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Does proficiency testing improve the quality of hantavirus serodiagnostics? Experiences with INSTAND EQA schemes

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ABSTRACT

Hantavirus infections in Germany appear periodically with peak numbers every 2–3 years. The reported cases in the years 2007, 2010 and 2012 exceeded many times over those in the years in-between. In order to reveal faults of certain in vitro diagnostic assays (IVDs), to harmonize the performances of the individual assays and to improve the users' competence in interpreting the results, the National Consiliary Laboratory for Hantaviruses and INSTAND e.V. (Society for Promoting Quality Assurance in Medical Laboratories e.V.) established an external quality assessment (EQA) scheme for proficiency testing of hantavirus serodiagnostics. The first EQA scheme (pilot study) started in March 2009 with 58 participating laboratories from Germany and neighboring countries. Twice a year four serum samples were sent out to the participants to investigate whether the sample reflects an acute or past infection and to distinguish between infections with the hantavirus types Puumala virus (PUUV) and Dobrava-Belgrade virus (DOBV), both endemic in Central Europe. In addition, samples negative for anti-hantavirus antibodies were tested in order to examine the specificity of the IVDs applied in the participating laboratories. An increasing number of laboratories participated, with a maximum of 92 in March 2014. When summarizing in total 2592 test results, the laboratories reached an overall specificity of 96.7% and a sensitivity of 95% in their detection of a hantavirus infection. A correct distinction between acute and past infections was forwarded in 90–96% of replies of laboratories. Exact serotyping (PUUV vs. DOBV) of the infection was reported in 81–96% of replies with the lowest accuracy for past DOBV infections; cross-reactivities between diagnostic antigens of the two viruses as well as persistent IgM titers in humans may interfere with exact testing. The EQAs revealed acceptable results for the serodiagnostic of hantavirus infection including serotyping but further improvement is still needed.

1. Introduction

Hantaviruses, members of *Bunyaviridae* family, are emerging viruses which cause Hemorrhagic Fever with Renal Syndrome (HFRS) in Asia and Europe and Hantavirus Cardiopulmonary Syndrome in the Americas (Vaheri et al., 2013). In Central Europe, Puumala virus (PUUV) and Dobrava-Belgrade virus (DOBV) are the two main pathogenic hantaviruses circulating in rodent hosts and transmitted to humans (Klempa et al., 2013). In Germany, a total of 7252 symptomatic HFRS cases were reported from 2007 to 2012 with increasing peak activities in the outbreak years 2007 (1687 cases), 2010 (2016 cases), and 2012 (2824 cases). During outbreak years, the hantavirus disease belongs to the group of the 5 most frequent notifiable virus diseases in Germany (www3.rki.de/SurvStat/QueryForm.aspx).

Large hantavirus outbreaks in Germany are caused by PUUV infections and a detailed molecular epidemiological characterization of PUUV strains from the different outbreak regions of the country has been established (Ettinger et al., 2012; Hofmann et al., 2008). Moreover, infections by the DOBV Kurkino genotype lead to additional cases of hantavirus disease (Hofmann et al., 2014). Since hantaviruses are strongly host-associated, infections of humans are linked to the particular virus type circulating in the geographic area where infected rodents live. Thus, PUUV infections appear mostly

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in South and Southwest Germany, but DOBV infections in the North and Northeast, corresponding to the natural habitats of the rodent hosts, the bank vole (*Myodes glareolus*) and striped field mouse (*Apodemus agrarius*), respectively.

Hantavirus diagnostic is based on seroassays, as enzyme immune assay (EIA), immunoblot, immunofluorescence assay (IFA), and rapid immunochromatographic strip assay. (Meisel et al., 2006; Vaheri et al., 2008). The antigen source in PUUV assays usually consists in a homologous (PUUV) nucleocapsid protein, while for DOBV assays – particularly in older IVDs – a heterologous (Hantaan virus) nucleocapsid antigen is used. DOBV and Hantaan virus nucleocapsid proteins share some homology in their amino acid sequences (Klempa et al., 2005), however, such cross-reactions complicate a reliable antibody particularly in low-titer sera (Meisel et al., 2006). Confirmatory assays – like the focus reduction neutralization test (FRNT) requiring BSL-3 conditions or RT-PCR assays – can be performed by highly specialized groups only but not by the majority of diagnostic laboratories. Moreover, whereas serotyping by FRNT is reported to be most specific when using sera of convalescent patients, the occurrence of detectable virus genomes in the blood is restricted to the very early phase after disease onset (Kruger et al., 2011). Therefore, the routine diagnostics is based on use of the above mentioned seroassays.

