

EPIDEMIOLOGY AND LABORATORY DIAGNOSIS OF HANTAVIRUS (HTV) INFECTIONS

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SUMMARY

Hantavirus (HTV) is a recently discovered "hemorrhagic fever virus" belonging to the Bunyaviridae family, which is spread throughout the world by wild rodents and/or laboratory rats. During an epidemic in the Belgian-French Ardennes in 1993, more than 200 acute cases were recorded of the milder European form of HTV-illness, otherwise known as Nephropathia epidemica (NE). This variant may be recognized by the sudden onset of fever, acute renal failure, thrombocytopenia and sometimes by ophthalmologic complications. The symptomatology is rather aspecific and diagnosis can only be confirmed by serologic tests, of which the best option nowadays seems to be: screening by IgG EIA, followed by IgM confirmation with a μ -capture EIA test. Some of the tests described allow an evaluation of the causative serotype or even the moment of infection.

Next to the "classic" serologic assays for detection of specific viral antibodies, we describe briefly our own experience with newer tests such as "high density particle agglutination" and "line immuno assay". Polymerase chain reaction for viral RNA genome typing and immunohistochemical colouring of the viral antigen in tissues seem to offer promising alternatives for the immediate future.

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INTRODUCTION

Hantavirus (HTV) is a newly discovered (1) hemorrhagic fever virus, genetically characterized as a new genus in the *Bunyaviridae* family (2). It possesses a tripartite, single-stranded, negative sense RNA genome, with segments designated as small (S), medium (M) and large (L). They encode respectively the nucleocapsid protein (NP), the two envelope proteins G1 & G2 and a virion-associated polymerase.

HTV is transmitted to man via aerosols of infected excreta (urine, faeces, saliva) from chronically infected, but apparently healthy small mammals, mainly wild rodents and/or laboratory rats. In a recent review of available literature, we listed evidence of HTV infection in 2 different classes of animals (mammals and birds), 8 different orders, 24 different families and a total of 164 different animal species (3). However, the order of *Rodentia*, and particularly the superfamily of *Muroidea*, are the most important vectors for HTV. Humans do not secrete HTV in substantial amounts during infection, and man-to-man transmission has not been documented so far.

Based on serotyping by plaque reduction neutralization tests (PRNT), and later confirmed by polymerase chain reaction (PCR) serotyping, a total of 8 important serotypes have been distinguished so far, 5 of which have a clinical significance (4-6). Each serotype has its own specific main rodent vector, its own geographical spread, and induces in man a more or less specific clinical picture (Table 1)(Fig.1).

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TABLE 1: MAJOR HANTAVIRUS SEROTYPES*
IN CHRONOLOGICAL ORDER OF ISOLATION

HANTAVIRUS SEROTYPE (year of isolation)	MAIN RODENT VECTOR (geographical spread)	HUMAN ILLNESS (type of spread)
1. Thottapalayam (TPM)** (1971)	<i>Suncus murinus</i> (shrew) (India)	not recorded
2. Hantaan 76-118 (HTN) (1976)	<i>Apodemus agrarius</i> (field mouse) (Asia, E-Russia and S-Europe)	severe: KHF, EHF, HFRS (rural)
3. Prospect Hill (PH) (1982)	<i>Microtus pennsylvanicus</i> (meadow vole) (USA) and <i>Microtus</i> sp. (Russia)	not recorded
4. Seoul (SEO) (1982)	<i>Rattus norvegicus</i> (brown rat) (worldwide)	intermediate HFRS (urban & rural)
5. Puumala (PUU) (1984) (POZ-M1) (1990)	<i>Clethrionomys glareolus</i> (red bank vole) (Europe, Russia and Korea) <i>Mus musculus</i> (house mouse)(Serbia)	mild: NE, HVD (rural) severe HFRS
6. Thailand (THAI) (1985)	<i>Bandicota indica</i> (bandicoot) (Thailand)	not recorded
7. Dobrava (DOB) (1992)	<i>Apodemus flavicollis</i> (yellow necked field mouse) (ex-Yugoslavia)	very severe HFRS (rural?)
8. Muerto Canyon Virus (MCV) (1993)	<i>Peromyscus maniculatus</i> (deer mouse) (S-W USA)	often lethal HPS

Legend to Table 1:

EHF: Epidemic hemorrhagic fever

HFRS: Hemorrhagic fever with renal syndrome
(current WHO denomination)

HPS: Hantavirus pulmonary syndrome

HTVD: Hantavirus disease
(proposed new common denomination)

KHF: Korean hemorrhagic fever

NE: Nephropathia epidemica

* A strain of a "serotype" is neutralized to more than 50% in plaque reduction neutralization tests (PRNT) by homologous antisera. A "serotype" is thus defined as having no cross reactions in PRNT with other strains or having a homologous- to -heterologous titer ratio of $\geq 1/16$ in both directions.

