Comparative Evaluation of an Enzyme-Linked Immunosorbent Assay (ELISA) To Detect Antibodies Directed against Glycoprotein I of Pseudorabies Virus and a Conventional ELISA and Neutralization Tests

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To determine whether a newly developed enzyme-linked immunosorbent assay (ELISA), which detects antibodies directed against glycoprotein I (gI) of pseudorabies virus, is suitable for serodiagnosis, it was compared with a conventional ELISA and two neutralization tests. The gI ELISA was 99.2% as sensitive and 100% as specific as the conventional ELISA. Antibody titters measured by the gI ELISA were lower than those measured by the conventional ELISA, comparable to those measured by the 24-h neutralization test, and higher than those measured by the 1-h neutralization test. After experimental infections in pigs, antibodies were first detected by the gI ELISA on postinoculation day (PID) 14, by both neutralization tests on PID 7, and by the ELISA on PID 10. All four tests showed the same rate of decline of maternal antibodies in pigs born to sows that had been vaccinated with inactivated vaccine. Thus, not only is the gI ELISA unique in being able to differentiate infected pigs from those vaccinated with gI-negative vaccines, but it is also as useful as a conventional ELISA and as neutralization tests for serodiagnosis.

Materials and Methods
gI ELISA. In the gI ELISA, antibodies directed against gI of PRV block the reaction of two monoclonal antibodies (MAbs) that are directed against different epitopes on gI. The test detects only those antibodies directed against the two epitopes recognized by the MAbs. The gI ELISA was conducted as described previously (13). Briefly, an ELISA plate was coated with one MAb directed against gI. The second MAb was coupled to horseradish peroxidase and used as conjugate. The conjugate was added to the MAb-coated ELISA plate just before the incubated antigen-serum mixtures were added. The serum had been incubated with antigen in a separate uncoated microtiter plate for 2 h at 37°C. After an incubation period of 1 h at 37°C, the plates were washed and a solution containing azino-bis(ethylbenz-thiazoline-sulfonic acid) and H2O2 was added as a substrate. After 2 h at room temperature, the plates were read spectrophotometrically. The cutoff value was 50% of the mean optical density (OD) value of four negative control serum samples tested on the same ELISA plate (13). A sample with an OD below the cutoff value was considered positive. The titers were read as log10 of the reciprocal of the highest final positive serum dilution. The sera were screened at a final dilution of 1:5, which was also the first dilution tested when sera were titrated. The relative sensitivity and specificity of the gI ELISA were determined as described earlier (9).

Conventional ELISA. A commercially available ELISA (8) was used according to the directions of the manufacturer (Enzygnost; Behringwerke AG, Marburg/Lahn, Federal Republic of Germany). Briefly, serum dilutions were incubated in an ELISA plate coated with antigen and control antigen for 1 h at 37°C. After the plates were washed, the conjugate was added to each well and the plates were incubated for 1 h at 37°C and washed again. Substrate solution was added to each well, and plates were incubated for 45 min at room temperature, after which the enzyme reaction was stopped and the results were read spectrophotometrically. The titer of a serum was read as the reciprocal of the highest dilution that gave an OD difference of >0.2 between antigen and control antigen. The initial dilution of a serum sample was 1:44.

Neutralization tests. Neutralization tests were performed in microtiter plates. Sera were diluted in duplicate in twofold steps and mixed with equal volumes (0.05 ml) of a virus suspension containing 100 to 200% tissue culture infective doses of the NIA-3 strain of PRV. The series of dilutions started with undiluted serum samples. Serum and virus were incubated for 1 or 24 h at 37°C (2). Thereafter, suspensions of secondary pig kidney cells were added to the microtiter
plates. After a 3-day incubation period at 37°C, cell monolayers were stained with amido black and the antibody titers were read. The neutralizing antibody (NA) titer (NA-1 and NA-24 titers are titers for serum-virus incubation periods of 1 and 24 h, respectively) was expressed as the reciprocal of the final serum dilution inhibiting the cytopathic effect in 50% of the cell cultures.

Test samples. Field sera were collected from 4- to 6-month-old pigs born to sows that had been repeatedly vaccinated with an inactivated PRV vaccine (Nobivac; Intervet, Boxmeer, The Netherlands). The piglets themselves were not vaccinated. The pigs were to be exported and therefore were quarantined. They were bled just before the 7-week quarantine period and again 5 weeks later. These sera were initially examined with the conventional ELISA. The sera used in this study were from two separate cases in which pigs had subclinical PRV infections during the quarantine period.

