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Original Article

Prevalence of anti-hepatitis E virus antibodies in domestic animal from three representative provinces of Burkina Faso



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ABSTRACT

Four major genotypes of Hepatitis E virus (HEV) have been documented worldwide (1–4) with genotypes 1 and 2 found in human in Sub-Saharan Africa. Human Hepatitis cases due to HEV genotype 3 and 4 are zoonotic with various animal identified as possible reservoirs. Recently, HEV genotype 3 was found in pigs and human beings in West Africa, which may change the epidemic in human. Here, we assessed the prevalence of HEV antibodies in various domestic and wild mammals in Burkina Faso. Random sampling was performed between 2015 and 2017 to collect serum from 100 rabbits (*Oryctolagus cuniculus*), 19 hares (*Lepus africana*), 72 cattle (*Bos taurus*), 75 sheep (*Ovis aries*) and 81 goats (*Capra aegagrus*) in three provinces in Burkina Faso. A multi-species ELISA was performed on serum samples from 328 domestic animals and 19 hunting hares. HEV total antibodies were identified in 121 out of 347 specimens (34.9% CI95% [29.9–39.9]). Sera from rabbits (60% CI95% [50.4–69.6]), hares (52.6% CI95% [30.2–75.1]), cattle (26.4% CI95% [16.2–36.6]), sheep (12.0% CI95% [4.6–19.4]), and goats (28.4% CI95% [18.6–38.2]) tested positive for antibodies anti-HEV. In this study we evidence presence of HEV antibodies in various mammals and highlight the importance of these species in the epidemiology of HEV infection in Burkina Faso.

1. Introduction

Hepatitis E virus (HEV) is one of the causative agent of acute hepatitis epidemic in human worldwide (Rein, Stevens, Theaker, Wittenborn, & Wiersma, 2012). HEV has a single-stranded, positive-sense RNA genome of approximately from 6.4 to 7.2 Kb (Purdy et al., 2017). Following the new classification, HEV belongs to the family of *Hepeviridae*, which consists of two genera, *Orthohepevirus* (with species A, B, C and D) and *Piscihepevirus* containing one known isolate derived from a trout (ICTV, 2014; Smith et al., 2015). *Orthohepevirus* A contains eight genotypes, the human-pathogenic genotypes HEV-1 to HEV-4

together with additional genotypes from wild boars (HEV-5 and HEV-6) and camels (HEV-7 and HEV-8) (Sridhar, Teng, Chiu, Lau, & Woo, 2017). In developing countries, HEV-1 or 2 appears to have an endemic pattern punctuated with epidemic outbreaks (Liu et al., 2013). HEV-3 and 4 widely found in animal reservoirs are thought to be zoonotic (Pavio, Meng, & Doceul, 2015; Y. Zhang et al., 2017). Indeed, HEV genotype 3 and 4 infections were reported in developed countries in individuals who had consumed raw meat or meat products from deer, wild boars, or pigs. Among the mammals, domestic pigs and wild boars are the main animal's reservoir of these genotypes (Schielke et al., 2009), but serological evidence of HEV infection was also documented

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in other animal species including chickens, rats, dogs, cats, cows, sheep, goats, buffalo, horses, cattle, camel and rhesus monkeys (Geng et al., 2011a,b; Izopet et al., 2012; Liang et al., 2014; Pavo et al., 2015; Wu et al., 2017; Zhang et al., 2017). Infection with one strain of HEV could confer cross-protection to other strains, even across genotypes 1 to 4, owing to the existence of a single serotype (Krain, Nelson, & Labrique, 2014; Zhang et al., 2017).

Initially HEV was described as the agent of severe forms of acute hepatitis with mortality level ranging from 1 to 4% in the general population to nearly 20% in pregnant women during epidemics, and especially in the third trimester (Worm, van der Poel, & Brandstatter, 2002). However, these epidemic pictures seem to be specific to HEV genotype 1 and 2 and it is also recognized that during inter-epidemic periods a large number of cases were not detected as they were self-limited infections with rapid viral clearance (Mirazo, Ramos, Mainardi, Gerona, & Arbiza, 2014). Most of these self-limited infections were due to genotype 3 and 4 even if severe hepatitis and chronic infection are now identified in at-risk population (Kmush, Nelson, & Labrique, 2015; Pas et al., 2012).