** TPM was first considered a novel arbovirus and only recently genetically confirmed as a distinct Hantavirus (4). For all the 8 serotypes listed here, a perfectly similar division in 8 distinct lineages was found by polymerase chain reaction (PCR)-genotyping (See Fig.1)(4-5).

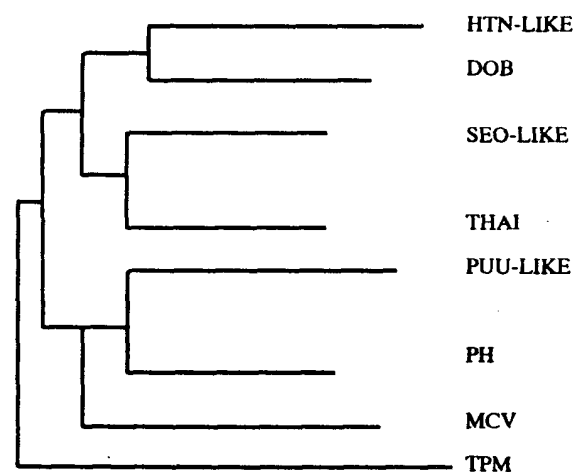


Fig. 1: Dendrogram with 8 lineages (HTN, DOB, SEO, THAI, PUU, PH, MCV, TPM) representing the genetic relationship between the 8 most important Hantaviral serotypes characterized so far and modified after the data given by Xiao et al (4,6). (With permission of the author). The length of the horizontal lines indicates approximately the respective differences in nucleotide sequences of the M or the S segment as obtained by PCR genotyping. Vertical lines are for clarity of presentation only. For the abbreviations used, see Table 1.

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CLINICAL PICTURE

The clinical presentation of the (mostly) mild European variant, induced by the *Puumala* (PUU) serotype and called *Nephropathia epidemica* (NE), consists of sudden fever, often severe lumbalgia (due to acute interstitial nephritis with renal swelling), acute renal failure (ARF) and thrombocytopenia (6). Early severe proteinuria (in almost 100% of the cases) and early ophthalmological symptoms (eye pain, conjunctival injection, blurred vision, acute glaucoma etc.) (24%) have additional diagnostic value (7). Hemorrhagic symptoms are rare (22%) and minor in NE (petechiae, nose bleeding etc), in contrast to the more severe forms in the Far East and in the Balkans, where severe hemorrhagic complications and shock often lead to death. In NE however, all these symptoms are mild and self-limiting, and complete restoration to normal within 2-3 weeks is the rule. Thus, except for the ophthalmological symptoms and the rare hemorrhagic complications, clinical presentation of NE is often aspecific or even totally absent (subclinical forms). Diagnosis can only be secured by serological tests. The most important differential diagnosis remains leptospirosis, particularly when the wild rat is suspected as the rodent vector (8). NE has a good prognosis, a low mortality (< 1%) and dialysis as a treatment for ARF is required in less than 5% of most European series (6,7).

In the USA, the recently isolated *Muerto Canyon virus* (See Table 1) was during a 1993 epidemic the cause of the so-called Hantavirus pulmonary syndrome (HPS) resulting in a severe form of "adult respiratory distress syndrome" (ARDS) with up to 60% mortality (9). For reasons not yet understood, in this "new" American form the lungs are more involved than the kidneys. "HPS" is not restricted to the USA however, and can exceptionally be seen also in European NE cases, albeit in milder forms (10).

