and swine tested in each farm is provided on the right axis. Detection of HEV RNA in swine pooled stool samples (+, pool positive; −, pool negative) is indicated on top of the corresponding farms.
HEV, hepatitis E virus.

presence of rabbits on the farm (8/27, 29.6% in the absence of rabbits vs. 3/7, 42.9% in the presence of rabbits; $p = 0.656$). Herd seropositivity was however significantly associated with the presence of pigs on the farm (0/10 in the absence of pigs vs. 11/24, 45.8% in the presence of pigs; $p = 0.014$).

Swine samples were also collected in 11 farms. The overall prevalence of anti-hepatitis E antibodies in pigs reached 80.7% (155/192). Seropositive pigs were detected in all farms investigated, with within swine herd seroprevalence ranging from 46.7% (7/15) to 100% (17/17; **Fig. 1**). Seropositive cattle were found in 5/11 farms. Rabbits were present in only one farm, in which both seropositive cattle and swine were found (farm H, **Fig. 1**). Viral RNA was also detected in pools of pig stools from 5/11 farms and the HEV strains belonged to genotype 3 (data not shown).

Zoonosis awareness and milk consumption habits

Among the 63 farmers interviewed, 84.4% (38/45) dairy farm owners and 100% (18/18) mixed dairy/meat farm owners were aware of the possibility of cattle to transmit diseases to human. All reported to consume milk from their own cattle. The main type of milk product consumed by dairy farm owners was fresh milk (26/45, 57.8%) followed by transformed milk products (11/45, 24.4%) and heat-treated milk (8/45, 17.8%). All mixed farm owners (n = 18) reported to preferentially consume transformed milk. Transformed milk products in the country consist of yogurt, curdled milk, fresh cheese or butter produced from fresh milk. All farmers sold raw milk to customers.

DISCUSSION

Since the first evidence of past exposure of cattle to HEV [17], other studies have also highlighted the presence of anti-HEV antibodies and, to a much lesser extent, of viral RNA in cattle. Although HEV strains from well characterized genotypes 4 and 3 have been sporadically sequenced from cattle [22-24,26,27], absence of HEV RNA detection by several PCR or next generation sequencing approaches in environmental samples nearby seroconverting animals led to the hypothesis of the existence of a viral species antigenically related to HEV, that remains to be identified [31]. Despite the need for further genetic and antigenic characterisation of HEV or HEV-like agents in cattle, geographic differences in seroprevalence in cattle seem to emerge. HEV has not been detected in Europe so far [32-35]. In Asia, where most surveillance studies have been carried out, the prevalence of anti-HEV antibodies in cattle serum ranged from 5.7% [17] to 47.2% [24]. In Africa, seropositive cattle have been identified in small sample cohorts in Egypt (21.6%, [21]), Ghana (23.8%; [20]) but not in Nigeria [36]. In Burkina Faso, a preliminary study taking place between 2015 and 2017 in Kodiogo Province found 19/72 (26.4%) seropositive cattle [28]. This number is 5 times higher than the seroprevalence rate observed in our study taking place in early 2017 in Houet Province. The observed difference cannot result from variations in sensitivity and specificity between serological assays since the same kit was used in the two studies. However, geographic differences, even within the same country, may exist as suggested by different seroprevalence rates of cattle from different localities in Egypt [21] or provinces in China [37]. In addition, seroprevalence rates in rabbits varied similarly between Houet (19.2%) and Kadiogo (80.4%) Provinces [28], suggesting that there might be a common, currently unknown, factor leading to lower virus circulation in Houet Province.

