Hantaviruses have been identified as an important threat for human health, especially in the Americas, Asia, and Europe. Since the first appearance of a new disease, the life-threatening hantavirus cardiopulmonary syndrome (HCPS), in 1993 in the Americas (32), several hantaviruses have been described (for reviews, see references 29 and 40). HCPS-causing New World hantaviruses, including the most prominent representatives, Sin Nombre virus (SNV) in North America and Andes virus (ANDV) in South America, are also caused and transmitted to humans by members of the rodent subfamily Sigmodontinae.

In contrast, hantavirus infections in Asia and Europe are mainly characterized by renal dysfunction and, in severe cases, by hemorrhages (hemorrhagic fever with renal syndrome [HFRS]). Several hantavirus species have been shown to cause HFRS in humans (for reviews, see references 24, 29, and 36). HFRS in Europe, are also caused by a DOBV lineage carried by the yellow-necked mouse, Apodemus flavicollis (2). This virus, DOBV-Af, has been detected by reverse transcription (RT)-PCR and neutralization assays in patients from various countries in southeastern Europe.

For a long time, a mild form of HFRS, nephropathia epidemica, has been known in northern Europe. The disease is caused by Puumala virus (PUUV), which is transmitted by the bank vole Clethrionomys glareolus (6). It is well established that PUUV is an etiological HFRS agent in many European countries. Moreover, mild courses of HFRS, at least in central Europe, are also caused by a DOBV lineage carried by the striped field mouse, Apodemus agrarius (48). In HFRS patients, infections by this virus, DOBV-Aa, were detected by RT-PCR and neutralization assays (22, 23).

DOBV is genetically and antigenetically related to HTNV; these viruses share the property of being transmitted by rodent reservoirs of the subfamily Murinae. In contrast, PUUV is more distantly related to this group; its reservoir host belongs to a different subfamily, Arvicolinae. Thus, the standard serodiagnosis of HFRS in Europe must enable the detection of antibodies against at least two hantaviruses, DOBV and PUUV. Moreover, it is important to determine whether all DOBV infections can be detected by recombinant antigen of the related HTNV or whether their detection requires the use of DOBV antigen.

Hantaviruses are enveloped viruses with a diameter of about 70 to 210 nm. The immunodominant nucleocapsid (N) protein associated with the tripartite RNA genome of negative polarity is encoded by the S genome segment. The hantavirus virion consists of a host-cell-derived envelope containing the viral glycoproteins G1 and G2. The glycoproteins are generated by cotranslational cleavage of a glycoprotein precursor encoded...
by the M genome segment and carry epitopes recognized by neutralizing antibodies. The replication and transcription of hantavirus is mediated by an RNA-dependent RNA polymerase encoded by the L genome segment (41).

Due to the difficult and only short-term detection of virus and viral nucleic acid in infected humans, the diagnostics of human hantavirus infections is mainly based on serological assays. Traditionally, the serological diagnosis of hantavirus infections was based on immunofluorescence assays (IFA). However, in the recent past, enzyme-linked immunosorbent assays (ELISAs), immunoblotting, and immunochromatographic rapid tests have been developed, allowing the highly sensitive and specific detection of hantavirus infections in humans (for reviews, see references 24 and 25).

For this purpose, viral antigens from hantavirus-infected cell cultures or recombinant proteins have been exploited. Several advantages make the recombinant N (rN) protein a preferential antigen for serological assays: (i) the N protein is highly immunogenic during natural infection in rodents and humans and in immunized laboratory animals; (ii) N-specific antibodies occur early after hantavirus infection in humans and persist for a long time, probably even lifelong; (iii) N protein can be expressed very efficiently in Escherichia coli, yeast, and insect cells; and (iv) due to its conserved nature, N protein is highly cross-reactive between different hantaviruses, allowing the detection of a majority of hantavirus infections also by a heterologous antigen.

Recently, we demonstrated the high-level expression of rN proteins of different Old and New World hantaviruses, i.e., PUUV, DOBV, HTNV, Seoul virus, SNV, and ANDV, in yeast cells (37, 42, 43, 44). Here, we report the development of ELISAs (Progen, Heidelberg, Germany) were used. This negative control serum characterized serum samples from DOBV- and PUUV-infected humans.

**Materials and Methods**

**Human sera.** To determine the specificity of the ELISAs, 504 sera from patients with documented hantavirus infections, such as Epstein-Barr virus, human cytomegalovirus, tick-borne encephalitis virus, and hepatitis A and B viruses.