THE 1993 HTV EPIDEMIC IN THE ARDENNES

From September 1992 on, and on both sides of the Franco-Belgian border in the Ardennes region, an outbreak of PUU-induced NE was noted, with more than 200 serologically confirmed cases through January 1994, confirming that NE is not to be considered a rare disease in our regions (7,11,12). In the Belgian part alone, a total of 62 serologically confirmed patients were registered so far: 44 in the Chimay region, 9 in Dinant, 4 in Lobbes, 2 in Philippeville, 2 in Charleroi and 1 in Loverval (7). Of these 62 patients, 43 (69.3%) had to be hospitalized, and a total of 55 (88.7%) medical records were available for analysis. A transient impairment of kidney function (S. creatinine $\geq 133 \mu\text{mol/L}$ or 1.5mg%) was noted in 46/55 (84%) patients with peak S. creatinines ranging between 97 and 964 $\mu\text{mol/L}$ (1.1 and 10.9 mg%). Thrombocytopenia ($\leq 100 \times 10^3/\text{ml}$) was noted in 39/55 (71%) patients with a range between 12 and 320 $\times 10^3/\text{ml}$. However, in our 10 years experience of more than 150 cases so far, thrombocytopenia is an early and transient phenomenon, which can easily be missed if the first blood examination is delayed for some days, as we have often witnessed in non-hospitalized patients.

THE MOUSE CONNECTION

In the epidemic region, i.e. mainly in the forested surroundings of Chimay, a conspicuously dense local population of the responsible vector the red bank vole (*Clethrionomys glareolus*) was noted end 1992-begin 1993. Sighting of bank voles around the habitats or working areas was often reported by the NE patients (7,11). This increase in vole numbers can be due to a rare combination of ecological factors such as the mild '92-'93 Winter, the abundant Spring '93 rains and the extraordinary abundance in the forests of beechnuts, the staple food of the bank vole (3,11). We performed in '92 and '93 successive rodent trappings in the

Chimay area and assessed the presence of Puumala-like antigen in the lungs of the trapped rodents by means of an antigen-capture ELISA technique (13). On a total of 163 examined bank voles, 10 (6%) were found antigen-positive, being 0/3 in May '92, 2/18 (11%) in June '93, 5/78 (6.4%) in September '93 and 3/64 (4.6%) in November '93 (14). All other captured rodent species (total number = 94) were negative. Rodent numbers decreased substantially end 1993-begin 1994, as did the patient numbers.

An even more convincing mouse-man connection was found during the 1993 Hantavirus pulmonary syndrome (HPS) epidemic in the USA. A common North-American small rodent, the deer mouse (*Peromyscus maniculatus*) was found in very high numbers (up to 10 times the normal), 30% of them showing in their lungs the presence of a novel HTV, as evidenced by PCR-genotyping. Nucleotide sequencing was exactly the same as in the PCR products obtained from human HPS autopsy material (15) and as in the responsible *Muerto Canyon Virus* (MCV), that was subsequently isolated (16). Comparative genotyping revealed that this novel MCV was close to, but not identical with *Prospect Hill* (PH), the only other indigenous serotype from N-America (See Table 1 and Fig. 1).

LABORATORY DIAGNOSIS

Since HTV can spread quickly, often in local outbreaks, or even in nation-wide epidemics with high mortality (in the Far East, and particularly in China), the need for rapid diagnostic tests is obvious. The principles and findings summarized in this paper focus mainly on one single class of viral hemorrhagic fevers (VHF), i.e. the so-called haemorrhagic fever with renal syndrome (HFRS) caused by Hantaviruses, but many conclusions as stated below may also be applicable to other classes of VHF as well.

Thus, rapid diagnostic tests for VHF should ideally be a) quick and simple to carry out, allowing if possible their use in "field condi-

tions", b) able to confirm serodiagnosis of a recent infection in 1 single sample, thus excluding the need of paired or serial follow-up samples. We will learn in the following discussion that the promise of both conditions a+b is rarely fulfilled.

The following techniques will briefly be reviewed:

Antibody detection methods

1) Immunofluorescent assay (IFA), 2) IgM Enzyme immuno assay (EIA), 3) Inhibition EIA with monoclonal antibodies (Abs) against structural proteins of Hantavirus, 4) μ -capture EIA, 5) Rapid avidity assay of Hantaviral IgG antibody, 6) High density particle agglutination (HDP), 7) Line immuno assay (LIA)

Antigen or viral RNA detection methods

1) Polymerase chain reaction (PCR), 2) Immunohistochemistry (IHC) staining.

