

Serological and molecular evidence of hepadnavirus infection in swine

Yasmine R Vieira¹, Marcelle FM Silva², Débora RL Santos³, Antônio A Vieira⁴, Janice R Ciacci-Zanella⁵, Gonzalo Barquero⁶, Bárbara V do Lago⁷, Selma A Gomes⁷, Marcelo A Pinto¹, Vanessa S de Paula¹

¹ Laboratory of Development Technological in Virology, Oswaldo Cruz Institute, FIOCRUZ, RJ, Brazil

² Laboratory of Comparative and Environmental Virology, Oswaldo Cruz Institute, FIOCRUZ, RJ, Brazil

³ Department of Veterinary Microbiology and Immunology, Federal Rural University of Rio De Janeiro, UFRRJ, RJ, Brazil

⁴ Department of Animal Production, Institute of Animal Science, Federal Rural University of Rio De Janeiro, UFRRJ, RJ, Brazil

⁵ Brazilian National Research Center, EMBRAPA Swine and Poultry, SC, Brazil

⁶ Tropical Sustainability Institute, TSI, SP, Brazil

⁷ Laboratory of Molecular Virology, Oswaldo Cruz Institute, FIOCRUZ, RJ, Brazil

Vieira YR, Silva MFM, Santos DRL, Vieira AA, Ciacci-Zanella JR, Barquero G, do Lago BV, Gomes SA, Pinto MA, de Paula VS. Serological and molecular evidence of hepadnavirus infection in swine. *Ann Agric Environ Med*. 2015; 22(1): 11–16. doi: 10.5604/12321966.1141361

Abstract

Introduction and objective. Recently, investigations in a swine herd identified evidence of the existence of a novel member of the Hepadnavirus family endemic in swine. The aim of this study was to investigate the serological and molecular markers of Hepadnavirus circulation in Brazilian domestic swine and wild boar herds, and to evaluate the identity with HBV and other Hepadnaviruses reported previously.

Materials and methods. For the study, 376 swine were screened for hepatitis B virus serological markers. Analyses were performed in serum samples using commercial enzyme-linked immunosorbent assay (ELISA) kits (DiaSorin®) for anti-HBc, HBsAg and anti-HBs. Reactive and undetermined swine serum samples were selected to perform DNA viral extraction (QIAamp DNA Mini Kit, Qiagen®), partial genome amplification and genome sequencing.

Results. From 376 swine samples analysed, 28 (7.45%) were reactive to anti-HBc, 3 (0.80%) to HBsAg and 6 (1.6%) to anti-HBs. Besides, more 17 (4.52%) swine samples analyzed were classified in the grey zone of the EIA test to anti-HBc and 2 (0.53%) to HBsAg. From 49 samples molecularly analyzed after serological trial, 4 samples showed a positive result for the qualitative PCR for Hepadnavirus. Phylogenetic reconstruction using partial genome sequencing (360 bp) of 3 samples showed similarity with HBV with 90.8–96.3% of identity.

Conclusions. Serological and molecular data showed evidence of the circulation of a virus similar to hepatitis B virus in swine.

Key words

diagnostic, hepadnavirus, swine

INTRODUCTION

Hepatitis B virus (HBV) infection constitutes a major public health problem globally. It is estimated that more than two billion people have been infected, and of these over 400 million people are chronically infected by this widespread pathogen [1]. Despite the availability of an effective vaccine, about one million people die each year due to HBV infection [2].

In most adults, HBV infections resolve spontaneously and a lifelong protective immunity response is maintained. However, most neonates and infants become chronically infected, with a high risk of developing life-threatening complications, such as chronic liver injuries, including cirrhosis and hepatocellular carcinoma (HCC) [3].

The main routes of HBV transmission are sexual, perinatal and percutaneous [4]. In endemic areas, like Asia countries, sub-Saharan Africa and amongst indigenous peoples in Central and South America, mother-to-child and horizontal transmissions constitute the predominant means of transmission [5]. In areas of low prevalence, the practice of drug injection and risky sexual behaviour represent the most frequent routes of infection [4].

Although HBV has not been described as a biological agent of zoonotic risk for farm workers [6], a possible occupational exposure as a form of infection should be considered since recently HBV and hepatitis E virus (HEV) have been included as work-related pathogens for slaughterhouse workers [7].

An HBV particle is 42 nm in diameter, enveloped, and presents a partially double-stranded circular DNA genome of about 3.2 Kb, with an intermediate RNA during replication. It belongs to the Hepadnaviridae family which is divided in 2 genera, Orthohepadnavirus and Avihepadnavirus, from mammals and birds, respectively [1, 8].

The Orthohepadnavirus genus includes HBV that naturally infects humans and some non-human primates, including chimpanzees, gibbons, gorillas, orangutans; WMHBV which infects woolly monkeys; and similar viruses which infect rodents, such as WHV (woodchuck) and GSHV/ASHV (squirrels). Avihepadnavirus genus includes viruses that have been reported infecting ducks (DHBV), geese (GHBV), herons (HHBV), and storks (STHBV) [9].