**Viral gene sequences.** Expression plasmids contained N-coding sequences of (38); HTNV, strain Fojnica (47); PUUV, strain Vranica/Hällnaès (38) or strain Sotkamo (51).

**N protein expression in yeast.** The generation of pFX7-derived expression plasmids encoding amino-terminal hexahistidine (His)-tagged, complete rN proteins (lacking only the first amino-terminal amino acid), their retransformation into the Saccharomyces cerevisiae wild-type strain FH4C, and the expression and purification of the rN proteins have been previously described (38). The purified rN proteins were characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting with the N-specific monoclonal antibodies (Mabs) A1C5, BSD9 (55), and 1C12 (27), produced from Progen (Heidelberg, Germany) and the Swedish Institute for Infectious Disease Control (Solna), respectively.

**Drospilia expression system.** To generate hantavirus antigens for the establishment of an IgM ELISA, the Drospilia melanogaster Schneider S2 cell expression system (Invitrogen, Groningen, The Netherlands) was used. The N protein-encoding sequences of DOBV and HTNV were PCR amplified from the template plasmids pCR1000/Jojica (47) and pCR2.1/DOB Slovakia 862 (48) by using the primers AM1 (5' -GGG TGA CTT ATG GCA ACA YTA GAG GAA CTC) plus AM2 (5' -ATT TGC GGC CCC GAA AGC TTA AGC GGC TCC TGA TT) and AM3 (5’-GGG GTA CCG ATG GCA ACT ATG GAG GAA TTA) plus AM4 (5’-ATT TGC GGC CCC GAG ATC AAA GGA GCT TGG TT), respectively. The PCR amplification products were cloned by using the Zero Blunt TOPO PCR cloning kit (Invitrogen). The N protein-encoding KpnI/Nol DNA fragments were subcloned into the plasmid pcA5.1/V5-HisA (Invitrogen), resulting in the generation of the expression plasmids pcA-S4 and pcA-F2, respectively. The N-encoding sequence of PUUV-Sot was subcloned by two subsequent steps into pcA5.1/V5-HisA with plasmid pCR-Blunt-II-TOPO-Sot-N (37). First, an XbaI (nucleotide [nt] 1651)/SspI fragment was inserted into XbaI/SstI-cleaved vector pcA.5.1/V5-HisA, resulting in the plasmid pcA-SoStSp. In the second step, a HindIII (nt 1236)/RsaI (nt 285) fragment from the initial cloning plasmid was used to replace a HindIII/EcoRI fragment in pcA-SoStSp. The resulting plasmid, pcA-Sot, was further used for transient transfection of S2 cells.

**SDS-PAGE and Western blotting.** Samples boiled in sample buffer were applied to a 10% SDS-polyacrylamide gel (about 0.5 or 1 µg of rN protein) and run in SDS-Tris-glycine buffer. Proteins were detected with SDS-PAGE by Coomassie blue staining. After separation by SDS-PAGE, the proteins were transferred to a cellulose nitrate membrane (Amersham, Buckinghamshire, England; Schleicher & Schuell) by semidry blotting. After blocking for 2 h in 5% dry milk-phosphate-buffered saline (PBS)–0.1% Tween 20, the filters were incubated overnight with the N-specific Mab 1C12, BSD9, or A1C5 at a concentration of 0.5 µg/ml or 1 µg/ml. For the detection of specific antibody binding, horseradish peroxidase (HRP)-labeled anti-mouse IgG (Dako) was used (45). IgG and IgA ELISAs for anti-DOBV and anti-PUUV detection. In general, all incubations were made at 37°C, unless otherwise stated. Between all incubations, washing steps were performed with washing buffer (0.01 M Tris, 0.3 M NaCl, 0.1% Tween 20 [pH 7.2]). For IgG and IgA ELISAs, microtiter plates (Maxisorp, Nunc, Wiesbaden, Germany) were coated overnight at 4°C with 100 µl of 1/200 in buffer A (50 mM sodium carbonate, pH 9.6) of Mab 1C12. Postcoating was conducted by the addition of 3% bovine serum albumin (BSA) (ph 7.0) in PBS for 1 h at room temperature. Thereafter, 100 µl (0.5 µg/ml in buffer A [1% BSA, 0.05% Tween 20 in PBS]) of the purified yeast-expressed rN protein of DOBV, HTNV, or PUUV was added. In parallel to each well replaced by coated with antigen, an additional well was used only with 100 µl of buffer A. After an incubation of 1 h, 100 µl of the serum sample was added at an initial dilution of 1:400 in buffer A. For the IgA and IgG ELISAs, the plates were incubated for 1 and 2 h, respectively. After the addition of 100 µl of HRP-labeled anti-human IgG (1 µg/ml in buffer A; Seramun, Dolgenbrodt, Germany), the plates were incubated again for 1 h. After the addition of 100 µl of TMB (3,3’,5,5’-tetramethylbenzidine [Seramun]), the peroxidase reaction was stopped by the addition of 10 µl of 1 M H₂SO₄. The optical density (OD) values were determined at 450 nm (reference, 620 nm). The OD value of each serum sample was determined as the difference between the OD value in the well with antigen added and that of the corresponding well without antigen.