In Burkina Faso the prevalence of Anti-HEV antibodies were 76% in pork butchers, and 47.8% in the general population in 2013 (Traore et al., 2015). Traore et al. also documented HEV antibodies (80%) in swine (Traore et al., 2015). The aim of this study was to assess the presence of anti-HEV antibodies in various wild and domestic mammals breed find in human food chain in Burkina Faso.

2. Material and method

2.1. Study area

We conducted an epidemiological survey in Kadiogo, Gourma and Houet, three (3) administrative provinces of the semi-arid country of Burkina Faso, West Africa. These provinces are located in the Sudanese agro-ecological area with rainfall ranging from 300 to 1200 mm³/year. The vegetation is represented by the wooded and shrubby savannah. The livestock systems are transhumant and sedentary livestock from the north to the south of the country. It is an agro-pastoral area with maize, sorghum, millet and cowpea as the main crops. The main culinary habits for meat is cooking at high temperatures (Drabo et al., 2009). Ouagadougou (province of Kadiogo) represents the most important city located in the center of the country (Fig. 1). The province of Kadiogo covered about 2805 square Km, with an estimated population of 2854,356 and an estimated livestock population of 22,779 rabbits, 224,056 sheep, 337,912 goats, 155,847 cattle, and other variable species of mammals and poultry (INSD, 2017). Evolution of controlled slaughter by species in province of Kadiogo is 102,384 for cattle, 84,040 for sheep, and 68,898 for goat (INSD, 2017).

2.2. Sampling and sample collection

Our study first focused on the most consumed animal species in the city of Ouagadougou (cattle, goats, sheep, and rabbits) except pork, which was studied in our previous survey. Five whole blood sample were collected weekly on red top tube from symptomless animals: 72 cattle (*Bos taurus*), 81 goats (*Capra aegagrus*), 75 sheep (*Ovis aries*), 100 rabbits (*Oryctolagus cuniculus*) and hares 19 (*Lepus africana*). Only rabbits are collected in the 3 area (Kadiogo ($n = 56$), Houet ($n = 26$) and Gourma ($n = 18$)) and Hares are collected in the 2 area (Houet ($n = 3$) and Gourma ($n = 16$)). Specimens were collected in February and August 2015, October and November 2016 and April and May 2017 at livestock farms, slaughterhouses, animal sale places and hunting areas for the species concerned. The slaughterhouse receives animals from different districts of Burkina Faso and is the most important source of meat supply in the city. Taking into account the literature, we considered that, in addition to pork, the real risks of HEV infection could come from lagomorphs (Liu et al., 2013), both for the high prevalence

of the virus in this mammalian order and for genotypes similar to those found in humans (Wang et al., 2013; Zhang et al., 2015). This motivates the extension of the study on rabbits to two other important provinces (Houet and Gourma) and on hares at the same time, since Houet and Gourma include hunting areas.

Serum was obtained by centrifugation at 2500 g for 5 min, aliquoted and stored at -20°C until testing.

2.3. Sample size determination

The sample size (n) was determined using an expected total prevalence within all the various species considered as a whole of 24.1% (p_{exp}) following the data from Junaid et al. acquired in a very close geographic area (Junaid, Agina, & Jaiye, 2014), with a 95% confidence interval level ($Z = 1.96$) and an error margin of 5% (d) using the formula developed by Fisher, Laing, and Stoeckel (1983). The expected sample size was thus $n = 281$. As our prospective study involved sampling of various animal species, the estimation of the minimum size was obtained using the lowest prevalence expected for each species reported in the literature (Table 3). Thus, the final target sample size were 50 for rabbits, 33 for hares, 30–86 for cattle, 30–144 for sheep and goats.

$$n = (Z^2 \times p^{exp} \times (1 - p^{exp})) / d^2$$

2.4. Detection of total HEV antibodies

All 347 Animal sera were tested following manufacturer instructions with the HEV ELISA 4.0v kit (MP Biomedicals, Eschwege, Germany). This commercially available double antigen sandwich ELISA (das-ELISA), developed strictly for veterinary use, is able to detect IgM, IgG and IgA directed against HEV in all animal species. According to the manufacturer, test sensitivity is 99.2% and specificity is 99.2%.

