Diagnostic Microbiology and Infectious Disease 85 (2016) 323–327

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Development and validation of a point-of-care test for detecting hantavirus antibodies in human and rodent samples

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ARTICLE INFO

Article history:
Received 8 March 2016
Received in revised form 11 April 2016
Accepted 14 April 2016
Available online 19 April 2016

Keywords:
Hantavirus
Hantavirus cardiopulmonary syndrome (HCPS)
Rapid test
Rodents
Human seroprevalence

ABSTRACT

Hantaviruses are etiologic agents of a zoonotic disease transmitted mainly from wild rodents to humans, causing Hemorrhagic Fever with Renal Syndrome in Eurasia and the Hantavirus Cardiopulmonary Syndrome in the Americas (HCPS), reaching a lethality rate of 40% in Brazil. Hantavirus diagnostic and seroprevalence are often based on the presence of IgM and IgG antibodies against the virus. Here we propose a rapid test assay able to identify hantavirus antibodies with sensibility and specificity similar to ELISA assays. We analyzed five groups of samples, including healthy human population and small mammals of endemic areas, suspected cases of HCPS, patients with non-related infections and a serum panel from a different geographical region. The test presented good rates of sensibility (87–100%) and specificity (97–100%) for all groups, being a promising tool suitable for both rodent and human hantavirus epidemiological surveys.

1. Introduction

Hantaviruses (family Bunyaviridae, genus *Hantavirus*) are a major class of zoonotic pathogens that cause two severe diseases in humans, the hemorrhagic fever with renal syndrome in Eurasia and the hantavirus cardiopulmonary syndrome (HCPS) in the Americas.

Although rodents have been considered to be the primary reservoir hosts of hantaviruses for a long time, these infectious agents are also carried by a more diverse range of mammalian species, particularly bats, moles and shrews (Guo et al., 2013; Holmes and Zhang, 2015). Accidental transmission to humans occurs mainly by the inhalation of virus in aerosolized rodent excreta. Person-to-person transmission have been reported for Andes hantavirus (Martinez-Valdebenito et al., 2014).

The emergence of hantavirus in human populations is often related to ecological interactions among reservoirs, virus and humans; influenced by rodent population density and structure, environmental factors related to virus survival outside the rodent host, and anthropogenic risk behavior factors that allows prolonged contact to host habitats and areas supporting virus survival, such as entering and cleaning of (closed) rodent infested places, camping, hunting, forestry, farming, living in close proximity to forest and the establishment of new agricultural practices including food and grain storage (Reusken and Heyman, 2013).

In the Americas, more than 40 hantavirus genotypes have been described and nearly half of them are pathogenic to humans. In Brazil, six different hantavirus genotypes have been associated with HCPS (Guterres et al., 2015). In the South region of the country, hantaviruses cluster with the Araucária (Juquitiba-like) or Jaborá genotypes (Raboni et al., 2012).

Since the first description of HCPS in Brazil in 1993 up to 2015, a number of 1,940 confirmed cases have been reported with a total of 765 deaths. The mean lethality rate is 40% and the majority of patients require hospital care. Of these, 227 cases were reported in Paraná State, with a lethality rate of 38.3% (Brazilian Health Ministry, 2016). The similarities of clinical hallmarks found in HCPS and in other viral hemorrhagic fevers make it difficult to diagnose the disease based only on clinical examination, therefore serologic and molecular diagnosis tests are important for the correct identification of the etiologic agent and proper treatment. Infection in humans result in very short-term viremia and patients have detectable IgM and IgG antibodies against the hantavirus nucleocapsid (N) antigen at the onset of clinical symptoms (Borges et al., 2006; Padula et al., 2000), thus serologic tests are often used for the detection of these antibodies classes (Vaheri et al., 2008).