Like most Hepadnavirus, HBV displays a narrow host range where it is possible to maintain the viral life cycle. The majority of these potential host species are not commonly used in medical research due to difficulty of availability, management in captivity and high cost maintenance [10]. Although host-specific, it is suspected that HBV ability of crossing specific barriers could hinder attempts to eradicate

Address for correspondence: Yasmine R Vieira, Laboratório de Desenvolvimento Tecnológico em Virologia, Pavilhão Hélio e Peggy Pereira - 2º andar - sala B220, Instituto Oswaldo Cruz, FIOCRUZ, Av. Brasil, nº 4365, Manguinhos - RJ, Rio de Janeiro, Cep: 21045-900, RJ, Brasil
E-mail: yasminerangel@ig.com.br

Received: 18 June 2013; Accepted: 05 September 2013

the human infection by immunization [11]. Furthermore, environmental circumstances, increase in travel, trade of animals and others factors, have been discussed as important influences in viral diseases behaviour, such as its emergence, distribution and evolution [12].

OBJECTIVES

Recently, researchers found evidence of the circulation of a new member of the Hepadnaviridae family endemic in swine [13] and in chickens [14] from China. However, it was not possible to determine whether the infection is actually caused by the same virus that causes infection in humans, or by a virus similar to HBV. The presented study aims to investigate evidence of Hepadnavirus circulation in Brazilian swine and to estimate the similarity with HBV and other Hepadnaviruses reported previously.

MATERIALS AND METHOD

Ethic Committee and Study Group. The study was approved by the Ethics Committee on Animal Use (CEUA) of the Oswaldo Cruz Foundation/FIOCRUZ, registered under license LW-49/11. 376 swine *Sus scrofa* from nursery to slaughter age (4–25 weeks) of both genders were investigated for serological markers of HBV. The study population was divided into groups A and B, corresponding to 288 domestic pigs from commercial herds and 88 wild boars, respectively.

Sample Collection. 5 mL of venous blood were collected from the marginal ear/cephalic vein with a 10 mL syringe and 18G needle, and transferred into vacuum tubes containing gel separator and clot activator (BD Vacutainer® SST II Advance). The tubes were kept refrigerated (4 °C) until processing. After 10–15 minutes of clot retraction, samples were centrifuged at 3,500 rpm for 5 minutes at room temperature (20–22 °C). Sera were separated and stored in aliquots at -20 °C and -80 °C until further analysis.

Evaluation of HBV serological markers. Serum samples were screened using commercial ELISA kits (DiaSorin®) for HBV serological markers (HBsAg, anti-HBc and anti-HBs). Interpretation of results was determined by the absorbance value (OD) at 450 nm of specimens to cut-off value, according to the manufacturer's recommendations. Samples in grey zone of the test (± 10 –20% of cut-off value) were classified as undetermined samples.

Molecular Tests. Three sets of primers were used: 2 sets for human HBV diagnosis (semi-nested PCR) and 1 set designed for this study. For the latter set, the optimum annealing temperature and limit of detection were established prior to testing the swine samples.

Orthohepadnavirus sequence alignment. In order to ensure amplification of Hepadnavirus-DNA in samples from animals not previously described in the literature, interspecific primers were designed for the most conserved genome region, equivalent to the S region of HBsAg from HBV. For this purpose, 110 public complete nucleotide sequences of Orthohepadnavirus available in GenBank were aligned using

the BioEdit 7.1.3 – ClustalW Alignment Multiple programme. This alignment included sequences of viruses similar to HBV that infect chimpanzees, orangutans, gibbons, woolly monkeys, woodchucks, squirrels and human HBV genotypes circulating in Brazil (HBV-A, D and F). Evaluation of the pair of mammalian primers (MF and MR) was performed with software OligoAnalyzer 3.1 for a region of 429 bp (Tab. 1).

Table 1. Primers used for partial genome amplification^(A), viral load quantification^(B) and partial genome sequencing^(C)