2.5. Ethic statements

Study protocol was approved and published by Burkina Faso Ministry of Environment and Fisheries in its deliberation n°2014 2015 – 001 / MERH/CAB. Blood samples were collected by licensed and certified veterinarians.

2.6. Statistical methods

Data were processed and analyzed using Microsoft Excel 2013 and GraphPad Prism version 7 (GraphPad Software, La Jolla, California USA). Only apparent prevalence were calculated for each species. Binary logistic regression analyses were carried out to determine which variables (Locality, sex and Species) were significantly associated with detection of HEV antibodies. Multivariate logistic regression was performed using R software version 2.13.0 (<https://www.r-project.org/>). $P < 0.05$ was considered significant. The lower and upper limits of the 95% confidence interval (CI) for a proportion were also calculated.

3. Results

3.1. Prevalence of anti-HEV antibody in different animal species

We detected anti-HEV total antibodies in 121 (34.9% CI95% [29.9–39.9]) out of the 347 mammals samples examined by ELISA.

Anti HEV total antibodies were detected in 60/100 rabbits (60.0% CI95% [50.4–69.6]); 10/19 hares (52.6% CI95% [30.2–75.1]); 23/81 goats, (28.4% CI95% [18.6–38.2]); 19/72 cattle (26.4% CI95% [16.2–36.6]) and 9/75 sheep (12.0% CI95% [4.6–19.4]) respectively as shown in Table 1. Fig. 2 presents the distribution of positive and negative cases according to the Optical Density (O.D.) of ELISA test and clearly highlights the indeterminate cases.

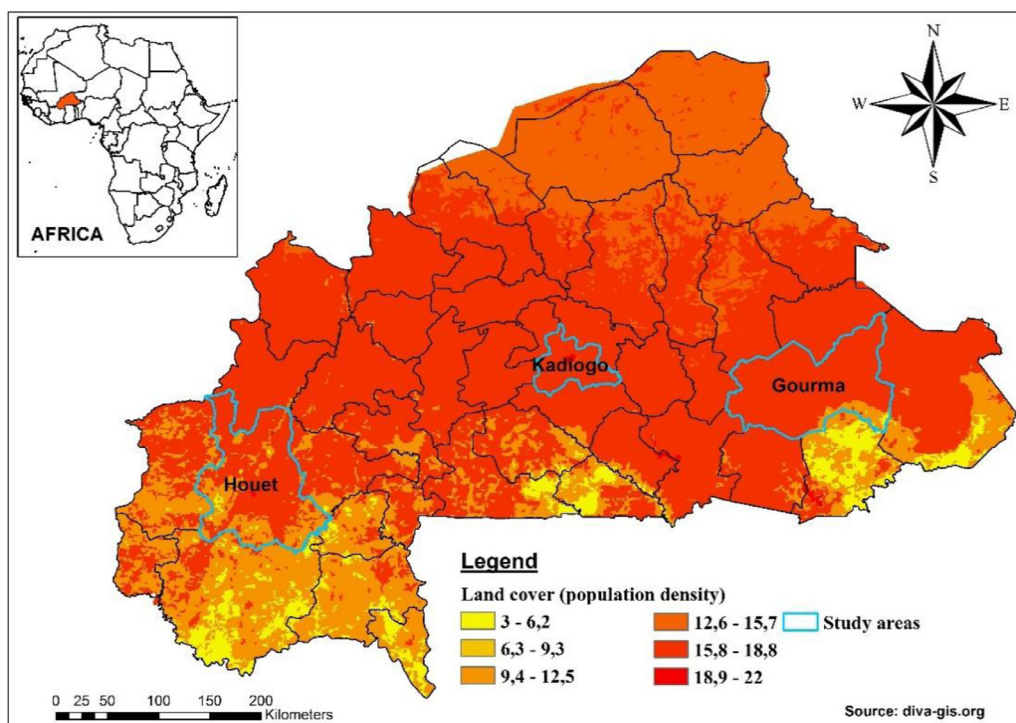


Fig. 1. Map of sampling sites
 Note: The map shows sampling sites (surrounded in green) that correspond to areas of high human population density and hunting areas. It presents three study regions stretching from East to West. The high human density is increasing from yellow to red. The density of the population varies from 3 to 22 inhabitants per hectare.

