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Seroprevalence of Hepatitis E Virus (HEV) In Goats from Germany

Dewi Murni[1] , Franziska Brüning[1], Christine Fast[1], Lisa Dahnert[1], Martin H. Groschup[1], Rainer G. Urlich[1]

[1]Friedrich Loeffler Institute, Institute for Novel and Emerging Infectious Diseases, Greifswald-Insel Reims.
Correspond to Dewi Murni. Telp/WhatsApp: +49 1748920440. E-mail: dewi_4access@yahoo.com

Abstract : Background: Hepatitis E Virus (HEV) cause an acute hepatitis in human. The disease is endemic in many developing countries and epidemic in some industrialized countries. The virus is being transmitted by food safety and it is recognized as zoonotic disease. Many animals act as reservoir of HEV infection, one of them is goat. HEV has four genotypes, genotype 1 and 2 of HEV are infectious to human, the genotype 3 and 4 of HEV are infectious to animals and these genotypes are *zoonotic*.Methodology: We collected plasma samples of goats from breeding farms in 12 federal states in Germany. Then we collected plasma from blood and created pools that contain five plasma samples and depending on quality of hemolytic of plasma samples, breed and owner of breeding farm in every federal state. After that, we analyzed the HEV antibody by Axiom-ELISA. Result: The average HEV sera prevalence in goats from Germany are 2.57% (44/1,710). The data were collected from several federal states from 2013 to 2015. The data with different ratio had given by the following: Baden-Wuerttemberg 4.00% (7/175), Bavaria 2.74% (6/219), Brandenburg 0.65% (1/154), Hesse 3.66% (3/82), Mecklenburg-Vorpommern 8.33% (7/84), North Rhine Westphalia 0.00% (0/91), Lower Saxony 0.84% (1/119), Rhineland-Palatinate 1.53% (4/262), Saxony 0.70% (1/143), Saxony-Anhalt 0.00% (0/49), Saarland 8.00% (4/50), Schleswig-Holstein 7.04% (5/71) and Thuringia 2.37% (5/211).Summary: We suspect that HEV related agents are circulating in the breeding farms due to the different ways of managements, according to the results.

Keyword: Hepatitis E Virus, HEV, goat plasma samples.

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I. INTRODUCTION

Hepatitis E Virus (HEV) causes the Hepatitis E disease with predisposition agent in liver. HEV has fecal-oral transmitted route. The disease was reported since 1955 in New Dehi, India (Kumar, 2013). HEV is causative agent for acute hepatitis in one-third of world's population and fulminant hepatitis in pregnant women (Zhu et al., 2010). The virion is relatively resistant to environmental conditions and remains infectious in event of sewage. Overall, the major route of HEV transmission is ingestion of fecal contaminated water (Teshale et al., 2010).

HEV is a single-stranded, positive sense, non-enveloped RNA virus that classified in the genus Hepevirus and include in Heperividae family (Purcell et al., 2008 and Meng et al., 2011). The Hepeviridae family has two genera with naming Orthohepevirus and Pescihepevirus. The Orthohepevirus genus is advanced divided into four species with different hosts. Orthohepevirus A attack to humans, pigs, wild boars, deer, mongoose, rabbit, and camel. Orthohepevirus B attack to chicken. Orthohepevirus C attack to rats and ferrets. Orthohepevirus D contains HEV isolated from bats. Pescihepevirus has one species that call Pescihepevirus A and it attack to trout species (Johne et al., 2014 and Smith et al., 2014).