**EM ELISAs for anti-DOBV and anti-PUUV detection.** For the IgM ELISA, anti-µ-coated microtiter plates (Medac, Hamburg, Germany) were used. After the addition of 100 µl of the serum sample (diluted 1:200 in buffer A), the plates were incubated for 1 h. Thereafter, 100 µl of diluted lystate of Drospilia Schnei
ders S2 cells (diluted 1:20 to 1:100 in buffer A) expressing the rN protein of DOBV, HTNV, or PUUV was added. In a parallel well for each serum, 100 µl of buffer A alone was added. After an incubation of 1 h, 100 µl (1 µg/ml in buffer A) of Mab 1C12 was added. The plates were incubated for 1 h, and finally HRP-labeled anti-mouse IgG (Dako) was added for 1 h. The peroxidase reaction was detected by the addition of TMB substrate for 10 min. The determination of OD values was performed as described above.

**Statistical Analysis.** For all statistical analyses, standard statistical software was used (45).
RESULTS

Primary characterization of recombinant hantavirus N proteins expressed in Drosophila Schneider S2 and yeast cells. The Drosophila Schneider S2 cell-expressed rN proteins of DOBV, HTNV, and PUUV were detected in crude lysates of cells in Coomassie blue-stained SDS-PAGE (data not shown). In the immunoblot, all three rN proteins of the expected molecular weights were recognized by the cross-reactive MAb 1C12 (Fig. 1A, lanes 5 to 7). In contrast, the PUUV- and HTNV-specific MAbs A1C5 and B5D9 detected only the rN proteins of PUUV (Fig. 1B, lane 7) and HTNV (Fig. 1C, lane 5), respectively.

The purified, yeast-expressed, His-tagged proteins of DOBV, HTNV, and PUUV reacted, in agreement with previous data (37), with these three different MAbs in the same pattern as did the S2-expressed proteins (Fig. 1A to C, lanes 2 to 4 and 5 to 7). As expected, E. coli-expressed, carboxy-terminally truncated hepatitis B virus core protein, used as a negative control, reacted with neither 1C12 nor A1C5 and B5D9 (Fig. 1A to C, lane 1) but with an anti-core protein antibody (data not shown).

Establishment of DOBV and PUUV IgG and IgA ELISAs based on yeast-expressed rN proteins and determination of cutoff values. Initial data obtained by comparison of indirect and MAb capture IgG ELISAs using yeast-expressed rN proteins demonstrated an approximately 10-fold higher sensitivity of the latter format (data not shown). Therefore, the IgG and IgA ELISAs were established on the basis of a MAb capture format. For this purpose, microtiter plates were coated with MAb 1C12 and subsequently loaded with the yeast-expressed rN protein of DOBV, PUUV, or HTNV. For each serum sample, two wells were used in parallel, one with the addition of antigen and one without. To estimate the cutoff values for the DOBV and PUUV IgG ELISAs, panels of 503 and 504, respectively, human anti-hantavirus-negative sera were tested in the corresponding MAb capture IgG ELISAs. In the DOBV and PUUV IgG ELISAs, the OD values for all negative serum samples were found to be lower than 0.10 and 0.11, respectively (Fig. 2A and B). Therefore, cutoff values of 0.10 and 0.11 were defined for the DOBV and PUUV IgG ELISAs. Similarly, the cutoff values for the DOBV and PUUV IgA ELISAs were determined to be 0.20 and 0.09, respectively (Fig. 2C and D).