Primers ^(A, B, C)	Sequence (5' → 3')	Location*	Length	GC%	T _m (°C)	Amplicon
Mammals F (MF) ^(A, C)	GAY TSG TGG TGG ACT TCT CTC	nt 251-271	21	54.8	55.9	MF-MR 429 bp
Mammals R (MR) ^(A, C)	TAA AYT GAG CCA DGA GAA ACG G	nt 679-658	22	44.7	54.8	
PS1 F ^(A, C)	CCA TAT TCT TGG GAA CAA GA	nt 2820-2839	20	40.0	49.6	PS1-S2 1.200 bp
S2 R ^(A, C)	GGG TTT AAA TGT ATA CCC AAA GA	nt 839-817	23	34.8	50.6	
SR R ^(A, C)	CGA ACC ACT GAA CAA ATG GC	nt 704-685	20	50.0	54.7	PS1-SR 1.100 bp
S10 F ^(C)	GTG GTG GAC TTC TCT CAA TTT TC	nt 256-278	23	43.5	53.8	
S3 R ^(C)	CCT TGA GCA GGA GTC GTG CAG GT	nt 543-521	23	60.9	63.3	
PS2 R ^(C)	GGT CCC CAG TCC TCG AGA AG	nt 143-124	20	65.0	59.2	
S14 F ^(B)	GGA CCC CTG CTC GTG TTA CA	nt 182-201	20	60.0	59.4	S14-S17 90 bp
S17 R ^(B)	GAG AGA AGT CCA CCM CGA GTC TAG A	nt 271-247	25	54.0	59.8	
Probe ^(B)	TGT TGA CAA RAA TCC TCA CAA TAC CRC AGA	nt 216-245	30	40.0	59.9	

*Nucleotides (nt) are numbered according to the HBV reference sequence available in GenBank (NC_003977.1)

PCR optimization. After establishing the pair of primers that anneal in Hepadnavirus from different animal species, its optimum annealing temperature was determined. Amplification assays were performed with a gradient of temperature ranging from 50 °C – 60 °C for HBV positive control samples. Furthermore, to confirm if the primers could amplify different HBV human genotypes, plasmids containing inserts of clones related to HBV genotypes A, D, E, F and G were subjected to amplification under the following conditions established in this study: 25 µL reaction volume consisting of 1 µL DNA, 0.2 mM dNTP, Buffer PCR 1X, 3 mM MgCl₂, 10 pmol primer MF-MR, 0.5 U Taq polymerase (Invitrogen®) and H₂O RNase free. The cycling conditions were initial denaturation at 94 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 54 °C for 40 s and extension at 72 °C for 2 min, and lastly, extension at 72 °C for 7 min.

Finally, to verify the detection limit, 2 positive controls were used, the first with 1.89×10^{10} copies/mL and the other with 9.7×10^4 copies/mL. Tenfold serial dilutions (1–10) from both controls were amplified and the result compared to that obtained using semi-nested PCR primers for human HBV diagnosis, according to the conditions established by the Laboratory of Molecular Virology, Oswaldo Cruz Institute, FIOCRUZ [15, 16].

Detection of Hepadnavirus-DNA in swine. After serological trial and PCR optimization, reactive and undetermined swine samples for HBV markers were submitted to DNA Purification Kit (QIAamp DNA Blood Mini Kit, Qiagen®), according to manufacturer's recommendations. The extracted DNA was eluted in 50 mL of AE Buffer and stored at -80°C until amplification assays.

The samples were amplified by 2 protocols. Initially, for human HBV diagnosis with primers PS1-S2 and PS1-SR (Tab. 1) to amplification of 1,100 bp of region from HBV surface (pré-S/S region of HBsAg). The conditions of PCR1 and PCR2 were the same as described in the literature. Then, for interspecific diagnosis, with primers MF-MR to amplification of 429 bp of the region corresponding to the S region of HBsAg according to conditions established in the presented study. Amplicons were analyzed by electrophoresis on 1.5% agarose gel, stained with ethidium bromide (0.2 $\mu\text{g}/\text{mL}$) and observed under UV Light.

Sequencing reactions. PCR products with expected size were purified using reagents and protocols of QIAquick Gel Extraction kit (Qiagen®) and sequenced using reagents and protocols of Kit Big Dye® Terminator 3.1 at sequencing platform of Oswaldo Cruz Institute and Ludwig Biotechnology Company. Samples were sequenced using primers previously used in PCR reactions and more three options of internal primers, like PS2R, S3 e S10 (Tab. 1).

Phylogenetic analysis. Phylogenetic trees were constructed with positive samples (360 bp) and sequences from GenBank by the neighbour-joining method (1,000 Bootstrap replications) and the Kimura 2-parameter model using software MEGA 5 Beta #6.1. With the same software, a nucleotide identity matrix was constructed using consensus nucleotide sequences for each Orthohepadnavirus used for the design of interspecific primers.

RESULTS

Serology. From 376 swine samples analyzed, 3 (0.8%) were reactive for HBsAg, indicating active infection, 28 (7.45%) were reactive for anti-HBc, indicating previous exposure to the virus, and 6 (1.60%) showed reactivity for anti-HBs,

indicating resolution of the infection or immunity. In addition, it was observed 17 more (4.52%) swine samples analyzed were characterized as undetermined to anti-HBc and 2 (0.53%) to HBsAg (Tab. 2).