Hantavirus virions are enveloped and contain three negative single-stranded RNA genome segments designated as large (L), medium (M), and small (S). These segments encode the viral RNA-dependent RNA polymerase, two envelope glycoproteins (Gn and Gc) and the nucleocapsid protein (N) (Elliot and Schmaljohn, 2013; Vaheri et al., 2013). Among the structural proteins, the nucleocapsid is the most abundant and antigenic protein in the virus particle, with immunodominant and

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http://dx.doi.org/10.1016/j.diagmicrobio.2016.04.010
0732-8893/

cross-reactive epitopes at the N-terminal domain. Hence diverse recombinant N proteins produced by various expression systems have been applied as diagnostic antigens to detect hantavirus-specific antibodies (Amada et al., 2013; Hujakka et al., 2001b; Koma et al., 2010; Raboni et al., 2007; Yoshimatsu and Arikawa, 2014a).

In the present study, we propose a new point-of-care test for the detection of hantavirus specific antibodies in human and rodent serum or whole blood samples. According to this evaluation, the rapid test proved to be a useful tool for the epidemiologic surveillance of the disease in human and rodent populations. Additionally it is quick, straightforward to perform and with no need of additional equipment or special storage conditions, thus allowing field studies.

2. Methods

2.1. Serum samples

This study is in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the National Ethical Committee (protocol no. 34257314.7.0000.5248) for the human samples, consent was obtained from each subject and all privacy rights were respected. The animal handling is in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, and was approved by the environmental local authority IAP/PR (environmental licensing no. 002/15). Serum samples were collected from healthy volunteers living in hantavirus endemic areas in South Brazil (Paraná State) or kindly provided by multiple public health laboratories and other external collaborators. Small mammal samples were obtained by trained staff in several municipalities of Paraná State, based on reported HCPS cases and/or on the environmental diversity of the locations. Animal capturing and sample processing were performed as previously described (Raboni et al., 2012). Human and animal serum samples were then assigned into five groups according to their origin and clinical features (Table 1). Groups I, II and III were tested with ICC EIA IgG and/ or IgM HANTEC (Raboni et al., 2007), and then assayed with the hantavirus rapid test for comparison purposes. Groups IV and V were tested with the hantavirus rapid test and compared to previously known results.

2.2. ICC EIA IgG and IgM HANTEC

The HANTEC EIA kits were developed and validated using the hantavirus nucleocapsid protein (Raboni et al., 2007). The GFP protein is used as a control for unspecific antibody binding. These kits are currently being distributed by the Brazilian Ministry of Health to the Reference Laboratories in hantavirus diagnosis. The ICC EIA IgG and IgM HANTEC kits were used as the initial comparison methods, and performed according to the manufacturer's instructions. For the IgM assay, a set of serum dilutions was performed (1:100 to 1:6,400), in order to improve specificity by eliminating false-positive samples. Then the absorbance difference between the hantavirus nucleoprotein and GFP control wells are summed. For the IgG assay, a cut-off value is obtained using controls, and the absorbance difference between the hantavirus nucleoprotein and GFP control wells should be divided by this cut-off. A sample is considered positive if it presents values above 1.1; samples between 0.9 and 1.1 are designated indeterminate and samples bellow 0.9 as negative.

Table 1
Human and small mammal's serum samples tested with the hantavirus rapid test.

Group	Number of samples	Description
I	131	Serum samples of healthy human individuals living in hantavirus endemic areas in South Brazil (Paraná state)
II	708	Wild small mammals serum/whole blood samples from hantavirus endemic areas in South Brazil (Paraná state)
III	68	Serum samples from patients with suspected HCPS[a]
IV	97	Panel of serum samples from patients with acute infections other than HCPS[a]
V	45	Human serum panel from a different geographical region[b]
Total	1,049	

[a] Provided by Brazilian Public Health Laboratories
[b] Pergamino, Argentina – provided by the Instituto Nacional de Enfermedades Virales Humanas "Dr. Julio I. Maiztegui".