Establishment of IgM ELISAs based on Drosophila cell-expressed rN proteins and estimation of their cutoff values. In preliminary experiments, yeast-expressed rN proteins of DOBV and PUUV were found to be less reactive with IgM-positive reference sera than was rN protein expressed in insect cells (data not shown). Therefore, for the establishment of IgM ELISAs we used rN proteins of DOBV and PUUV expressed in Drosophila Schneider S2 cells. In the DOBV and PUUV IgM ELISAs, all sera of the control serum panels (n = 200 and 80, respectively) resulted in OD values lower than 0.13 and 0.15, respectively. Therefore, the cutoff values for the PUUV and DOBV IgM ELISAs were defined accordingly (Fig. 2E and F).

Specificity and sensitivity of the ELISAs. As mentioned above, no anti-hantavirus-negative serum reacted false-positively with the rN proteins of DOBV and PUUV in any of the ELISAs. This demonstrates a specificity of 100% for all six ELISA tests.

To determine the sensitivity of the ELISAs, a total of 304 reference sera from DOBV- or PUUV-infected HFRS pa-
FIG. 2. Determinations of cutoff values, specificities, and sensitivities for PUUV and DOBV IgG, IgA, and IgM ELISAs. (A and B) IgG ELISAs based on DOBV (A) or PUUV (B) rN antigens. For determinations of cutoff values and specificities, 503 (A) or 504 (B) sera from blood donors and patients with viral infections other than hantaviruses were used. The sensitivities were determined with 60 and 69 sera originating from DOBV- and PUUV-infected HFRS patients, respectively. (C and D) IgA ELISAs based on DOBV (C) or PUUV (D) rN antigens. For determinations of cutoff values and specificities, 112 sera each from blood donors and patients with viral infections other than hantaviruses were used. The sensitivities were determined with 29 and 23 sera originating from DOBV- and PUUV-infected HFRS patients, respectively. (E and F) IgM ELISAs based on DOBV (E) or PUUV (F) rN antigens. For determinations of cutoff values and specificities, 200 (E) and 80 (F) sera from blood donors and patients with viral infections other than hantaviruses were used. The sensitivities were determined with 35 and 88 sera originating from DOBV- and PUUV-infected HFRS patients, respectively.
Patients and reconvalescents were used. All sera of DOBV (60 sera) and PUUV (69 sera)-infected HFRS patients were detected by the corresponding IgG ELISAs (Fig. 2A and B). Similarly, all tested acute- and convalescent-phase sera of DOBV (29 sera) and PUUV (23 sera)-infected patients were detected by the corresponding IgA ELISAs (Fig. 2C and D). All acute-phase sera of DOBV (35 sera) and PUUV (88 sera)-infected patients were detected by the PUUV and DOBV IgM ELISAs, respectively (Fig. 2E and F). Taken together, the DOBV- and PUUV-specific IgG, IgM, and IgA ELISAs were found to exhibit a sensitivity of 100%.

Follow-up of antibody detection. For 34 patients with acute DOBV (n = 17) and PUUV (n = 17) infections (proven by serotyping with c-FRNT), we examined IgM, IgA, and IgG responses in follow-up sera for up to 3 years. This study demonstrated the long-lasting presence of IgG antibodies in both DOBV- and PUUV-infected HFRS patients. IgM and IgA antibodies decreased below the detection limit in all PUUV-infected patients and about half of the DOBV-infected patients after about 3 months (Fig. 3A and B). Interestingly, and in contrast to PUUV-infected individuals, for eight and nine DOBV-infected patients, IgM and IgA antibodies, respectively, were found as late as about 100 days after the onset of disease. In three of these patients, IgM antibodies persisted (with very low titers, 1:400) for even 2 to 3 years. Three patients (two of them identical with the IgM-positive patients) exhibited detectable IgA antibodies over this longer period (Fig. 3A).

Cross-reactivity of human sera in the DOBV and PUUV IgG ELISAs. Seventy-four and 35 sera identified by c-FRNT to be sampled from DOBV- and PUUV-infected patients, respectively, with confirmed HFRS were compared for cross-reactiv-

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**FIG. 3.** Time courses of the reciprocal titers of hantavirus-specific IgM, IgA, and IgG antibodies in 17 DOBV-infected (A) and 17 PUUV-infected (B) German HFRS patients during the acute and convalescent phases, starting at the time of hospitalization.
ity in our IgG ELISAs using rN proteins of PUUV, DOBV, and HTNV (Fig. 4). As expected, the OD values (at a standard dilution of 1:400) for anti-PUUV- and anti-DOBV-positive sera were clearly different when homologous versus heterologous rN antigens were used (Fig. 4A). To get more detail, 31 out of 35 anti-PUUV-positive sera were endpoint titrated against our rN proteins of DOBV and PUUV; they always demonstrated a significantly higher endpoint titer for the homologous antigen (data not shown). Similarly, the endpoint titer of 20 anti-DOBV-positive sera (out of 74) was determined and always found to be much higher for the rN proteins of DOBV and HTNV than for that of PUUV, including 6 totally lacking reactivity with the PUUV rN protein (data not shown). However, the reactivities of anti-DOBV-positive sera with the rN proteins of DOBV and HTNV could not be differentiated (Fig. 4B).