The variations of HEV seroprevalence rates in cattle observed in different studies may arise from husbandry practices and host interactions such as mixed farming of multiple species. Frequent mixed farming of cattle and swine in rural China, lower seroprevalence rates in cattle compared to swine in the same region, together with the close phylogenetic relationship between cattle and swine strains [24,26] suggest inter-species transmission. In Egypt, Sayed et al. [22] identified a genotype 3 strain in one cattle milk sample from a herd with confirmed seroconversion of some animals at follow-up, indicating that cattle are permissive to genotype 3 in addition to genotype 4 [23,24,26,27]. High seroprevalence rates in pigs in all the mixed cattle/swine farms sampled in our study, as well as in slaughtered animals [7], point to HEV enzooticity in swine in Burkina Faso. This together with the presence of HEV genotype 3 in swine stools or livers [7] highlights the high degree of exposure of cattle to genotype 3 strains, at least in mixed cattle/swine farms. The association between pig presence and cattle herd seropositivity also suggests the importance of swine as virus reservoirs for cattle. However, sequencing data is still necessary to confirm this hypothesis. Lower seroprevalence in cattle compared to pigs may indicate lower permissivity and/or transient seropositivity. Transient seroconversion, already observed in calves and adult cows in a longitudinal study [31], may also explain the absence of association between age and seropositivity in our study.

Rabbits were bred only in 1/11 mixed cattle/swine farms where both cattle and swine were tested (**Fig. 1**) and no association between rabbit presence and anti-HEV antibodies in cattle was observed. However, interspecies transmissions involving rabbits in addition to pigs should not be dismissed. Future studies would benefit from longitudinal and combined serological and molecular investigations in all species present on site, given the wide HEV host range.

HEV transmission routes in animals include direct fecal-oral transmission by contact with fecal matter [38]. Assuming that HEV infections of pigs contributes to exposure of cattle in the same farm, age-structure of the swine population—with young animals being most susceptible and excreting highest viral quantities—, type and intensity of inter-species contacts as well as hygiene on the farm may explain the range of anti-HEV antibody prevalence in cattle observed in our study. Indirect transmission by contact with contaminated water [38] may also be an additional factor for cattle exposure. In most of the cattle farms visited, we observed that farmers use water wells without a rim. During the rainy season, the animals are also taken to pasture and drink in backwaters. Backwaters and wells without rims receive run-off water and can then be contaminated by washed-off faeces from multiple animals or humans. Environmental sampling within the farm and in pasturing area are warranted to further investigate these inter-species transmission routes.

Human infections through the consumption of contaminated swine, wild boar or deer products have been well documented [6,15,16]. To our knowledge, zoonotic transmission through cattle meat products has not been identified so far, but the recent detection of HEV positive bovine livers [27] suggests that it is a possibility. In our study, the absence of seropositive animals at slaughter coupled with the overall low seroprevalence currently indicates a low risk for the population. However, further investigations are necessary to compensate the limited sample size. The recent discovery of infectious HEV particles in cow milk samples in Asia [26] suggests that fresh milk consumption is yet another route for zoonotic transmission. The detection of HEV RNA in the breast milk of a woman suffering from acute HEV infection [39], and in goat, sheep and donkey milk [40,41] suggest that viral excretion in milk during acute infection is not uncommon. In our study, the vast majority of cattle owners (55/63, 87.3%) reported to consume preferentially fresh milk or dairy products transformed from raw milk and milk was always sold raw. Raw milk consumption is frequent in Burkina Faso since raw milk is considered tastier. While few farmers reported to heat milk prior to consumption, time and temperature are not controlled during the process and may not be optimal to inactivate a rather thermo-tolerant virus such as HEV [42]. Although seroprevalence of HEV antibodies in dairy cows was low in Burkina Faso, our results nevertheless highlight that the presence of HEV or HEV-like agent in cattle in the country needs to be monitored in the future.

In conclusion, we showed presence of antibodies against HEV or HEV-like agent in cattle in Houet Province in Burkina Faso, with concomitant presence of pigs on cattle farms as risk factor for seropositivity. Despite low HEV seroprevalence rates in cattle in the region, our study showed frequent consumption of raw dairy products, highlighting the need to raise awareness on the general microbiological risk of raw milk consumption. Further studies on the frequency and duration of viral excretion in milk as well as factors promoting virus transmission to cattle are necessary to refine risk assessment.

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