2.3. Hantavirus point-of-care test (POC HANTEC)

The POC HANTEC is a lateral flow immunochromatographic assay that uses the hantavirus nucleocapsid recombinant protein as the antigen (test line) (Raboni et al., 2007). Protein A (Invitrogen) is composed of five homologous Ig-binding domains, and each domain is able to bind proteins from many mammalian species, most notably IgGs (Frank, 1997), for these characteristics it was used as the test control line and conjugate. The test was performed by adding 5μl of serum or whole blood into the sample well followed by three drops of sample buffer (100μl). After 10 to 15 min of incubation, the result was visually read by at least two people. The control line must be present in all tests, otherwise it was considered invalid and repeated (Suppl. Fig. 1).

2.4. Additional comparative methods

Discrepant results between the two assays (EIA and POC HANTEC) were confirmed by immunoblot and/or RT-PCR (Johnson et al., 1999). For the immunoblot, 5μg of the hantavirus recombinant nucleoprotein were separated by SDS-PAGE and transferred to nitrocellulose membranes. Individual blocked nitrocellulose strips were incubated for 1 hour with each serum diluted 1:100. After three washes with Tris-buffered saline (TBS), a second 1 hour incubation with anti-mouse IgG, anti-human IgG or anti-human IgM alkaline phosphatase conjugated was performed. Following three washes with TBS, membranes were revealed with BCIP/NBT reagent (Promega).

3. Results

3.1. POC HANTEC

The newly developed point-of-care test was performed with 5 μl of serum or whole blood samples and visually read after 10 to 15 min. A red line in the test window indicated a positive result and the absence of this line a negative result. Some samples presented a faded test line and were considered as indeterminate. The test was considered invalid if no control line was observed.

To validate the POC HANTEC test, we evaluated a total of 1,049 serum samples divided into 5 groups: healthy individuals living in endemic areas, wild small mammals captured in endemic areas, patients with suspected HCPS, patients with acute infections other than HCPS, and a blind panel of human serum from a different geographic region (Table 1).

3.2. Hantavirus specific antibody detection in human and wild small mammals samples from endemic areas

Combined results from the HANTEC EIA and immunoblot assays were used to ascertain the sample final status as negative/ positive or indeterminate. A total of 131 serum samples from healthy individuals living in hantavirus endemic areas were tested with the POC HANTEC and the results are shown in Table 2. From the 64 positive samples, the test was able to correctly identify 58. Three samples were indeterminate and another three were false negative. The sensibility and specificity for group I was 95.1% and 100%, respectively.

Regarding the results obtained for whole blood/ sera from wild small mammals samples (group II), the POC HANTEC presented a sensibility

Table 2
Hantavirus IgG detection by POC HANTEC in serum/whole blood from small mammals and healthy human individuals from endemic areas.

Group	Origin	POC HANTEC	Sample final status[a]			Total
			IgG positive	Negative	Indeterminate	
I	Human	Positive	58	0	0	58
		Negative	3	63	0	66
		Indeterminate	3	1	3	7
		Total	64	64	3	131
II	Small mammals	Positive	39	1	0	40
		Negative	1	667	0	668
		Total	40	668	0	708

[a] HANTEC EIA and immunoblot combined results

of 97.5% and a specificity of 99.9% (Table 5). The two conflicting results were tested negative by RT-PCR for hantavirus.

Among the 40 confirmed hantavirus positive samples, 26 were positive by RT-PCR and sequenced as the genotypes Jaborá (11 samples) and Araucária (Juquitiba-like) (15 samples). The POC HANTEC test proved to be useful to detect antibody against hantavirus in different rodent genera such as *Akodon* sp., *Oxymycterus* sp., and *Oligoryzomys* sp. The test was also valid for samples of the rodent genera *Brucepattersonius* sp., *Juliomys* sp., *Micromys* sp., *Mus* sp., *Necromys* sp., *Nectomys* sp., *Oryzomys* sp., *Rattus* sp., *Rhipidomys* sp., *Sooretamys* sp., *Thaptomys* sp.; and the marsupial *Monodelphis* sp. Four samples out of 20 obtained from *Calomys* sp. were invalid.