Figure 1 shows the reactivity of the samples for the markers analyzed, observing ratios between absorbance values (OD)/cut-off. For the direct assay of HBsAg screening (Fig. 1a), ratio OD/cut-off from HBsAg reactive samples ranged from 1.11–3.95. For the competitive assay of anti-HBc screening (Fig. 1b), the ratio from anti-HBc reactive samples ranged from 0.14–0.90. Finally, for the direct assay of anti-HBs screening (Fig. 1c), the ratio from anti-HBs reactive samples ranged from 1.71–15.96. According to the manufacturer, analytical sensitivity is < 0.03 PEI U/mL (HBsAg), < 0.5 PEI U/mL (anti-HBc) and < 10 UI/L (anti-HBs).

Molecular diagnosis. All swine samples classified as reactive or undetermined in serological assay were tested to molecular diagnosis to investigate the presence of Hepadnavirus-DNA. From a total of 49 samples selected after serological trial, only 2 samples (SW216, JV45) were positive in semi-nested PCR commonly used to human HBV diagnosis, with primers PS1-S2 and PS1-SR (1,100 pb). In interspecific PCR, using primers MF-MR (429 pb), 4 samples were positive by PCR (SW216, JV45, SW170, SW203) (Tab. 3).

Phylogenetic analysis. Among the 4 samples positive in qualitative PCR, the presence of a virus similar to HBV was confirmed in 3 of them (SW216, SW170, SW203) by partial genome sequencing. The PCR products from these samples were sequenced 4 times using a different set of primers. Once the nucleotide mutations were reproducible in each sample, there were no similarities between the samples sequenced, and no sample was identical to positive control, the possibility of contamination was therefore discounted. The sequences of samples positives were deposited in GenBank (Access Nos. JX439919 [SW216], JX439920 [SW170] and JX439921 [SW203]). Since sample JV45 showed a weak band PCR product and low viral load, it was not possible to confirm the HBV detection by sequencing.

Analyzing the nucleotide identity matrix, the similarity between swine nucleotide sequences ranged from 90.8–96.3% compared to human HBV consensus sequence, from 84.1–90.8% with non-human primates consensus sequence, and

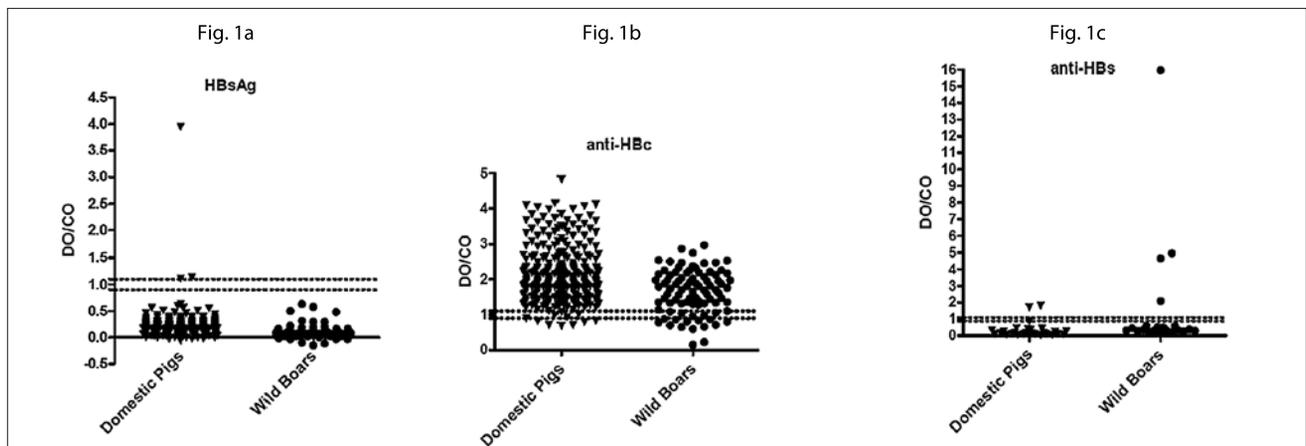


Figure 1 (a, b, c). Degree of reactivity of swine samples to HBV markers analyzed. Between dot lines, the grey zone, corresponding to the interval between ± 10 –20% of cut-off value, for which samples are classified as indeterminate. As the assay for anti-HBc is competitive (Fig. 1b), the reagent samples are below the grey zone. For direct assays for HBsAg (Fig. 1a) and anti-HBs (Fig. 1c), reagent samples are above grey zone

Table 2. Prevalence of HBV serological markers in swine from Brazil

Group	Herd	N	HBsAg, n (%)		Anti-HBc, n (%)		Anti-HBs, n (%)	
			+	IND	+	IND	+	IND
A – Domestic Pigs	Rio de Janeiro	38	0	---	0	---	0	---
	Santa Catarina	250	3 (1.2)	2 (0.8)	11 (4.4)	13 (5.2)	2 (0.8)	---
B – Wild Boars	São Paulo	88	0	---	17 (19.3)	4 (4.5)	4 (4.5)	---
Total		376	3 (0.80)	2 (0.53)	28 (7.45)	17 (4.52)	6 (1.60)	---

IND – Samples in gray zone of EIA (± 10-20% of cut-off value)

from 70.7–72.4% with rodents consensus sequence (Tab. 4). The phylogenetic tree (360 bp) shows that swine sequences are closely related to human sequences, followed by non-human primates and rodents (Fig. 2). When analyzed with human HBV strains, 2 swine sequences were close to human samples belonging to genotype A (SW216, SW203) and 1 to genotype G (SW170).