3.2. Prevalence of anti-HEV antibodies in study areas

Within a specie, anti-HEV antibodies positivity rates varied according to different locations (Table 2); rabbit in the province of Kadiogo are more likely to be infected than those in province of Gourma and Houet ($p < 0.05$).

Comparing domestic and wild animals within an area where both lagomorph species were tested, prevalence in hares (10/19 (52.6%)) is higher than that in rabbits (15/44 (34.1%)) in both Gourma and Houet provinces ($p > 0.05$) (Table 1).

Considering the positive cases in a locality without distinction of species (Table 2), the highest seroprevalence was detected in Gourma (52.9%, CI95% [36.2–69.7]) and in Kadiogo area (33.8%, CI95% [28.3–39.3]). The lowest value of 24.1% (CI95% [8.6–39.7]) was recorded for the province of Houet.

Considering all animals species, sex distribution of HEV infection were 62/169 (35.9% CI95% [28.7–43.2]) in males and 61/182 (33.9% CI95% [27.0–40.8]) in females. There was no correlation between gender and HEV seropositivity ($p > 0.5$).

4. Discussion

Evidence of endemic HEV infections in Burkina Faso was demonstrated through the evaluation of prevalence or incidence of HEV in human population (blood donor and pregnant women) and in asymptomatic domestic swine (Traore et al., 2015, 2016). To the best of our

knowledge, this is the first study that investigates anti-HEV antibodies in other mammals that are important part of local food habits in three provinces of Burkina Faso.

HEV antibodies were detected in 34.9% of tested animals belonging to five (05) mammalian species, which indicate that HEV viruses or HEV infections occur in a wide range of mammals. These data match findings from a study conducted in Nigeria that found anti-HEV antibodies in pigs, goats, sheep and cattle (Junaid et al., 2014).

Table 3 summarizes previously reported HEV prevalence rate in rabbits, which varies from 3.4% (Di Bartolo et al., 2016) to 72.5% (Liu et al., 2017). Three studies conducted with laboratory developed tests determined prevalence rate of 36.0% (Cossaboom, Cordoba, Dryman, & Meng, 2011), 3.4% (Di Bartolo et al., 2016) and 62.5% (Liu et al., 2017). Five other studies using commercial kit from Wantai Biopharmaceutical reported prevalence rates of 57.0% (Zhao et al., 2009), 15.4% (Geng et al., 2011c), 54.6% (Geng et al., 2010a,b,c, Geng et al., 2011b), 37.3% (Hammerschmidt et al., 2017) and 72.5% (Liu et al., 2017). Our results in Burkina Faso are close to the highest rates previously reported. We strongly feel/believe that lowest prevalence rates can't solely be explained by genotypes variation or immunological characteristics. Other factors such as breeding hygiene conditions, rabbit species, and transmission mode in different regions should also be considered (Liu et al., 2017). During our study we noticed poor hygiene and breeding space management conditions that allow cross-contamination between animals and wide spread of HEV. This can explain the high prevalence rate of HEV found in our study.

Table 1
 Comparison of prevalence of total antibodies anti-hepatitis E 477 virus in the different animal species and the three provinces of Burkina Faso.

Species	Seroprevalence (%) and 95% CI	Seroprevalence (%) in provinces and 95% CI		
		Kadiogo	Houet	Gourma
Rabbits	60.0 [50.4–69.6]	80.4 [70.0–90.8]	19.2 [4.1–34.4]	55.6 [32.6–78.5]
Hares	52.6 [30.2–75.1]	NS	66.67 [13.3–100.0]	50.00 [25.5–74.5]
Cattle	26.4 [16.2–36.6]	26.4 [16.2–36.6]	NS	NS
Sheep	12.0 [4.6–19.4]	12.0 [4.6–19.4]	NS	NS
Goats	28.4 [18.6–38.2]	28.4 [18.6–38.2]	NS	NS

Note: CI = confidence interval. NS = No Sample.