3.3. Hantavirus specific antibody detection in suspected cases of HCPS

Despite the suspicion of HCPS in all patients from group III, 5 samples were tested negative by all assays (Table 3). It was possible to correctly detect anti-hantavirus antibodies in 50 positive sera. Six samples were considered indeterminate and seven samples gave false negative results.

3.4. POC HANTEC with other acute disease samples

We investigated cross-reactivity by testing 97 serum samples from patients with non-HCPS acute disease (Suppl. Table 1). This panel included sera positive for Toxoplasmosis (5 samples), Epstein–Barr virus (EBV) (14), Venereal Disease Research Laboratory test – VDRL (22), Cytomegalovirus – CMV (12), CMV/EBV (2), Chagas disease (5), Leptospirosis (7), Leishmaniosis (1), and Dengue (29). Although the HANTEC IgM EIA gave a false positive result for one acute Chagas disease serum sample, no cross-reactivity was observed when tested with the POC HANTEC test.

3.5. Serum panel from a different geographical region

We assessed the applicability of the POC HANTEC test with samples from Argentina, another South American country with confirmed hantavirus circulation. Results show that it was able to detect all hantavirus positive samples (Table 4), indicating that there is a cross-reactivity of the recombinant Araucaria nucleoprotein with sera from patients infected with another genotype of hantavirus (Andes virus). The test was also able to detect the Maripa virus from French Guiana (data not shown). Furthermore the test showed no cross-reactivity for samples from patients with acute Argentina hemorrhagic fever, Yellow fever, Dengue and Saint Louis encephalitis.

In summary, the POC HANTEC had a sensibility of 88–100% and specificity of 97–100% for the five groups of samples (Table 5).

4. Discussion

Hantavirus infection prevalence studies in wild rodents and humans have been done mainly by serological detection of hantavirus-specific antibodies using enzyme linked immunosorbent (ELISA) and immunofluorescent antibody assays (IFA) (Pini et al., 2003; Raboni et al., 2012; Yoshimatsu and Arikawa, 2014b). However, more rapid, simple and safer diagnostic methods are required for these surveys, and rapid tests are of great potential for this purpose.

Information about the serological status of the human population and the potential rodent reservoirs regarding hantavirus exposure is important, once the seroprevalence data and the distributions of seropositive reservoir species will assist health authorities in adjusting their prevention policies and defining a permanent surveillance program for the entire region (Raboni et al., 2012).

The first application of a immunocromatographic rapid test for hantavirus detection was the POC Puumala® test for detection of Puumala virus (PUUV) (Hujakka et al., 2001a, 2001b), followed by POC Dobrava-Hantaan® (Hujakka et al., 2003). Both tests use a recombinant N protein for the detection of specific human IgM antibodies. Regarding the hantavirus reservoir hosts a serological immunochromatographic rapid test was developed for the detection of anti-PUUV IgG antibodies in rodents (Sirola et al., 2004). More recently, a rapid strip test was settled for detection of Seoul virus (SEOV) IgG antibody in blood from *Rattus* spp (Amada et al., 2013). To our knowledge, the POC HANTEC is the first reported test to detect antibodies specific for South American hantavirus in both, human and rodent samples.

Since 2013, the POC HANTEC test was challenged in 18 field expeditions that reached 21 municipalities throughout Paraná State, with the valid testing of 14 wild rodent genera and the marsupial *Monodelphis* sp., showing its potential usefulness with a wide range of mammalian species. *Calomys* sp. represented an interesting variable, since it gave some invalid results (4 of 20), probably due to *Calomys* IgG weak binding to protein A, as previously described (Favoreto-Junior et al., 1998). Despite this, the test with the wild animals panel showed high specificity and sensibility (Table 5) proving that it is a reliable tool for epidemiological surveys in reservoirs populations.