In an additional study, up to 26 acute-phase/early convalescent-phase and 26 convalescent-phase serum samples from 17 German DOBV-infected HFRS patients were tested (Fig. 5A). All sera were detected in the IgG ELISAs by at least DOBV

![Fig. 4.](https://example.com/fig4.png)

**FIG. 4.** Levels of cross-reactivity (estimated as OD) of 74 anti-DOBV-positive sera (open circles) and 35 anti-PUUV-positive sera (closed circles) from German HFRS patients in IgG ELISAs based on rN proteins of PUUV versus DOBV (A) and HTNV versus DOBV (B). For calculations of \( r^2 \) values as a measure of the goodness-of-fit of linear regression, SPSS 12 software (SPSS Inc., Chicago, IL) was used. \( P \) values were found always to be <0.001.

![Fig. 5.](https://example.com/fig5.png)

**FIG. 5.** Cross-reactivity patterns of acute-phase/early convalescent-phase and convalescent-phase sera of 17 DOBV-infected (A) and 17 PUUV-infected (B) German HFRS patients in respective IgM, IgA, and IgG ELISAs.
and HTNV rN antigens. However, in the IgM and IgA assays, two and one sera, respectively, could be identified only by use of the homologous (DOBV) antigen. From 17 PUUV-infected patients, up to 23 acute-phase/early convalescent-phase and 15 convalescent-phase sera were used for the cross-reactivity study (Fig. 5B). Again, all sera were detected by the IgG ELISAs (mostly cross-reacting with all three antigens), but two acute-phase/early convalescent-phase sera and one convalescent-phase serum became detectable only when the homologous (PUUV) rN antigen was used. In the IgM and IgA assays, the cross-reactivity was even lower; 10 out of 19 sera found to be IgM positive and 6 out of 20 sera found to be IgA positive required the homologous PUUV antigen for detection.

**DISCUSSION**

The diagnosis of a hantavirus infection is mainly based on serological assays. In recent years, IFA, ELISA, and immunoblot tests using native or recombinant antigens have been developed (for reviews, see references 24 and 25). For ELISA, preferentially complete or truncated rN proteins expressed in *E. coli* have been used (10, 11, 13, 34, 49, 55, 56, 57). However, eukaryotic expression systems might prevent unspecific reactivities of human sera due to antibodies raised against *E. coli* (49). Therefore, rN proteins of different viruses have been generated in insect cells using baculovirus- and plasmid-driven expression systems (7, 18, 19, 39, 52). In addition, mammalian cell expression systems and synthetic peptides originated from N proteins have been exploited as tools for hantavirus serodiagnostics (5, 17, 20, 26).

Here, we have established highly specific and sensitive IgG, IgM, and IgA ELISAs using yeast- and insect cell-expressed complete rN proteins of PUUV and DOBV as useful tools for efficient serodiagnosis of hantavirus infections in Europe. Hantavirus N proteins can be expressed at high levels in yeast and can be easily purified. These proteins are highly stable during long-term storage and possess high hantavirus antigenicity (37). Furthermore, problems with ELISA specificity caused by anti-*E. coli* antibodies can be prevented, as recently evidenced by the establishment of highly specific and sensitive ELISAs based on yeast-expressed rN proteins of SNV and ANDV for the detection of SNV- and ANDV-specific antibodies in humans (43, 44).

With a large panel of human anti-hantavirus-negative and -positive reference sera, the specificities and sensitivities of the PUUV and DOBV MAb capture IgG ELISAs described here have been found to be 100%. The high sensitivity and specificity of the IgG ELISAs was confirmed by analysis of a blinded serum panel in an interlaboratory test (4). The cutoff values were defined as 0.11 and 0.10 for the PUUV and DOBV IgG ELISAs, respectively. Since the IgG response in HFRS patients is sometimes delayed, detection of IgM is the method of choice for serodiagnosis of acute infections (for a review, see reference 24). The developed IgM ELISAs based on insect cell-expressed rN proteins of PUUV and DOBV were found to have specificities and sensitivities of 100%. Furthermore, the DOBV and PUUV IgM ELISAs identified correctly all blinded serum samples in a ring test (4).