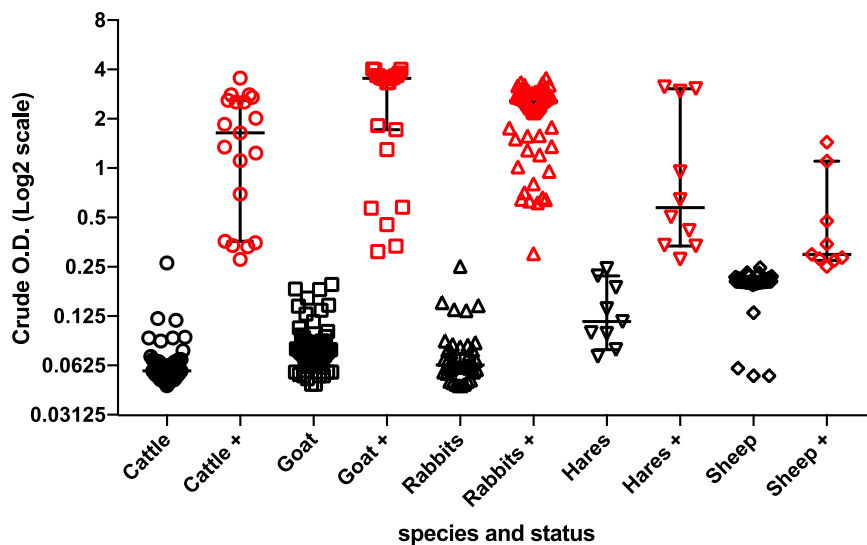


Fig. 2. Distribution of the positive and negative cases according to the Optical Density
 Note: Distribution of the positive and negative cases according to the Optical Density (OD) of samples from the various species studied. Positive cases are in red and negative cases are in black. Positive samples were defined according to the Kit CUT-OFF value according to the internal standard of each assay.

Further studies are needed in Burkina Faso to explore zoonotic transmission of HEV and the molecular epidemiology of the virus.

Our survey shows a HEV seroprevalence of 52.6% (IC 95%; [30.2-75.1]) in hares (Table 1), suggesting that hares could be a reservoir of the virus in the wildlife in West Africa. In contrast, a low seroprevalence rate of 2.2% (Using Axiom test corresponding to the assay produced by Wantai) has been reported in Germany although for different species of hare (Hammerschmidt et al., 2017). This low prevalence, associated with those of two other studies carried out on the same species in Italy (Mazzei et al., 2015; Serracca et al., 2015), could indicate assumed minor importance of European hare in the epidemiology of HEV (Hammerschmidt et al., 2017). This discrepancy needs to be clarified by conducting more studies about HEV infection status in different districts. To that end, different habitats and biological behaviors should be considered.

The anti-HEV seroprevalence rate of 26.4% found in cattle samples was higher than those found in cattle from China or USA (Table 3). In these areas, HEV genotype 4d that is also found in human seems to circulate in the cattle (Yan et al., 2016). If the same strains were found in Burkina's cattle, as infectious HEV can also be excreted into milk, this can cause a new potential zoonotic risk, considering the Burkinabe population's consumption of the unpasteurized milk. In addition, HEV in milk seems not to be inactivated by pasteurization (Huang et al., 2016). However, recent data suggest in cattle a potential antigenic cross-reaction of the antibodies with anti-HEV responsiveness with a related, but as yet unknown agent (Yugo et al., 2019). We thus must be very cautious about the possible role of cattle in HEV transmission until new methods like deep sequencing of transcriptome or proteome of

these "HEV positive" cattle may allow to identify the real antigen identity.