Moreover, fast results, easy manipulation and flexibility for sample use (serum, plasma and whole blood) makes it highly recommended for field studies. The small sample volume required to run the POC HANTEC enables the use of blood from the animal tail tip or saphenous vein puncture, making possible the release of non-infected animals,

Table 3
Hantavirus IgG/M detection using POC HANTEC in serum samples from patients with suspected HCPS.

POC HANTEC	Sample final status[a]					Total
	Positive			Negative	Indeterminate	
	IgM	IgG	IgM/IgG			
Positive	2	7	41	0	0	50
Negative	4	1	2	5	0	12
Indeterminate	0	2	3	0	1	6
Total	6	10	46	5	1	68

[a] HANTEC EIA and immunoblot combined results.

Table 4
Serum panel from a different geographical region tested with the Hantavirus POC HANTEC.

Samples	POC HANTEC		Total	Concordance
	Positive	Negative		
Argentina hemorrhagic fever	0	14	14	100%
Yellow fever	0	1	1	100%
Dengue virus	0	2	2	100%
Saint Louis encephalitis	0	1	1	100%
Hantavirus IgM	6	0	6	100%
Hantavirus IgG	1	0	1	100%
Hantavirus IgM/ IgG	5	0	5	100%
Negative	1	14	15	93.34%

Table 5
Sensibility and specificity of the POC HANTEC.

	Groups				
	I	II	III	IV	V
Number of samples*	124	708	62	97	45
Sensibility	95.08	97.50	87.72	-	100.00
Specificity	100.00	99.85	100.00	100.00	96.97
Efficacy	97.54	98.68	93.86	100.00	98.48

* Indeterminate results were excluded from the analysis.

thus allowing long-term studies and preventing biodiversity loss, which can lead to increase of reservoir population density and consequently, higher incidence of illness (Suzán et al., 2009).

An easy method of IgG detection is also important for epidemiological surveys in human population living in areas of viral circulation. To verify the test performance in this context, a panel of samples of healthy individuals living in hantavirus endemic areas was tested, and the POC HANTEC also showed high sensibility and specificity rates (Table 5). Such panel represents a special challenge for the test sensibility, since these individuals are likely to exhibit low anti-hantavirus IgG levels. Human hantavirus seroprevalence surveys allows the estimation of viral circulation among a population, and ELISA-based assays are the most common choice to perform the initial screenings (Badra et al., 2012; De Souza et al., 2011; Witkowski et al., 2015). However, low hantavirus seroprevalence is reported worldwide (1.7 to 4.7%), suggesting that an *in loco* assay should be suitable to improve logistics, such as to simplify sample storage. Additionally, it is important to note that hantavirus is mainly related to rural areas with difficult access and an on-time result could favor control and prevention measures.

When tested with samples from patients with suspected HCPS, the POC HANTEC could correctly identify anti-hantavirus antibodies in 50 out of 69 sera. Among the discrepant results, five indeterminate and seven negative sera in the POC HANTEC were reagent in the immunoblot assay, showing a sensibility issue especially for exclusively IgM-positive sera. This result can be explained by the fact that protein A has low affinity to human IgM (Frank, 1997). Therefore negative results with the POC HANTEC for early acute patients do not exclude the possibility of hantavirus infection. According to diagnostic guidelines, serological tests must be carefully considered together with clinical and epidemiological evaluations to diagnose the disease (PAHO, 1999). Finally, since no cross-reaction with a panel of 11 acute infections was observed, added to the ability of detection of antibodies against different hantavirus genotypes, the POC HANTEC represents a real potential of use for the anti-hantavirus antibody detection not only in Brazil, but also in other South American countries.

5. Conclusion

The POC HANTEC test for hantavirus specific antibody detection presented good rates of sensibility (87–100%) and specificity (97–100%). It can be transported, kept and run under field conditions with no need of equipment or electricity, yielding results in 10 to 15 minutes. This rapid, sensitive, and cost-effective test can be used for epidemiological surveys and help focus target control measures with the goal of reducing the disease burden. The test enabled the detection of antibodies to Jaborá, Araucária (Juquitiba like) and Andes viruses, and has a potential use for other regions of Brazil and other South American countries. Currently we are improving the test sensibility to exclusively IgM-positive serum and broadening our sample panel from other regions of Brazil and South America.