More cumbersome tests such as plaque reduction neutralization tests (PRNT) (2,4), and Western Blotting (WB)(17), will not be discussed here.

Antibody detection methods

1. Immunofluorescent Assay (IFA)

IFA remains for many laboratories the "golden standard" diagnostic assay for the detection of Hantavirus (HTV) specific antibodies. By comparing the titers obtained upon screening with several HTV antigens, IFA can give an indication of the HTV-serotype involved in an infection. Theoretically, PRNT or even newer techniques such as PCR can give a definitive answer as to the HTV-serotype concerned (4,15). PRNT, which detects neutralizing antibodies induced by envelope glycoproteins G1 & G2, is however a labour intensive technique requiring a P3 biosafety level. On the other hand, the full array of primers necessary for PCR may not be available in many labs. The fact that HTV's, as originally used in Vero E 6 cells for the IFA technique, also require a P3 containment

would perhaps seem to form a hinderance for simple and quick diagnosis. However, heat inactivation of viral antigen preparations for 1 hour at 60°C has enabled their use in less stringent lab conditions (18). Alternatively, antigens may be inactivated by gamma irradiation (19) or by treatment with β -propiolactone (20). The inactivated HTV screening antigens representing the major HTV serotypes may be fixed with acetone (20°C) or with ethanol (-70°C) onto microscope slides. The presence of the HTV-specific antibodies is detected indirectly in the sera to be tested using a suitable conjugate labelled with fluorescein isothiocyanate (FITC). The test although indirect, is still rapid and retains a certain flexibility in that either IgG or IgM HTV-specific antibodies can be detected. The screening for IgM anti-HTV antibodies by IFA is however less sensitive than enzyme immunoassay (EIA) techniques (21,22). Interference from rheumatoid factor can be minimized by treatment with anti-human Fc specific conjugate.

The WHO has recommended that the prototype *Hantaan* (HTN) virus (isolated from *Apo-demus agrarius coreae*, the striped field mouse) and the *Puumala* (PUU) virus (isolated from *Clethrionomys glareolus*, the bank vole) should be used as routine screening antigens (23). HTN has the broadest one-way cross-reactivity with the other HTV antigens, and has been used worldwide as a screening antigen (2,5). However, we have found that the sensitivity of the IFA assay can still be improved upon if a rat-derived *Seoul* (SEO) HTV strain is included in the battery of IFA screening antigens (24). Indeed, despite the close relationship between HTN and SEO, (4, 5) (see Fig.1), we found that IFA screening with HTN alone resulted in false negatives in cases of SEO-induced HFRS in Brazil (25), in N.Ireland (24) and in a recently confirmed SEO-case in Bosnia with a clear history of rat exposure (8). All these cases were at the onset mistaken for leptospirosis .

Consideration must be given to the other limits of the IFA test, being the requirement of a

fluorescent microscope. IFA is not ideal for the screening of large batteries of sera (an advantage given by automated ELISA's), since the test is not readily adaptable for automation. Similarly, the interpretation of the test requires a considerable degree of experience to discern the specific dot-like staining found in the cytoplasm of infected cells, from the more diffuse "atypical" staining that may be present at lower serum dilutions (26). Again interpretation of ELISA assay optical density data is much less subjective. The simplicity of the IFA technique will however probably continue to favour its use under many laboratory conditions.

2. Indirect IgM Enzyme Immunoassay (EIA)

Since in man as in rodents, IgG antibodies seem to persist for life, IFA-seropositivity for IgG may not always reflect a recent infection. In highly endemic countries, such as certain areas in Sweden, IgG seroprevalence can be as high as 30% (27). Moreover, a diagnostic rise in IgG titers has not always been observed in recent clinical cases (22,27). Specific anti-Hantaviral IgA and particularly IgM EIA are accepted now as the serologic prerequisite for confirming suspected clinical cases (22,26,28,29). The assay is based upon a sandwich technique in which HTV-infected crude cell lysates, purified virus or even recombinant viral proteins serve as screening antigen. The latter technique obviates the need for P3 biosafety levels in the preparation of the antigen, which is a major advantage (28).

These IgM EIA techniques were shown to have a sensitivity greater than IgG IFA to pick up recent clinical NE cases, giving positive results from the first day after onset of symptoms, up to 6 months afterwards with cell lysates as antigen (22) and up to 50 days afterwards with a recombinant antigen (28).