Since hantavirus disease is notifiable in Germany according to the Infection Protection Act, a country-wide network of qualified laboratories is needed which uses assays of high reliability. We have established an external quality assessment (EQA) system and determined the diagnostic performance of more than 50 laboratories in Germany and neighboring countries twice a year.

2. Material and methods

Serum samples were obtained from patients who were diagnosed in the National Consiliary Laboratory to be either IgG+/IgM− or IgG+/IgM+ seropositive for either PUUV or DOBV. After approval of the physician in charge, the patients were asked for giving a blood donation. The patients completed a disease-specific questionnaire, including potential infection source, clinical course, travel activities, which was later used as additional information for the participating laboratories. Certain acutely infected patients were sampled again one year later providing valuable follow-up sera. In any case all clinical samples were taken from the patients after informed consent including clarification that the samples are exclusively used for EQA schemes and completely anonymized in order to exclude any traceability to personal data of the patients. Donated blood materials were processed at the GBD Gesellschaft für Biotechnologische Diagnostik mbH, Berlin. The resulting sera were characterized at the National Consiliary Laboratory and the GBD and were tested by PCR to be negative for hantavirus RNA in order to exclude infectivity especially in samples representing acute hantavirus infection (DOBV or PUUV). These well-investigated samples were sent to up to eight experienced target value laboratories for serological confirming the sample properties. If concordant results were obtained, the individual sample was included into the proficiency testing panel. In addition, samples negative for anti-hantavirus antibodies were tested in order to examine the specificity of the IVDs applied by the participating laboratories. According to the amount of the test material certain samples were used in more than one panel. The results reported by the participating laboratories and the correct interpretations were made available (in anonymous form) to every individual laboratory and also to non-participants on the homepage of INSTAND (http://www.instandev.de/en/eqas.html). Thus, information on the performance of single assays was distributed publicly.

3. Results

Each EQA scheme comprised four samples from PUUV-infected, DOBV-infected, or non-infected individuals. Table 1 summarizes the number of participating laboratories (increasing from 58 in the pilot study of March 2009 to 92 in the EQA scheme of March 2014), the composition of each 4-sera panel, and the assays used by the participants during the 11 EQA schemes. The number of participating laboratories increased by about one-third after 2012, the year with multiple outbreaks and the highest number of reported cases.

Fig. 1 gives an overview about the assays used by the participants. More than 50% of all analyses were performed exclusively by immunoblot assays, numerically followed by a combination of EIA and immunoblot (16% of analyses). The proportion of the single

Table 1
Number of participants, characteristics of the panel composition, and IVDs used in the EQAs from March 2009 to March 2014.

	Pilot study	Sep 09	Mrz 10	Sep 10	Mrz 11	Sep 11	Mrz 12	Sep 12	Mrz 13	Sep 13	Mrz 14
	Mrz 09										
Participants (*n*)	58	55	64	64	71	69	71	71	83	81	92
Sera provided in the panel											
Acute PUUV	2	1	1	0	0	0	1	0	0	0	1
Past PUUV	0	0	1	1	2	2	0	1	1	1	1
Acute DOBV	1	1	0	2	1	1	1	2	1	2	1
Past DOBV	0	1	2	1	1	1	1	1	1	1	0
Negative	1	1	0	0	0	0	1	0	1	0	1
Assays used by participants											
EIA only	9	8	9	11	9	9	8	10	10	9	10
IFA only	5	5	3	3	4	3	3	3	2	3	3
Blot only	28	26	32	32	37	35	36	34	44	40	50
EIA + IFA	3	2	2	3	2	2	2	2	1	1	1
EIA + RT	1	1	1	0	0	1	0	1	0	0	1
EIA + Blot	7	6	8	7	10	8	11	13	15	16	17
IFA + Blot	3	3	2	3	3	4	3	3	4	3	4
EIA + IFA + Blot	2	4	4	3	3	3	3	4	4	5	3
EIA + RT + Blot	0	0	1	0	1	2	2	1	1	1	1
EIA + IFA + RT	0	0	0	0	1	1	1	0	0	0	0
RT + Blot	0	0	0	0	0	0	1	0	0	2	2
RT + EIA + IFA + Blot	0	0	0	0	0	0	1	0	0	0	0
IFA + RT	0	0	1	1	1	1	0	0	2	0	0
IFA + RT + Blot	0	0	1	1	0	0	0	0	0	1	0

PUUV: Puumala virus, DOBV: Dobrava-Belgrade virus, EIA: enzyme immunoassay, IFA: immunofluorescence assay, RT: rapid test.