Acknowledgements

The authors are grateful to Giselia Burigo Guimarães Rubio, Tania Portella Costa, Edison do Santos, Luiz Eduardo Loyola Gonçalves Pereira, Josiane dos Santos Raymundo, Ricardo Matsuo and Joel Lopes da Silva from the Public Health Secretary of the State of Paraná for their assistance in field expeditions; Fiocruz/Bio-Manguinhos team for technical support and Daisy Maria Strottmann for assistance with the recombinant protein production. This work was supported by the following funding sources: CNPq, Fundação Araucária, CAPES and PROBIO.

Conflicts of interest: None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.diagmicrobio.2016.04.010.

References

Amada T, Yoshimatsu K, Yasuda SP, Shimizu K, Koma T, Hayashimoto N, et al. Rapid, whole blood diagnostic test for detecting anti-hantavirus antibody in rats. J Virol Methods 2013;193(1):42–9.
Badra SJ, Maia FGM, Figueiredo GG, dos Santos Junior GS, Campos GM, Figueiredo LTM, et al. A retrospective serologic survey of hantavirus infections in the county of Cássia dos Coqueiros, State of São Paulo, Brazil. Rev Soc Bras Med Trop 2012;45(4):468–70.
Borges AA, Campos GM, Moreli ML, Souza RLM, Aquino VH, Saggioro FP, et al. Hantavirus cardiopulmonary syndrome: immune response and pathogenesis. Microbes Infect 2006;8(8):2324–30.
Brazilian Health Ministry. Hantavirose [Internet]. cited 2016 Feb 2. Available from: http://portalsaude.saude.gov.br/index.php/o-ministerio/principal/secretarias/svs/hantavirose, 2016.
De Souza WM, Machado AM, Figueiredo LTM, Boff E. Serosurvey of hantavirus infection in humans in the border region between Brazil and Argentina. Rev Soc Bras Med Trop 2011;44:131–5.
Elliot RM, Schmaljohn CS. Bunyaviridae. In: Knipe DM, Howley PM, editors. Fields Virol. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2013. p. 1244–82.
Favoreto-Junior S, Ferro EA, Clemente D, Silva DA, Mineo JR. Experimental Infection of Calomys callosus (Rodentia, Cricetidae) by Toxoplasma gondii. Mem Inst Oswaldo Cruz 1998;93(1):103–7.
Frank M. Antibody Binding to Protein A and Protein G beads. In: Frank M, editor. Mol Biol Protoc. Oklahoma; 1997.
Guo WP, Lin XD, Wang W, Tian JH, Cong ML, Zhang HL, et al. Phylogeny and Origins of Hantaviruses Harbored by Bats, Insectivores, and Rodents. PLoS Pathog 2013;9(2).
Guterres A, de Oliveira RC, Fernandes J, Schrago CG, de Lemos ERS. Detection of different South American hantaviruses. Virus Res, 210. Elsevier B.V.; 2015. p. 106–13.
Holmes EC, Zhang Y-Z. The evolution and emergence of hantaviruses. Curr Opin Virol, 10. Elsevier B.V.; 2015. p. 27–33.
Hujakka H, Koistinen V, Eerika P, Kuronen I, Mononen I, Parviainen M, et al. New Immunochromatographic Rapid Test for Diagnosis of Acute Puumala Virus Infection. J Clin Microbiol 2001a;39(6):2146–50.
Hujakka H, Koistinen V, Eerikäinen P, Kuronen I, Laatikainen A, Kauppinen J, et al. Comparison of a new immunochromatographic rapid test with a commercial EIA for the detection of Puumala virus specific IgM antibodies. J Clin Virol 2001b;23(1–2):79–85.