3. Inhibition EIA

An inhibition EIA was developed, using monoclonal antibodies (Abs) with specific

tions for Hantaviral group determinants on glycoproteins G1, G2 and nucleocapsid (NC) protein (30). The solid phase is coated with HTN antigen, and the binding of HTN-positive human Ig serum Abs inhibits the reactivity of HTN-specific monoclonal Abs directed against the respective structural proteins of HTN. It was shown in Belgian and in Dutch clinical cases that serum Abs appeared against the G1 epitope in the acute (0-2 weeks) and early convalescent (3-5 weeks) periods, just before Abs to the NC epitope developed. In contrast, Abs to the G2 epitope were not found before the convalescent period. Thus, ratio's between G1 specific Abs on the one hand and Abs to either of the other proteins on the other hand, may be indicative for the period that has elapsed after initial HTV-infection, allowing serological confirmation of a recent infection in one single serum sample (30).

Determination of the time of infection with one single serum sample may also be estimated using inhibition EIA for quantification of different classes and subclasses of Ig antibodies formed after infection (29).

4. μ -Capture EIA

In IgM-specific EIA assays, false positive results can occur (by the presence of rheumatoid factor) as well as false negative results (by the presence of high levels of IgG Abs). These potential problems are circumvented by the so-called capture technique, whereby the solid phase of a microtiter plate is coated with antibody capable of capturing human IgM or IgA from the test serum, indicative of a recent infection. These specific viral IgM or IgA antibodies bind to the viral screening antigen which again can be a cell lysate, a purified antigen (22,29) or a recombinant protein (31,32). The antigen thus binded can be detected upon addition of animal hyperimmune anti-viral antibody followed by anti-species marked antibody and the appropriate substrate. Zöller et al (31,32) described a novel μ -capture IgM EIA test, in which two *E. coli*-expressed recombinant nucleocapsid pro-

teins were used as antigen, one derived from *Hantaan* (HTN) 76-118, and another from the *Puumala* (PUU) strain CG 18-20 (see Fig.1 and Table 1). The assay was said to have a sensitivity of up to 100% for detecting HTN or PUU-induced disease (32). The authors described positive IgM-detection with this test up to 2 years after NE infection (32). We found positive IgM levels in several Belgian patients more than 1 year after confirmed NE infection (unpublished own observations). An unexpected side-effect of this increased sensitivity would be that a positive result is more difficult to interpret by the clinician in his quest to confirm recent clinical cases by a single test. Moreover, in our hands up to 43% (6/15) of N-Irish cases, apparently all infected with a rat-induced SEO-strain, remained negative with this assay (24). Further investigation is needed as to the sensitivity of screening SEO-cases with HTN and PUU recombinant antigens. It seems clear from now however that this assay is superior to IgM solid-phase EIA and to IFA for screening clinical NE cases, both in terms of sensitivity and specificity (32). This assay also has the added advantage of lacking a pre-incubation step of serum samples with anti-rheumatoid factor.

5. Rapid avidity assay of Hantaviral IgG antibody

This single-serum sample test measures the avidity or functional affinity of IFA-IgG Abs against PUU virus, and is said to be almost four times more sensitive than conventional IFA IgG serology (33). Treatment with 8 mol/L urea solutions of PUU-infected Vero E-6 cell-slides, after incubation with the test serum causes elution of low avidity IgG Ab; ratios of IFA IgG titer differences without urea/with urea measures the degree of avidity (the greater the avidity, the lower the magnitude of the ratio). IgG antibody appeared to have a low avidity in the acute phase and high avidity in the convalescent phase. Thus high (≥ 32) titer ratios (low avidity) are diagnostic of acute NE, whereas low (≤ 4) ratios (high avidity) rule out NE during the pre-

vious months. Recently, a more suitable IgG EIA version of this assay has been introduced, using recombinant (*E.coli*-expressed) PUU (Sotkamo strain) nucleocapsid protein as an antigen, instead of infected Vero E 6 cells (34).

6. High Density Particle Agglutination (HDP A)

HDP A was introduced as a quick (1 hour) and simple test, based on a passive agglutination reaction, using high density composite particles of silica coated with a color layer and with Hantaviral antigen (Korean strain HTN 84/105) (35). Readings of the test results can easily be done with the naked eye. HDP A permits detection of both IgM and IgG Abs, however, no discrimination can be made between these 2 Ab classes. In addition, since no specific conjugate is required in the assay, this technique can also be easily used to screen animal sera.