The sera used for studying the kinetics of the antibody response after infection were from four pigs of the specific-pathogen-free herd of the Central Veterinary Institute. The pigs had been inoculated intranasally at the age of 10 weeks with 10^6 PFU of the mildly virulent Sterksel strain of PRV (10). The sera used for studying the decline of maternal antibodies were from four pigs of the same herd; these had been born to four sows repeatedly vaccinated with an inactivated PRV vaccine (Nobivac; Intervet). Serum and colostrum samples were collected from the sows on postpartum day (PPD) 1. Colostral whey was collected after centrifugation at 40,000 × g for 30 min and then was assayed for antibody. Blood samples were collected from the piglets at 2-week intervals beginning with PPD 1. At 18 weeks after birth, the piglets were intranasally inoculated with 10^6 PFU of the virulent NIA-3 strain of PRV, as described previously (4).

RESULTS

Comparison of field sera. A total of 273 samples were tested. Of these, 150 serum samples had OD values above the cutoff value, which was 0.895, and were thus negative for antibodies to gI of PRV (Fig. 1). Of these 150 serum samples, 149 were negative both by the ELISA and by the NA-1 test and 132 were negative by the NA-24 test. The 18 positive serum samples had NA-24 titers of ≥0.6. These low NA-24 titers were probably caused by maternal antibodies, because pigs 4 to 6 months of age still can have low levels of maternal NAs (see Fig. 4). Of the 149 serum samples that were negative by the ELISA, 147 had ODs by the gI ELISA well above the cutoff value while 2 serum samples had ODs of 1.105 and 1.182. One serum was negative by the gI ELISA but positive by the ELISA; its OD by the gI ELISA was 0.988, which is just above the cutoff value, and its NA-24 titer was 1.8.

The remaining 123 serum samples were positive by the gI ELISA as well as by the ELISA, and their antibody titers
were ≥1.5 by the NA-24 test. The sera that were positive by the gI ELISA had ODs well below the cutoff value. The four serum samples with OD values between 0.6 and 0.8 (Fig. 1) all had OD values below 0.7. Thus, the gI ELISA was 99.2% as sensitive and 100% as specific as the ELISA.

The set of 273 serum samples comprised paired sera of 125 pigs. The ELISA and the NA tests detected seroconversion in 124 of 125 pigs, whereas the gI ELISA detected seroconversion in 123 pigs. The serum sample that had an OD value of 0.988 (mentioned above) was the second one of the pig in which no seroconversion was detected with the gI ELISA.

To gain more insight into the sensitivity of the gI ELISA, 40 of the 123 serum samples positive by the gI ELISA were randomly selected to determine the antibody titers by the various tests. Antibody titers by the gI ELISA were usually 10 to 20 times lower than those measured by the conventional ELISA; they were comparable to the NA-24 titers and higher than the NA-1 titers (Fig. 2).

Comparison of antibody development in experimentally infected pigs. The development of the antibody responses after intranasal inoculation with the mildly virulent Sterksel strain of PRV were compared (Fig. 3). The gI ELISA first detected antibody in sera on postinoculation day (PID) 14, the ELISA first detected antibody on PID 10, and both NA tests first detected antibody on PID 7. Antibody titers by all four tests markedly increased until PID 21 and after that remained relatively stable. After PID 21, antibody titers measured by the gI ELISA and the NA-24 test were comparable. The ELISA measured the highest antibody titers, and the NA-1 test measured the lowest titers.

Comparison of the decline of maternally derived antibodies. The decline of maternally derived antibodies against PRV was compared (Fig. 4). By all four tests, the antibody titers for colostrum collected on PPD 1 were higher than those for the sera of the sows of PPD 1. Sera collected from piglets on PPD 1 had higher titers than sera of sows. Antibody titers measured by the gI ELISA were comparable to those measured by the NA-1 test and lower than those measured by the ELISA or the NA-24 test. The decrease of maternal antibodies was more or less parallel by all four tests. Antibodies were detected by the gI ELISA until 3 to 4 months after parturition. At the time of inoculation (18 weeks after birth), all four pigs were gI seronegative, two were still seropositive by the ELISA, and all were seropositive by the NA tests. After inoculation with the NIA-3 strain of PRV, antibody titers markedly increased in all tests. The

FIG. 3. Antibody development in pigs (n = 4) inoculated with a mildly virulent strain of PRV, measured by gI ELISA (●), conventional whole-virus ELISA (○), NA-24 test (□), and NA-1 test (■).