In Orthohepevirus A has seven different genotypes. HEV-1 and HEV-2 are hosts confined to humans, while HEV-3 and HEV-4 zoonosis with pigs that serve as reservoirs. HEV-5 and HEV-6 are found in wild boars, and HEV-7 is found in camels (Anonimous, 2015). Johne et al., (2014) trialed of experimental with seven genotype of hepevirus infections to different mammal species and chicken. The genotype of hepeviruses were GT1, GT2, GT3, Rabbit GT3, GT4, rat-HEV and avian-HEV and all of them were trialing to monkeys, pigs, rabbits, gerbils, rats, mice and chicken. From seven genotypes of hepeviruses were infected to monkeys and pigs, every genotype got infection successful except with rat-HEV and avian-HEV, but only avian-HEV without trial to the animal model pigs. When three genotypes were infected to the rabbits with naming the GT3, rabbit-GT3 and GT4 HEVs, only rabbit-GT3 HEV got infection successful. Thus, getting successful infected of GT4 HEV to Gerbil and only rat-HEV got successful to the rats so it was with avian-HEV to the chicken was successful.

In mammalian, HEV has 7 recognized genotypes. Genotype 1 HEV outbreaks to human in Asia. Genotype 2 HEV outbreaks to human in Africa and Mexico. While infectious to human, genotype 3 HEV (Emerson *et al*., 2005), the genotype was isolated in deer (Takahashi *et al.,* 2004), swine (Takahashi *et al.,* 2004; Meng *et all*., 1997), mongooses (Nakamura *et al*., 2006), rats (Lack et al., 2012), rabbits (Cossaboom *et al*., 2011 and Zhao *et al*., 2009). This genotype is associated with infectious HEV in human (Meng, 2013).

HEV is transmitted via the fecal-oral route through contaminated food or water and typically causes an acute icteric disease known as hepatitis E (Meng 2010; Purcell 2001). This route is the cause of most clinical cases of the disease so that cases of hepatitis E are associated with poor sanitation, where the virus is expelled in infected feces to reach drinking water sources. Other transmission routes have been identified and considered for impact, but the number of clinical cases in humans is much smaller. These transmission routes include: the consumption of cooked meat or meat products derived from infected animals; transfusion of infected blood products; and vertical transmission from pregnant women to their fetuses and consumption of raw or raw shellfish can be a source of sporadic cases in endemic areas. Given this research information, it is possible that the source of drinking water contaminated by animals is a source of transmission as well.

The definite transmission of HEV from animal to human (Takahaski *et al*., 2004) had been reported that the people consumed the meats of animal production. On the other hand, many countries consume the meat products of goat, sheep and cattle what were infected by HEV (Arankalle *et al*, 2001; Peralta *et al*., 2009). Lately, the cases of HEV infection in developing countries were reported to be associated with sanitation of the life of people.

HEV infectious to the pigs, cattle, goats and sheep (Junaid *et al*., 2014), human-non-primates, rabbits, turkeys and rodents (Yogo *et al*., 2014), and camels (Woo, 2014). The virus is infectious also to the ferrets, rats, rabbits, chicken and bats (Johne *et al*., 2014). Infectious to the animals and human, these suspected genotypes are zoonosis transmission (Dalton *et al*., 2008; Michitaka *et al.*, 2007; Takahashi *et al.*, 2004). Manifestation clinics of HEV are neurogic (Kamar *et al*., 2011) and liver inflamation such as jaundice, pruritus and upper abdominal pain in human (Pischke, 2014) but asymptomatic to the animals.

Seroprevalence of HEV in human from industrialized country, mainly 20% in United States (Kuniholm *et al*., 2009), 14.1% Southern France (Temmam, 2013), 13.55% in Austria (Fischer, 2015) and 17% in Germany (Pischke, 2014).

Here, we are interested to know what kind of relationship amongst the disease, human and goats in Europe especially in Germany because the people in Germany follows traditional breeding, keeping landscape management, and the people like to consume meat and milk of goat production. Then we will be able to know the prevalence of HEV in goats from Germany.

II. MATERIAL AND METHODS

Blood and plasma samples

A total of 1940 plasma samples of goats from several breeding farm in thirteen federal states of Germany were collected during 2013, 2014 and 2015 agreement from owner (samples from Franziska Brüning and Christine Fast). Then collecting plasma from blood and created pools that containing five of plasma samples depending on quality of hemolytic of plasma samples, breed, and owner of breeding farm in every federal state. All of plasma samples were stored in -70°C and using dry ice when transportation.