DISCUSSION

Initial steps of HBV infection related to attachment of viral particles to hepatocytes have a critical role in the specificity of host species [17]. Although previous studies indicate that HBV infection is host-specific [9], the literature reports its circulation in humans and some apes [18]. Recently, infection by a virus similar to HBV was described in pigs from China by ELISA, immunohistochemical staining, and transmission electron microscopy (TEM) [13], but was not molecularly confirmed. In this scenario, the aim of the presented study was to investigate the Hepadnavirus infection in swine from Brazilian herds, and to evaluate the identity with HBV and other Hepadnaviruses reported previously.

Table 3. Results of serological and molecular tests from swine positive to hepatitis B

ID Animal	Result			
	Serological		Molecular	
	Anti-HBc	HBsAg	Anti-HBs	Qualitative PCR
SW 216	+	+	-	1.100 bp and 429 bp
JV 45	+	-	+	1.100 bp and 429 bp
SW 170	UND	-	-	429 bp
SW 203	UND	-	-	429 bp

UND – Undetermined – Samples in gray zone of EIA (20% of cut-off value)

According to Centers for Disease Control and Prevention (CDC), different serological markers or combinations of markers are used to identify HBV acute or chronic infection in human samples. HBsAg marker is the earliest sign of HBV infection, useful for identifying active infection. Anti-HBc marker is useful for epidemiological studies, and indicates present contact or previous exposure to the virus. In the case of the window period and occult HBV infection, since HBsAg levels are undetectable and the viral load is low, molecular diagnosis has been described as a useful tool for the detection of infection, in addition serological trial [19]. For