We applied HDP A-screening after preliminary heat inactivation at 56°C for 3 min. on a total of 29 acute or convalescent sera previously confirmed as being PUU-induced NE by positive IFA and PRNT (courtesy of Dr.J.LeDuc, USAMRIID, USA). We found an excellent sensitivity of 100% (no false negatives). Moreover, the highest HDP A titers (range 1280-2560) were demonstrated in the 5 acute Belgian NE cases, whereas the lowest titers were found in the late retrospective NE cases. In a negative control group consisting of 421 leptospirosis-suspected cases, a total of 13 cases (3.0%) were found with false positive HDP A results, resulting in a specificity of 97.1% (unpublished own observations). More data are needed however in the future to see if all clinically relevant HTV-strains (see table 1) are sufficiently picked up by HTN as the only screening antigen used so far in HDP A.

7. Line Immuno Assay (LIA)

LIA is a recently developed test (36) in which multiple antigens are extracted, purified and fixed in parallel lines onto nitrocellulose strips. Following incubation with test serum, antibo-

dies to the various antigens can be detected with specific anti-human conjugate and a suitable substrate. Readings by the naked eye of seropositivity are rapid and simple, without the need for special equipment (dipstick method). Readings are mostly possible after only ± 3 hours and can be measured quantitatively if a densitometer or scanner are used. Sera analysed via LIA were considered positive if the coloured reaction that was obtained with the Hantaviral antigen was stronger than that of the control antigen.

This technique has been successfully applied to the serological detection of malaria (37), syphilis (38) and AIDS infection (39,40). LIA could be of particular importance in the field of HTV serology, since it allows in a single experiment the presence of antibodies against different HTV serotypes to be determined. In a preliminary study, comparing in NE sera the LIA results for seven different HTV-serotypes with IFA as the golden standard, we found an overall correlation between these two assays of 85.3% (unpublished own observations). This promising test awaits further industrial elaboration in view of the high technical demands for antigen purification and application to strips.

Antigen or viral RNA detection methods

1. Polymerase Chain Reaction (PCR)

PCR is aimed at detecting viral RNA in blood and tissues, i.e. the presence of the infectious HTV itself, at least in theory (41).

In a first 1992 clinical study by Grankvist et al (42), nested primer PCR was reported to detect PUU virus RNA in urinary and respiratory tract cells and in peripheral blood mononuclear cells of patients with serologically proven NE, despite the fact that all virus isolation attempts from these patients in Vero E-6 cells remained negative by IFA screening. PCR holds excellent potential for laboratory detection and specific identification of isolates. Amplification of a 333-base pair region of the Medium (M) segment of 30 different HTV-isolates by reverse

transcription PCR and subsequent comparison of the obtained nucleotide sequences, allowed the construction of a phylogenetic dendrogram representing the six important lineages HTN, DOB, SEO, THAI, PUU, and PH, exactly as found already by PRNT and other serological techniques (4)(Table 1 and Fig.1). Subsequent characterization of TPM and MCV added 2 more lineages to this tree. This new technique has also proven to be extremely useful for epidemiological purposes as well, since it can demonstrate a genetic link between viruses detected both in man and in rodents (See The Mouse Connection).

The enormous possibilities of PCR genotyping were further demonstrated during the recent "Hantavirus Pulmonary syndrome" (HPS) epidemic in the USA. A recombinant nucleoprotein antigen for the screening of suspected HPS-cases was used (43). This screening antigen was obtained by acid guanidium thiocyanate-phenol-chloroform extraction from the lungs of deceased HPS patients, followed by reverse transcriptase PCR amplification and expression in *E. coli*. This "tour de force" of genetic molecular biology was accomplished even before the responsible Muerto Canyon virus was actually isolated (16), and has been aptly called "virology without a virus".