Detection of anti HEV antibodies

Commercial Enzyme-Linked Immunoassay (ELISA), the product of AXIOM from Germany according to modified protocols instructions, detected anti-HEV antibodies. Collecting 12 µl of plasma sample to get 60 µl in one pool. After that, homogenous of plasma sample and detecting antibody of pool. Positive result of pools would be repeating assay to one by one of plasma samples and dubious result would be repeating assay of pool.

III. RESULT

For initial screening pools of five samples of plasma each were generated. For all pools with a result of ≥ 0.8 calculate sample of the pool was tested individual.

Table 1. Prevalence of goatHEV

Nr.	Plasma of Goats from Germany		Positive	Prevalence of goatHEV
	City	Total Count	Sera	
1	Baden-Wuerttemberg	175	7	4.00%
2	Bavaria	219	6	2.74%
3	Brandenburg	154	1	0.65%
4	Hesse	82	3	3.66%
5	Mecklenburg-Vorpommern	84	7	8.33 %
6	North Rhine-Westphalia	91	0	0.00 %
7	Lower Saxony	119	1	0.84%
8	Rhineland-Palatinate	262	4	1.53%
9	Saxony	143	1	0.70%
10	Saxony-Anhalt	49	0	0.00%

11	Saarland	50	4	8.00%
12	Schleswig-Holstein	71	5	7.04%
13	Thuringia	211	5	2.37%
		1710	44	2.57%

The prevalence range of anti-HEV antibodies in goats from 0.00% (0/91 and 0/49) to 8.33% (7/84). Overall, the prevalence of anti-HEV antibodies was 4.00% (7/175) in Baden-Wuerttemberg, 2.74% (6/219) in Bavaria, 0.65% (1/154) in Brandenburg, 3.66% (3/82) in Hesse, 8.33% (7/84) in Mecklenburg-Vorpommern, 0.00% (0/91) in North Rhine Westphalia, 0.84% (1/119) in Lower Saxony, 1.53% (4/262) in Rhineland-Palatinate, 0.70% (1/143) in Saxony, 0.00% (0/49) in Saxony-Anhalt, 8.00% (4/50) in Saarland, 7.04% (5/71) in Schleswig-Holstein and 2.37% (5/211) in Thuringia. Here, the lowest prevalence of goatHEV was 0.00% in North Rhine Westphalia and Saxony-Anhalt. Then the highest prevalence was 8.33% in Mecklenburg-Vorpommern (Table 1).

IV. DISCUSSION

Hepatitis E virus (HEV) is a small, spherical and non-enveloped, positive-sense single stranded RNA virus with icosahedral capsid symmetry. It belongs to the genus *Orthohepevirus* in the family *Hepeviridae.*

Hepatitis E Virus is pathogenic agent of Hepatitis E Disease with predisposition in the liver. The symptoms of this disease are similar with Hepatitis A and B. Therefore, we call the disease as enterically transmitted non-A**,** non-B **(**ET-NANB**).** Dissemination of the virus usually spread by the wrong sanitation management in one area, and then the virus would contaminate the food and water and the virus would be transmitted to the host by fecal-oral. Clinical manifestations of HEV is asymptomatic in animals.

This study uses commercial ELISA with identified IgG and IgY antibody and sensitive to the general genotype of HEV. We have 1940 plasma samples, but only 1710 plasma samples have identified by ELISA because 230 of plasma samples were not clear enough to identify. In this study, used of modified manuscript protocol that made pools and every pool contain five of plasma samples with different federal states. Then the plasma pools screened by ELISA. Every positive results of plasma pools would be retesting by ELISA again. The prevalence of anti-HEV antibody of goats in Germany was 2.57% but every state in Germany has a difference of prevalence. The prevalence range of anti-HEV antibodies in goats from 0.00% (0/91 and 0/49) to 8.33% (7/84). In sequence, the prevalence rate of HEV disease in goats in several cities in Germany were 4.00% (7/175) in Baden-Wuerttemberg, 2.74% (6/219) in Bavaria, 0.65% (1/154) in Brandenburg, 3.66% (3/82) in Hesse, 8.33% (7/84) in Mecklenburg-Vorpommern, 0.00% (0/91) in North Rhine Westphalia, 0.84% (1/119) in Lower Saxony, 1.53% (4/262) in Rhineland-Palatinate, 0.70% (1/143) in Saxony, 0.00% (0/49) in Saxony-Anhalt, 8.00% (4/50) in Saarland, 7.04% (5/71) in Schleswig-Holstein and 2.37% (5/211) in Thuringia. Here, the lowest prevalence of 0.00% in North Rhine Westphalia and Saxony-Anhalt, then the highest prevalence of 8.33% in Mecklenburg-Vorpommern (Table 1).