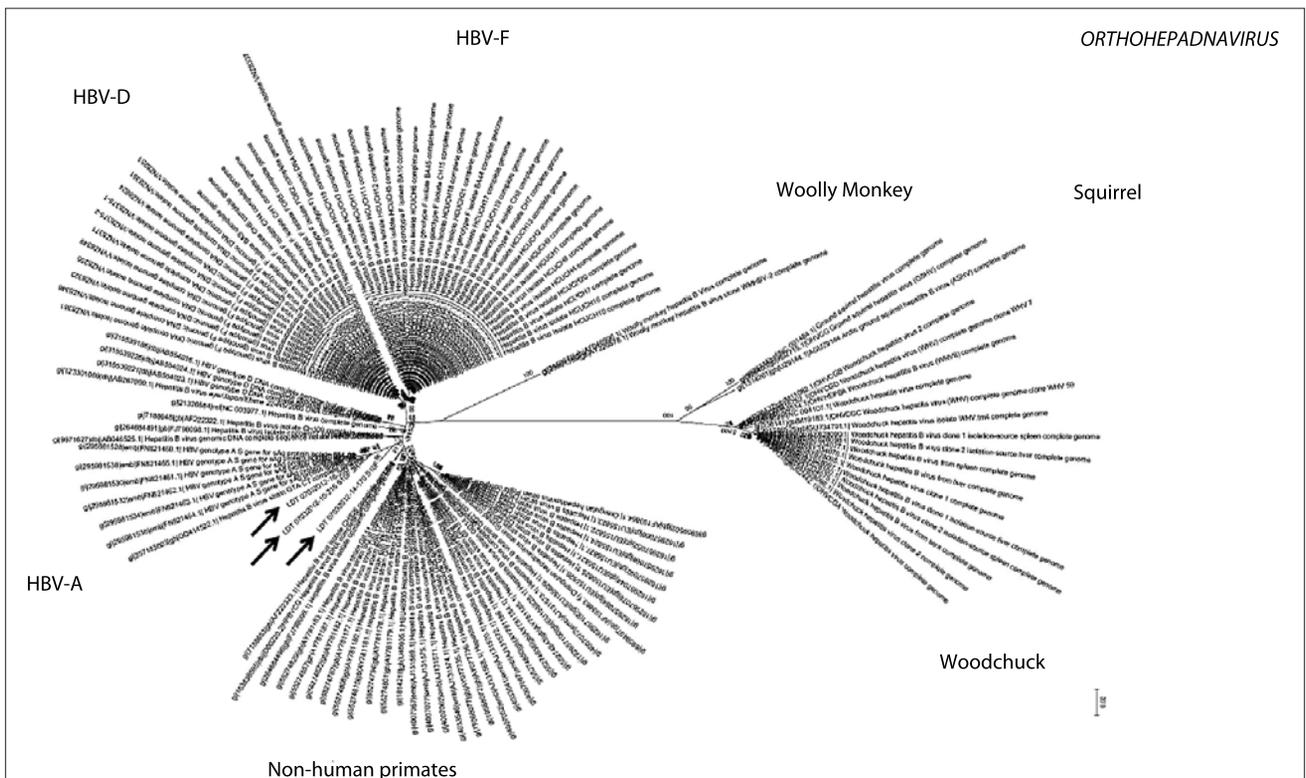


Figure 2. Phylogenetic analysis of *Orthohepadnaviruses* based on partial genome sequences. Number at nodes indicate the percentage of bootstrap replications (1000 sets) supporting the clusters. Identified groups of mammals related to *Orthohepadnaviruses*: human (HBV), non-human primates (HBV), rodents (WHV and GSHWASHV) and woolly monkey (WMHBV). Sequences from swine are pointed out with arrows

this reason, serological and molecular tests were performed in swine samples.

Serological screening by anti-HBc revealed that 7.45% among swine were exposed to the virus. If the samples classified as undetermined by EIA were considered, the prevalence could increase by more than 4.52%. Using the HBsAg test, only 0.8% of samples were positive and 0.53% were undetermined. According to the literature, reactive anti-HBc associated with non-reactive HBsAg could indicate that there is an ongoing lifelong HBV infection hidden from the immune system, with consequent low levels of HBV quantification in the circulation, reflecting a non-reactive HBsAg result [19]. Concerning anti-HBs evaluation, 1.60% of swine were seroreactive; however, depending on the period of infection, the neutralizing antibody titer is not detectable, as occurs in the case of the window period [19].

Initial serological data demonstrate that Brazilian pigs were previously exposed to a virus similar to HBV, as observed in herds from China where 63.9% of swine analyzed showed anti-HBc reactivity [13]. Given the similar rates of infection between humans and animals in the same region, researchers discuss whether pigs may also contribute as reservoirs for the maintenance of virus circulation, as occurs with non-human primates from endemic areas [9, 20].

The samples analyzed in the presented study originate from the southern and southeastern regions of Brazil, where the prevalence of human acute HBV infection ranges 0.3–0.5%. Although characterized as low endemicity areas, these regions concentrate 68.2% of notifications in this period [21].

In order to confirm the possibility of infection, viral genomic detection was performed in all reagent or undetermined samples to ELISA tests. From the total of 49 samples tested by PCR, 2 samples exhibited expected products of 1,100 bp using primers to human HBV diagnosis. Comparing genomic detection with serology (Tab. 3), 1 sample was characterized as positive for anti-HBc and HBsAg (SW216), indicating hepatitis B infection; and another sample (JW45) was reagent to anti-HBc and anti-HBs, showing immunity for hepatitis B, a past infection.

Using interspecific primers, 2 additional samples (SW170, SW203) exhibited expected products of 429 bp. Both samples were characterized as undetermined in EIA test for anti-HBc marker. The presence of anti-HBc indicates previous or ongoing HBV infection in an undefined time frame.

In 3 of the samples (SW216, SW170, SW203) it was possible

to confirm the presence of Hepadnavirus-DNA by sequencing of 360 bp. Although the sample JW45 had been amplified by both strategies, in this case sequencing was not possible due to the low concentration of DNA detected. The PCR results and serological marks found in this swine suggest that this animal was at the end of infection.

Phylogenetic reconstruction indicated that Hepadnavirus sequences obtained from pigs in this study are closely related to human HBV sequences. Nucleotide identity matrix confirmed a higher similarity of Hepadnavirus from pigs with human HBV (90.8–96.3%), than Hepadnavirus from non-human primates (84.1–90.8%) and rodents (70.7–72.4%). However, it is noteworthy that the sequencing product corresponds to a region of high conservation on the Orthohepadnavirus genus, and its length was equivalent to about 11.2% of the HBV genome.

Curiously, when a phylogenetic tree was constructed to compare the Brazilian swine sequences to human HBV strains previously genotyped, the samples were related to 2 different genotypes groups. Two of them were close to the samples from genotype A, the most prevalent genotype in Brazil [22]. Another was similar to samples that belong to genotype G, which is common in countries like USA, Mexico and France, and described in cases of coinfections with other genotypes, mainly genotype A [23]. However, the size of the fragment amplified in this study is not useful for genotyping HBV. The complete sequencing of pre-S/S region, rather than only the S region, could provide more accurate information and allow hypotheses about the evolutionary origin. The amplification of the complete genome or the gene encoding the surface antigen is required for classification of HBV in genotypes [24].

The presented study shows for the first time molecular evidence of a virus similar to HBV circulating in swine from commercial herds, detected by qualitative PCR and confirmed by partial sequencing of the virus genome. Similarity to human HBV was confirmed by phylogenetic analysis and by cross-reactivity in non-host specific commercial serological assays.