Hujakka H, Koistinen V, Kuronen I, Eerikäinen P, Parviainen M, Lundkvist A, et al. Diagnostic rapid tests for acute hantavirus infections: specific tests for Hantaan, Dobrava and Puumala viruses versus a hantavirus combination test. J Virol Methods 2003;108(1):117–22.
Johnson AM, De Souza LTM, Ferreira IB, Pereira LE, Ksiazek TG, Rollin PE, et al. Genetic investigation of novel hantaviruses causing fatal HPS in Brazil. J Med Virol 1999;59(4):527–35.
Koma T, Yoshimatsu K, Pini N, Safronetz D, Taruishi M, Levis S, et al. Truncated hantavirus nucleocapsid proteins for serotyping Sin Nombre, Andes, and Laguna Negra hantavirus infections in humans and rodents. J Clin Microbiol 2010;48(5):1635–42.
Martinez-Valdebenito C, Calvo M, Vial C, Mansilla R, Marco C, Palma RE, et al. Person-to-Person Household and Nosocomial Transmission of Andes Hantavirus, Sourthern Chile, 2011. 2014;20(10):1629–36.
Padula PJ, Rossi CM, Della Valle MO, Martinez PV, Colavecchia SB, Edelstein A, et al. Development and evaluation of a solid-phase enzyme immunoassay based on Andes hantavirus recombinant nucleoprotein. J Med Microbiol 2000;49(2):149–55.
PAHO. Hantavirus in the Americas: Guidelines for Diagnosis, Treatment, Prevention, and Control. Washington ; 1999.
Pini N, Levis S, Calderon G, Ramirez J, Bravo D, Lozano E, et al. Hantavirus infection in humans and rodents, northwestern Argentina. Emerg Infect Dis 2003;9(9):1070–6.
Raboni SM, Levis S, Rosa EST, Bisordi I, Delfraro A, Lemos E, et al. Hantavirus infection in Brazil: development and evaluation of an enzyme immunoassay and immunoblotting based on N recombinant protein. Diagn Microbiol Infect Dis 2007;58(1):89–97.
Raboni SM, Delfraro A, De Borba L, Teixeira BR, Stella V, De Araujo MR, et al. Hantavirus infection prevalence in wild rodents and human anti-hantavirus serological profiles from different geographic areas of South Brazil. Am J Trop Med Hyg 2012;87(2):371–8.
Reusken C, Heyman P. Factors driving hantavirus emergence in Europe. Curr Opin Virol, 3 (1). Elsevier B.V; 2013. p. 92–9.
Sirola H, Kallio ER, Koistinen V, Kuronen I, Lundkvist A, Vaheri A, et al. Rapid field test for detection of hantavirus antibodies in rodents. Epidemiol Infect 2004;132(3):549–53.
Suzán G, Marcé E, Giermakowski JT, Mills JN, Ceballos G, Ostfeld RS, et al. Experimental evidence for reduced rodent diversity causing increased hantavirus prevalence. PLoS One 2009;4(5).
Vaheri A, Vapalahti O, Plyusnin A. How to diagnose hantavirus infections and detect them in rodents and insectivores. Rev Med Virol 2008:277–88.
Vaheri A, Strandin T, Hepojoki J, Sironen T, Henttonen H, Mäkelä S, et al. Uncovering the mysteries of hantavirus infections. Nat Rev Microbiol, 11 (8). Nature Publishing Group; 2013. p. 539–50.

Witkowski PT, Leendertz SAJ, Auste B, Akoua-Koffi C, Schubert G, Klempa B, et al. Human seroprevalence indicating hantavirus infections in tropical rainforests of Côte d'Ivoire and Democratic Republic of Congo. Front Microbiol 2015;6.

Yoshimatsu K, Arikawa J. Antigenic properties of N protein of Hantavirus. Viruses 2014a; 6(8):3097–109.

Yoshimatsu K, Arikawa J. Serological diagnosis with recombinant N antigen for hantavirus infection. Virus Res 2014b;187:77–83.