FIG. 4. Antibody development in pigs (n = 4) born to sows (n = 4) repeatedly vaccinated with an inactivated PRV vaccine. Piglets were inoculated with a virulent strain of PRV at 18 weeks after birth. Titers were measured by gI ELISA (●), conventional whole-virus ELISA (○), NA-24 test (□), and NA-1 test (■). C, Mean antibody titer in colostral whey; S, mean antibody titer in sera of sows.
gl ELISA measured higher titers than the NA-1 test at 2 and 3 weeks after inoculation.

**DISCUSSION**

The study of the field sera demonstrated that the sensitivity and specificity of the gl ELISA were comparable to those of the ELISA and the NA tests. All sera that were negative by the ELISA were also negative by the gl ELISA, and the gl ELISA detected antibodies in all but one of the serum samples that were positive by the ELISA. This sample, the second of a pair, had an OD value of 0.988 by the gl ELISA (close to the cutoff value) and was positive by both NA tests. This serum sample was probably collected between 1 and 2 weeks after infection, when antibodies can be detected by NA tests and the ELISA but not by the gl ELISA (Fig. 3), because this pig seroconverted as determined by the other three tests and was among 65 quarantined pigs, 64 of which seroconverted as determined by all four tests.

Eighteen of the first serum samples that were negative by both the gl ELISA and the ELISA were low positive (≤0.6) by the NA-24 test and negative by the NA-1 test. The low NA titers were probably caused by maternal antibodies, because pigs 4 to 6 months of age can still have low levels of maternal antibodies, which can be detected by NA tests but not by the gl ELISA (Fig. 4) or the conventional ELISA; in the latter test, the initial serum dilution is 1:44. Another, less likely explanation is that the low antibody titers resulted from an infection that occurred approximately 1 week before samples were collected. Around 1 week after infection, antibodies to PRV can be detected by NA tests but not by ELISAs (Fig. 3). It is also possible that the results of the NA-24 test were false-positive.

In both the naturally occurring and experimentally induced infection, antibody titers measured by the gl ELISA were lower than those measured by the ELISA; they were comparable to those measured by the NA-24 test but higher than those measured by the NA-1 test. In contrast, the titers of maternal antibody in the piglets born to vaccinated sows were lower by the gl ELISA than by the NA-24 test but were comparable to titers measured by the NA-1 test. In this case, the gl ELISA titers are lower because vaccination, particularly with inactivated vaccines, does not induce antibody titers against gl as high as those induced by infection with wild-type PRV (12).

The patterns of the various antibody responses in the experimentally infected pigs and in the pigs with maternal antibodies were similar. The antibodies appeared, however, at different times after infection. The gl ELISA did not detect antibodies until PID 14, 1 week after NAs were detected. Even the NA-1 test, which measured antibody titers that were much lower than those obtained with the gl ELISA, detected antibodies 1 week earlier. Thus, antibodies directed against gl may be produced later than others, or antibodies induced early during infection may have a low affinity for the antigens and consequently may not be able to compete with MABS used in the gl ELISA (3, 16). Whereas NAs were detected on PID 7, antibodies were not detected by the ELISA until PID 10. These results do not agree with those of previous studies (1, 6, 8), which found that antibodies were detected by the ELISA at the same time as the NA tests or even earlier. The most likely explanation for this difference is that unlike the other investigators, we considered a serum sample positive by the neutralization test if the undiluted serum inhibited cytopathic effect in one of two cultures.

The results also confirmed an earlier report that the gl ELISA clearly discriminates between infected and uninfected pigs (Fig. 1) (14). Because OD values of all serum samples but one were well below or above the cutoff value, results can be regarded as conclusive, an important feature when the test is used in control programs.

For serodiagnosis of Aujeszky’s disease, the gl ELISA appeared to be as sensitive as the ELISA and the NA tests, as evidenced by the results of paired serum samples. However, when the gl ELISA is used for diagnosis, serum samples should be collected at slightly longer intervals than the 2- to 3-week interval that is usual in serological diagnosis, because antibodies directed against gl cannot be detected as early as other antibodies. Because all field strains of PRV appear to express gl (11), the gl ELISA can be used for the routine diagnosis of Aujeszky’s disease.

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**REFERENCES**

DETECTION OF ANTIBODIES AGAINST PRV gi