Further studies are needed to reveal if Hepadnavirus detection in swine may represent a potential risk for human health. Although the transmission route is unclear, domestication could favour a mutual share of Hepadnavirus between humans and animals. This hypothesis is supported by HBV ability to cross specific barriers [11] and to alter its pathogenicity and transmission through errors in the replicative cycle and interspecific recombination [13]. These

Table 4. Nucleotide Identity Matrix

Sequence	216 (S10F)	170 (S10F)	203 (S10F)	HBV-A	HBV-D	HBV-F	Chimpanze	Orangutan	Gibbon	Woolly Monkey	Woodchuck	Squirrel
216 (S10F)												
170 (S10F)	0.949											
203 (S10F)	0.988	0.955										
Consensus HBV-A	0.958	0.93	0.963									
Consensus HBV-D	0.919	0.93	0.924	0.913								
Consensus HBV-F	0.908	0.916	0.913	0.905	0.933							
Consensus Chimpanze	0.891	0.896	0.896	0.908	0.91	0.905						
Consensus Orangutan	0.899	0.894	0.908	0.899	0.908	0.883	0.888					
Consensus Gibbon	0.863	0.869	0.869	0.888	0.871	0.866	0.908	0.88				
Consensus Woolly Monkey	0.841	0.841	0.846	0.835	0.849	0.835	0.824	0.827	0.813			
Consensus Woodchuck	0.71	0.724	0.715	0.715	0.727	0.721	0.699	0.696	0.688	0.701		
Consensus Squirrel	0.707	0.715	0.71	0.713	0.713	0.721	0.707	0.707	0.696	0.671	0.858	

events have been described among HBV strains from human and non-human primates, and between hepadnavirus strains from birds of different subfamilies [9]. Therefore, the closer contact of humans and swine compared to other animals of naturally occurring HBV could favour these episodes.

The ability to cross the barrier of different species is widely known for the Influenza virus, Hantavirus, Bluetongue and West Nile fever [6, 12]. Focusing on Influenza A virus, researchers discuss the competence of this virus circulating in mammalian hosts to rapidly evolve due to mutations during replication and reassortment with animal viruses. Concerning the role of swine in the zoonotic circulation, they could act as reservoirs for mammals adaptation of the avian viruses, either by reassortment with human or swine viruses, or through adaptive changes [22]. However, the emergence of strains among the human population requires that the novel strain should be able to replicate and efficiently to transmit between humans to deserve attention and monitoring.

Hepatitis E virus (HEV), another classic hepatotropic virus, has proved to be common in Brazilian swine herds [26, 27]. National studies indicate a probable zoonotic transmission of swine strains in urban areas from the southeastern region of Brazil, in sporadic human cases of acute hepatitis [28].

Pig farming is of economic importance to the Brazilian food industry. However, the swine health checks by official inspectors are limited to reviews of visible lesions present in animal herds, and post-mortem gross macroscopic examinations in slaughterhouses. Perhaps preventive sanitary efforts should be applied in mammal hosts such as domestic swine.

The confirmation that swine are also natural hosts of Hepadnavirus may contribute to disclosure of the mechanisms of replication, pathogenesis, and new antiviral drugs in a new experimental animal model for the study of HBV, which is currently restricted to chimpanzees. Due to anatomical and physiological similarities with humans, the species *Sus scrofa* presents a significant role as an experimental animal for biomedical investigation.

CONCLUSIONS

Serological and molecular data indicate evidence for the circulation of a virus similar to HBV in Brazilian swine. However, these data do not elucidate whether we are facing a new swine virus or a human virus circulating in swine. Further studies are needed to determinate the likely infectivity of this agent, as well as evaluating possible epidemiological risks. Due to this, the presented study brings new perspectives for zoonotic risk evaluation, infectivity studies in non-human primates and HBV studies in the swine host, since in addition to its abundant supply, these animals are already widely used in medical research.

Acknowledgements

The authors express their thanks to Marcia Terezinha Baroni de Moraes e Souza for donating the positive controls.