2. Immunohistochemistry (IHC)

Using monoclonal Abs to nucleoprotein epitopes common to all Hantaviruses, Immunohistochemistry (IHC)-detection of HTV antigen was possible in formalin-fixed autopsy tissues by means of an immunoalkaline phosphatase staining method (15,44). This technique allows even retrospective diagnosis in cases that died years ago (44). Since protein denaturation is not a hindrance for IHC (as it is for PCR), even Bouin-fixed biopsy material (e.g. kidney biopsies) could be used for retrospective HTV-diagnosis. Immunostaining with gold-labelled monoclonal or polyclonal Ab particles seems

also to be an interesting alternative for the future (45).

CONCLUSION.

The following scheme appeared in our experience the most rewarding for serodiagnosis of recent clinical HTV-cases, up to 6 months after onset of symptoms:

- 1) Screening for the presence of IgG Ab with an IgG EIA assay, using both HTN and PUU antigens, *and* preferably also a SEO antigen.
- 2) Confirmation and titration of Ab levels may be followed by IgG IFA using the same 3 antigens from a starting dilution of 1/16 on. However, this former "golden standard" is not any more an absolute prerequisite.
- 3) Confirmation with solid-phase IgM EIA or preferably with μ -capture EIA using both HTN and PUU antigens *and* if possible also a SEO antigen.
- 4) High density particle agglutination (HDP) with HTN antigen may be a quick and easy one-step alternative, which allows however no distinction between IgG and IgM seropositivity.
- 5) PCR and/or IHC seem the methods of choice for detecting viral RNA, respectively viral antigen in frozen or even (for IHC) in fixed tissue. So far, PCR was disappointing however as screening technique for sera, perhaps due to the fact that in most human HTV-infections the phase of viremia is very short-lived.

SAMENVATTING

Hantavirus (HTV) is een recent ontdekt "hemorragische koorts virus" behorende tot de familie van Bunyaviridae, dat wereldwijd wordt verspreid door wilde knaagdieren en/of laboratorium ratten. Tijdens een epidemie in 1993 in de Belgisch-Franse Ardennen werden meer dan 200 acute gevallen genoteerd van de milde Europese variant van HTV-ziekte, ook genoemd Nephropathia epidemica (NE). Deze wordt

gekenmerkt door plotse koorts, acute nierinsufficiëntie, thrombocytopenie en soms ook ophthalmologische tekens. Deze symptomatologie is echter dikwijls aspecifiek, en de diagnose kan enkel bevestigd worden door serologische technieken, waarvan de beste oplossing ons thans lijkt: screening door IgG EIA, gevolgd door IgM confirmatie met een μ -capture techniek. Sommige van de beschreven tests laten een evaluatie toe van het oorzakelijke serotype, of zelfs van het ogenblik van infectie.

Naast "klassieke" serologische tests voor detectie van specifieke virale antistoffen, beschrijven we in het kort onze eigen ervaring met nieuwe varianten zoals "high density particle agglutination" en "line immuno assay". Polymerase chain reaction voor typering van het viraal RNA-genoom en immunohistochemische kleuring van het viraal antigeen in weefsels, lijken veelbelovende alternatieven voor de onmiddellijke toekomst.

RESUME

Appartenant à la famille des Bunyaviridae, l'Hantavirus (HTV) est un nouveau membre des "virus de fièvre hémorragique", propagé sur une échelle mondiale par des rongeurs sauvages ou par des rats de laboratoire. Lors d'une épidémie dans les Ardennes franco-belges en 1993, plus de 200 cas aigus de la variante bénigne Européenne, appelée aussi Nephropathia epidemica (NE) ont été détectés.

Cette forme se caractérise par une fièvre et une insuffisance rénale aiguës, une thrombocytopenie, et parfois par des signes ophtalmologiques. La symptomatologie est toutefois souvent aspécifique et le diagnostic repose donc sur des tests sérologiques, dont la meilleure séquence nous paraît pour l'instant: un premier dépistage en IgG EIA, suivi d'une confirmation pour l'IgM en μ -capture EIA. Quelques-unes des techniques décrites permettent en outre une évaluation du sérotype incriminé ou, même, du moment de l'infection.

Après les tests sérologiques "classiques" de détection d'anticorps anti-viraux spécifiques, nous décrivons brièvement notre expérience avec des variantes plus récentes comme la "high density particle agglutination" ou encore le "line immuno assay". Le typage du génome RNA viral à l'aide de la "polymerase chain reaction" et la coloration immunohistochemique de l'antigène viral dans les tissus semblent être des alternatives très prometteuses dans un avenir proche.

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