From 13 federal states, there were 11 federal states suspected with goatHEV and two federal states with nothing of goatHEV. The clearing HEV state were North Rhine-Westphalia and Saxony-Anhalt. Contradiction of sera prevalence HEV in each breeding farm in every federal state in Germany, probably, because every farm has different management to handle their farms. In addition, visitor to the breeding farm who has positive HEV would be in contact and can contaminate to the breeding. Whit in the transportation of animal from one breeding farm to another breeding farm are suspected, the contact between rodents to the facility of breeding farm would be suspected also but Johne et al., (2014) reported that the genotype of rat HEV is not infect to the ruminant and mono gastric animals.

There are some reports of HEV sera prevalence of goats in some developing countries. It has been reported that there are cases of goats infected by HEV in China with a prevalence of 12% (12/50) (Zang et al., 2008) and 1.6% (11/700) of sera prevalence (Gang et al., 2010). Wang et al., 2002 said that the level of prevalence could be found more widely in China. In addition, cases of HEV disease in Virginia occur also with the level of HEV sera prevalence in goats by 16% (13/80) (Sanford et al. (2014) and in Pateau in State-Nigeria by 37% (32/43) (Junaid et al., 2014). Approximately, HEV sera prevalence in goats from Germany with an average of 2.57% (44/1710) has been found it. This study is the first reported case of HEV on goats from Germany so far.

However, HEV disease control is very important; it can avoid the spread or expansion of hepatitis C infection. Therefore, it is necessary to monitor animal health especially against HEV infection. Thus, genetic identification of HEV species in animals is essential to know the ability to show cross-HEV infection of animals. It can tell the diversity of HEV and the extent to which the host infects. The presence of HEV needs to be anticipated, especially GT-3 and GT-4 HEV because these genotypes are zoonotic. One way is to provide healthy animal products. Goats are commodities of consumption livestock that can serve as reservoirs against HEV. Direct contact with infected goats and other animals and the consumption of contaminated animal meat and meat products poses a risk of HEV infection.

Given this HEV, disease shows asymptomatic in animals that are unknown and unseen where there is no vaccine to caring their immunity, while the disease is potential zoonosis and very dangerous for fetal security in the womb of women it is necessary to conduct an animal health management program in the environment. Animal health management program in question is to maintain the cleanliness of the environment and equipment of cages and slaughterhouses, providing hygiene drinking and eating, limiting the contacts of goats in different farms and limiting the presence of visitors to the stables and farms. In addition, farm workers must maintain personal hygiene.

Hygiene in drinking water (Emerson, 2004) and food (Said, 2009) should be the prevention of HEV infection. For travelers from endemic areas should recover from contaminated areas and should avoid eating raw meat of animal products. Special care should be taken when cooking some animal products. Thus, pasteurization of drinking water and milk from animal products is recommended.

V. CONSLUSSION

Seroprevalence is approximately 2.57% (44/1710) HEV in goats in Germany. The thirteen federal states have different percentages of seroprevalence, two of 13 federal states were free of HEV although the country had many developer farms in the state of North Rhine Westphalia and Saxony-Anhalt. Therefore, we suspected that HEV-related agents were being circulated on developer farms according to the various ways of management, according to the results.

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