Like the PUUV and DOBV IgM and IgG ELISAs, our respective IgA ELISAs demonstrated high sensitivity and specificity. Besides hantavirus-specific IgM, IgA antibodies represent an additional marker for the detection of acute hantavirus infections. There was a good correlation (94%) between the IgM and IgA responses during the acute phase. For one patient without detectable IgM, the evidence of acute infection was confirmed by IgA detection accompanied by increasing IgG titers in follow-up. In previous studies, another MAb capture IgA ELISA (specificity 100%) was found to detect 91% of IgM anti-N-positive sera used (8). In agreement with previous observations (8, 12, 15, 34), the N-specific IgA response has been found to be decreased in convalescent- and late-convalescent-phase sera. However, particularly for certain DOBV-infected patients, a persistence of IgM and/or IgA antibodies was observed in four persons (three of them IgM positive and three of them IgA positive) even until the end of the observation period of 2 to 3 years. Taken together, these data suggest that combined exploitation of IgM and IgA ELISAs should be useful for highly sensitive detection of acute hantavirus infection.

N protein-specific antibodies induced by natural infection in human and experimental infections and immunizations in rodents have been found to be highly cross-reactive between different hantaviruses (9, 11, 12, 14, 37). However, we observed that 2 and 1 out of 23 acute-phase sera from DOBV-infected patients reacted exclusively with the IgM and IgA ELISA, respectively, based on the DOBV rN protein but were not detected by use of the related HTNV rN protein. In the case of PUUV-infected patients, an even higher percentage of sera could not be detected by use of the more distantly related DOBV and HNTV antigens. These data are in agreement with previous observations that the homologous antigen is needed for highly sensitive detection of DOBV and SNV infections (13, 43, 50).

For serotyping of hantavirus infections (i.e., specification of the particular hantavirus species involved), FRNT is commonly used. In contrast to ELISA tests using cross-reacting N protein, neutralization assays detect a subpopulation of glycoprotein-specific antibodies. In convalescent-phase sera, the cross-reactivity of neutralizing antibodies is reduced, permitting typing of the hantavirus most likely representing the causative agent of infection (28). A major drawback of FRNT is the necessity of performing it in a biosafety level 3 containment laboratory. Alternatively, assays based on pseudotypes (33) or truncated rN derivatives (1, 30, 31, 54) have been developed for serotyping of hantavirus infections.

As already known, a primary indication of infections with distantly related hantaviruses can be observed when the OD values of the corresponding ELISAs are compared (56; also this study). Discrimination between infections with distantly related hantaviruses might be obtained by endpoint titration of human sera in an ELISA format, as demonstrated by Morii et al. (31). In this and other studies, for DOBV-infected patients (as compared with the PUUV IgG ELISA) a higher OD value was always observed in the DOBV test and the endpoint titration demonstrated an at least 10-fold-higher titer against DOBV antigen. Accordingly, for most PUUV-infected patients a higher ELISA reactivity has been found with PUUV antigen; however, in very rare cases, identical endpoint titers in the PUUV and DOBV IgG ELISAs prevented a differential diagnosis (H. Meisel, unpublished data). It should be mentioned that in very rare cases human infections by Tula han-
Hantaviruses have been observed in central Europe (21, 46, 53); those sera should mostly cross-react with PUUV antigen.

In conclusion, IgA and IgG ELISAs based on yeast-expressed n proteins of PUUV and DOBV have been developed; they demonstrate sensitivities and specificities of 100% each. IgM ELISAs using insect cell-expressed n proteins of PUUV and DOBV permit the detection of hantavirus-specific IgM antibodies with the same sensitivity and specificity. These ELISAs can be used for efficient serodiagnosis of hantavirus infections in central Europe.

ACKNOWLEDGMENTS

We are very grateful to Brita Auste, Heike Lerch, Beate Ziaja, and Inge Krahm for excellent technical assistance. We thank Tatjana Voronkova, Judith Koch, and Matthias Niedrig for support and long-lasting cooperation.

These experiments were supported by grants from the Bundesministerium für Gesundheit (grant 325-4771-02-40), the Deutsche Forschungsgemeinschaft (grant KR1293/2-4), DLK-International Büro des BMBF (grants LTI 00 0001 and LVA 00 0001), and the Charité Medical School.

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