In this study, a prevalence of 12.0% (9/75) was recorded in sheep. This was in accordance with prevalence from similar studies in Nigeria and China (Geng et al., 2010a,b,c; Junaid et al., 2014). Higher prevalence has been recorded with 29.0% to 35.2% in China (Wu, Kang, Bai, & Bai, 2010; Wu, Si, Jiang, Li, & Jin, 2015), and 31.8% from Nigeria (Shuaibu et al., 2016). The high prevalence recorded in China could be linked to the sheep breed studied, there specific strains and the geographic area. The low prevalence of 12.0% observed in the sheep of our study can be explained by the presence of indeterminate cases. These indeterminate cases are samples that have an Optical Density value close to or superimposed on those of the cut-off (Fig. 2). In China, phylogenetic analysis based revealed that HEV strains isolated from sheep belong to genotype 4, subtype 4d (Wu et al., 2015), a genotype that can be transmitted from animals to humans. However, the zoonotic potential of HEV in sheep is still under discussion (Junaid et al., 2014).

Our study showed a prevalence of 28.4% (23/81) in goat which was also in accordance with similar studies in Nigeria and China (Table 3). To note, 100% (86/86) of goats in India were tested positive (Shukla, Chauhan, Naik, Anderson, & Aggarwal, 2007), although in the absence of molecular characterization of these putative HEV infection we cannot relate that to the repeated human HEV outbreaks recorded in India. In contrast, other studies demonstrated no anti-HEV antibodies in goats (Vital et al., 2005). Therefore, HEV infection in goats may be infrequent, or the diagnostic methods used (in-house test) were not totally appropriate for this species (Arankalle et al., 2001).

The main limitation of our study is the usage of the single MP

Table 2

Comparison of prevalence of total antibodies Anti-HEV in domestic and wild animals in three developed provinces and two rural provinces of Burkina Faso according to different animal species, genders and localities.

	Nr positive/nr tested	Total antibodies anti-HEV%	p-value	Odds ratio	IC95% Odds ratio	
Rabbits	60 (/100)	60.0 ± 9.6	Ref	Ref	Ref	Ref
Cattle	19 (/72)	26.4 ± 10.2	1.3.10 ⁻⁸	11,6	[5.2-28.2]	<0.001***
Goats	23 (/81)	28.4 ± 9.8	1.9.10 ⁻⁸	10.5	[4.8-24.9]	<0.001***
Hares	10 (/19)	52.6 ± 22.5	0.6	0.8	[0.2-2.6]	>0.05
Sheep	9 (/75)	12.0 ± 7.4	3.2.10 ⁻¹²	31.3	[12.5-87.5]	<0.001***
Female	61 (/180)	33.9 ± 6.9	Ref	Ref	Ref	Ref
Males	60 (/167)	35.9 ± 7.3	0.5	0.8	[0.5-1.4]	>0.05
Houet	7 (/29)	24.1 ± 15.6	Ref	Ref	Ref	Ref
Gourma	18 (/34)	52.9 ± 16.8	0.04	3.3	[1.0-11.2]	<0.05*
Kadiogo	96 (/284)	33.8 ± 5.5	2.4.10 ⁻⁶	13.9	[4.9-44.4]	<0.001***

Note: OR, odds ratio; CI, confidence interval; *, statistically significant. Statistically significant analyzes in the study were shown in the figure with an asterisk (*); **, statistically significant and P value < 0.05.

Table 3
Comparison of seroprevalence rates per animal species between seroprevalences recorded in different countries and the results of our study.