REFERENCES

- Grimm D, Thimme R, Blum HE. HBV life cycle and novel drug targets. *Hepatology*. 2011; 5(2): 644–653.
- Xie YH, Hong R, Liu W, Liu J, Zhai JW. Development of novel therapeutics for chronic hepatitis B. *Virology*. 2010; 25(4): 294–300.
- Shi YH, Shi CH. Molecular characteristics and stages of chronic hepatitis B virus infection. *World J Gastroenterol*. 2009; 15(25): 3099–3105.
- Dienstag JL. Hepatitis B virus infection. *N Engl J Med*. 2008; 359(14): 1486–1500.
- Andre F. Hepatitis B epidemiology in Asia, the Middle East and Africa. *Vaccine* 2000; 18(Suppl 1): 20–22.
- Dutkiewicz J, Cisak E, Sroka J, Wojcik-Fatla A, Zajac V. Biological agents as occupational hazards – selected issues. *Ann Agric Environ Med*. 2011; 18(2): 286–293.
- Haagsma JA, Tariq L, Heederik DJ, Havelaar AH. Infectious disease risks associated with occupational exposure: a systematic review of the literature. *Occup Environ Med*. 2012; 69(2): 140–146.
- Patient R, Hourieux C, Roingeard P. Morphogenesis of hepatitis B virus and its subviral envelope particles. *Cell Microbiol*. 2009; 11(11): 1561–1570.
- Yang J, Xi Q, Deng R, Wang J, Hou J, Wang X. Identification of inter-species recombination among hepadnaviruses infecting cross-species hosts. *J Med Virol*. 2007; 79(11): 1741–1750.
- Dandri M, Volz TK, Lutgehetmann M, Petersen J. Animal models for the study of HBV replication and its variants. *J Clin Virol*. 2005; 34(Suppl 1): 54–62.
- Makuwa M, Souquiere S, Clifford SL, Mouinga-Ondeme A, Bawe-Johnson M, Wickings EJ, et al. Identification of hepatitis B virus genome in faecal sample from wild living chimpanzee (*Pan troglodytes troglodytes*) in Gabon. *J Clin Virol*. 2005; 34(Suppl 1): 83–88.
- Jimenez-Clavero MA. Animal viral diseases and global change: bluetongue and West Nile fever as paradigms. *Front Genet*. 2012; 3: 105.
- Li W, She R, Liu L, You H, Yin J. Prevalence of a virus similar to human hepatitis B virus in swine. *Virology*. 2010; 7: 60.
- Tian J, Xia K, She R, Li W, Ding Y, Wang J, et al. Detection of Hepatitis B Virus in Serum and Liver of Chickens. *Virology*. 2012; 9(1): 2.
- Motta-Castro AR, Martins RM, Yoshida CF, Teles SA, Paniago AM, Lima KM, et al. Hepatitis B virus infection in isolated Afro-Brazilian communities. *J Med Virol*. 2005; 77(2): 188–193.
- Niel C, Moraes MT, Gaspar AM, Yoshida CF, Gomes SA. Genetic diversity of hepatitis B virus strains isolated in Rio de Janeiro, Brazil. *J Med Virol*. 1994; 44(2): 180–186.
- Chouteau P, Le Seyec J, Cannie I, Nassal M, Guguen-Guillouze C, Gripon P. A short N-proximal region in the large envelope protein harbors a determinant that contributes to the species specificity of human hepatitis B virus. *J Virol*. 2001; 75(23): 11565–11572.
- Grethe S, Heckel JO, Rietschel W, Hufert FT. Molecular epidemiology of hepatitis B virus variants in nonhuman primates. *J Virol*. 2000; 74(11): 5377–5381.
- Allain JP, Cox L. Challenges in hepatitis B detection among blood donors. *Curr Opin Hematol*. 2011; 18(6): 461–466.
- Starkman SE, MacDonald DM, Lewis JC, Holmes EC, Simmonds P. Geographic and species association of hepatitis B virus genotypes in non-human primates. *Virology*. 2003; 314(1): 381–393.
- Health Ministry, Brazil. www.aids.gov.br/pagina/hepatites-virais-enumeros (access: 2012.07.16)
- Becker CE, Mattos AA, Bogo MR, Branco F, Sitnik R, Kretzmann NA. Genotyping of hepatitis B virus in a cohort of patients evaluated in a hospital of Porto Alegre, South of Brazil. *Arq Gastroenterol*. 2010; 47(1): 13–17.
- Santos AO, Alvarado-Mora MV, Botelho L, Vieira DS, Pinho JR, Carrilho FJ, et al. Characterization of hepatitis B virus (HBV) genotypes in patients from Rondonia, Brazil. *Virology*. 2010; 7: 315.
- Norder H, Courouce AM, Magnius LO. Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. *Virology*. 1994; 198(2): 489–503.
- Horimoto T, Kawaoka Y. Influenza: lessons from past pandemics, warnings from current incidents. *Nat Rev Microbiol*. 2005; 3(8): 591–600.
- dos Santos DR, Vitral CL, de Paula VS, Marchevsky RS, Lopes JF, Gaspar AM, et al. Serological and molecular evidence of hepatitis E virus in swine in Brazil. *Vet J*. 2009; 182(3): 474–480.
- Vitral CL, Pinto MA, Lewis-Ximenez LL, Khudyakov YE, dos Santos DR, Gaspar AM. Serological evidence of hepatitis E virus infection in different animal species from the Southeast of Brazil. *Mem Inst Oswaldo Cruz*. 2005; 100(2): 117–122.
- Lopes Dos Santos DR, Lewis-Ximenez LL, da Silva MF, de Sousa PS, Gaspar AM, Pinto MA. First report of a human autochthonous hepatitis E virus infection in Brazil. *J Clin Virol*. 2010; 47(3): 276–279.