Species /Expected genotype	Prevalence from literature		Method ELISA		Method PCR		Seroprevalence in our study			
	Percent	Positive / Total	Method ELISA Used	Percent (%)	Positive / Total	Ref*	Countries / Year of study	Percent	Positive/ Total	IC 95%
Rabbit Gen: 3	54.6	65/119	Commercial kit (Wantai)	6.9	8/115	Geng et al. (2011a)	China (North) / 2010	60.0	60/100	[50.4–69.6]
	37.3	47/126	Commercial kit (Wantai)	17.1	28/164	Hammerschmidt et al. (2017)	Germany / 2016			
	36.0	31/85	In-house test	16.0	14/85	Cossaboom et al. (2011)	USA (Virginia) / 2011			
	57.0	191/335	Commercial kit (Wantai)	7.6	11/144	Zhao et al. (2009)	China (North) / 2009			
Hare	15.4	169/1094	Commercial kit (Wantai)	2.0	22/1094	Geng et al. (2011a)	China (North; Northeast; North; South East; South) / 2010			
	3.4	7/206	In-house test	0.0	0/206	Di Bartolo et al. (2016)	Italy / 2015			
	72.5 and 62.5	58/80 and 50/80	Commercial kit (Wantai) for human and In-house test for swine sera	46.1	24/52	Liu et al. (2017)	China (Center) / 2017			
	2.2	14/624	Commercial kit (Axiom corresponding to the assay produced by Wantai.)	0.0	0/624	Hammerschmidt et al. (2017)	Germany / 2016	52.6	10/19	[30.2–75.1]
Cattle Gen: 4d	0.0		Commercial kit (DIA.PRO)	–	–	Mazzei et al. (2015)	Italy / 2015			
	15.0	52/346	Commercial kit (Wantai)	–	–	Geng et al. (2010a)	China (North) / 2010			
	28.2	257/912	Commercial kit (Wantai)	0.8	7/912	Geng et al. (2010a)	China / 2008 (center, North, South, East and West)	26.4	19/72	[16.2–36.6]
Sheep Gen: 4d	6.0	6/100	Commercial kit (Wantai)	0.0	0/100	Zhang et al. (2008)	China (East) / 2004-2006			
	15.0	174/1156	In-house test	–	–	–	Dong et al. (2011)	USA (Virginia) / 2011		
	100.0 And 77.5%	58/58 And 45/58	Genelabs Diagnostics (Commercial kit) and In-house test	0.0	0/58	Shukla et al. (2007)	India / 2006	12.0	9/75	[4.6–19.3]
Goat Gen: 3c	10.5	2/19	Diagnostic Automation (commercial kit for human)	–	–	Junaid et al. (2014)	Nigeria / 2012			
	31.8	56/176	ID-vet (commercial kit)	–	–	Shuaibu et al. (2016)	Nigeria / 2016			
	35.2	176/500	Commercial kit (Wantai)	5.3	4/75	Wu et al. (2015)	China (West) / 2014			
	100.0	86/86	Genelabs Diagnostics (Commercial kit) and In-house test	0.0	0/86	Shukla et al. (2007)	India / 2006	28.4	23/81	[18.6–38.2]
	10.4	73/700	Commercial kit (Wantai)	1.6	11/700	Geng et al. (2010)a	China (center, North, South, East and West) / 2008			
	37.2	16/43	Diagnostic Automation (commercial kit for human)	–	–	Junaid et al. (2014)	Nigeria / 2012			
	16.0	13/80	In-house test	0.0	0/80	Sanford et al. (2013)	USA (Virginia) / 2002			
	24.0	12/50	Commercial kit (Wantai)	0.0	0/50	Zhang et al. (2008)	China (East) / 2004-2006			
	0.0	0/5	In-house test	–	–	Vitral et al. (2005)	Brasil / 2005			

Note: * references.

Diagnostics HEV ELISA 4.0v, that is an enzyme-linked immunosorbent assay intended for the detection of antibodies to Hepatitis E Virus in serum or plasma. The second limitation of this study is that was a prerequisite for complementary study that will focus on molecular detection of the virus RNA. Fig. 2 presents a distribution of the positive and negative cases according to the Optical Density (OD) of each sample. Positive cases are in red and negative cases are in black. Some positive specimens seem stackable to negative; this is explained by the fact that some samples, particularly in sheep, have an OD value close to or equal to the CUT-OFF value. These samples could correspond to indeterminate cases and should be re-examined by another test such as Western Blot, in order to eliminate false positive cases caused by potential agents antigenically-related to HEV (Sridhar et al., 2019; Yugo et al., 2019).

5. Conclusion

Finally, the observed anti-HEV seroprevalence were considerably high in rabbits and local hares an already identified HEV reservoir in other countries, and was significantly lower but still present in cattle, sheep and goats. Our results could suggest that antigenic markers of HEV circulates among several animal species that are in the food chain in Burkina Faso. Further investigations are underway to identify the HEV genome in these animals as well as in sewage samples of animal origin for the dispersion capacity of the virus.

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Declaration of Competing Interest

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