Preliminary Report on an Agar-Gel Immunodiffusion Test for Pseudorabies

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Pseudorabies is a disease which may affect North Dakota hog production if infected animals are introduced to the state. The test for Pseudorabies now in use is difficult and expensive. An agar-gel immunodiffusion (AGID) antigen for Pseudorabies testing is described. Results obtained from AGID proved to be at least as reliable as the currently approved test while reducing testing time and difficulty.

Introduction

Increasing concern over the possibility of pseudorabies infection in North Dakota has spurred the State Veterinarian to require pseudorabies testing of all hogs entering the state. While pseudorabies does not currently appear to be a problem in the state, pork producers would like to insure that new animals introduced into their herds are free of the pseudorabies virus.

The current approved test for pseudorabies is a serum neutralization procedure that measures serum antibody produced in response to previous exposure. Unfortunately, the test procedure is complicated and requires the specialized skills and materials found only in diagnostic virology laboratories.

This report describes a new approach to pseudorabies testing which, when approved by the proper regulatory agencies, should lower the cost of testing while increasing the accuracy of reports. The test utilizes an agar-gel immunodiffusion (AGID) technique to detect pseudorabies antibody. The AGID test is based on the ability of specific antibody to precipitate its antigen in an agar medium. A well in the agar plate is filled with concentrated antigen (pseudorabies viral protein) and another well is filled with porcine (hog) serum. If the serum contains antibody to pseudorabies virus, a visible precipitation will occur between the virus and antibody as they migrate through the agar. The reliability of the AGID test depends to a large extent upon the properties of the antigen (Crowle, 1973). This report describes an antigen preparation that was found to be suitable for gel diffusion and makes a comparison to the serum neutralization test now in use.

Agar-Gel Immunodiffusion Antigen:

The AGID antigen was prepared with the Shope strain of pseudorabies virus obtained from the National Animal Disease Laboratory, Ames, Iowa. Virus was grown in a continuous cell line of porcine kidney (PK-15). A concentration of $8x10^9$ TCID/2 mls of pseudorabies virus was used to innoculate a roller bottle of stationary phase PK-15 cells (Tokumaru 1965, Watson, et al. 1966, 1967). Cells were harvested 18 hours after innoculation by vigorous shaking. The medium was removed by centrifugation at 1000 x g for 10 minutes. The cell pellet was resuspended in 8 mls of distilled water and frozen and thawed once to break up cellular material while releasing virus and viral proteins from the cells. The suspension was heated at 56° C for 90 minutes to inactivate infective virus. Cellular debris was removed from the antigen preparation by centrifugation at 3000 x g for 20 minutes. Antigen was stored at 5°C with 0.02 per cent sodium azide added to prevent bacterial growth.

Agar plates were prepared as described by LeJeune et al. (1977), modified to use 1 per cent agar. A template of 6 peripheral wells (serum) and 1 center well (antigen) was used. All wells were 3 mm apart and 6 mm in diameter. Alternating peripheral wells were filled with pseudorabies positive control serum.

Antibody:

Porcine serum with a high titer of pseudorabies antibody was obtained by repeated innoculations (4) of a baby pig with inactivated pseudorabies virus in Freunds Adjuvant, followed by an intranasal innoculation with 2 mls live pseudorabies virus containing 10^6 TCID/.025 ml. The resulting serum had a 1:128 serum neutralization titer for pseudorabies virus and was used as a positive control serum in the AGID test.

Serums to be tested for pseudorabies antibody were diagnostic cases submitted to the North Dakota State University Veterinary Diagnostic Laboratory for pseudorabies testing and samples obtained from the Nebraska Veterinary Diagnostic Laboratory, Lincoln, Nebraska.

Serum Neutralization:

A standard micro-titration serum neutralization test using 100-1000 TCID/.025 mls pseudorabies virus was employed (Johnson & Hill, 1976) for comparison with the AGID test. All serums were screened for pseudorabies antibody at a final dilution of 1:4, as recommended by the National Animal

[1]These samples were obtained from the Nebraska Veterinary Diagnostic Laboratory because very few Pseudorabies positive samples were available from North Dakota herds.

Disease Laboratory, Ames, Iowa. Positive serums were then titered by micro-titration. Serums that were found to be slightly inhibitory at 1:4 were repeated using undiluted serum. If no additional viral inhibition occurred, they were scored as negative.

Results

The ability to detect pseudorabies antibody in 21 porcine serums[1] by serum neutralization and AGID was compared (Table 1). All serums that had a 1:4 or greater serum neutralization titer for pseudorabies were also found to be positive using the AGID technique. Of the six serums that had a less than 1:4 titer by serum neutralization, five were found to be negative using AGID and one was found to be positive for Pseudorabies antibody.

A total of 272 porcine serums submitted by veterinarians to the North Dakota State Diagnostic Laboratory were examined for pseudorabies antibody between January 1, 1977 and July 15, 1977 (Table 2). These samples were examined by serum neutralization for certification purposes and by AGID to evaluate the reliability of the new test. The great majority (259) were negative by both methods, but pseudorabies antibody was detected in five serums by both methods. Six serums could not be successfully reported by serum neutralization due to toxicity from serum contamination or degradation. Of these six serums, five were negative and one was found to contain pseudorabies antibody using the AGID technique. Some viral inhibition was seen in two serums run by serum neutralization, but when these serums were rerun undiluted, they were judged to be negative. Both these serums were negative by AGID.

Discussion

The agar-gel immunodiffusion technique was found to be at least as sensitive as the serum neutralization test for detection of antibody to Pseudorabies virus. It appears (Table 1) that the AGID test may be able to detect low levels of antibody sometimes missed by serum neutralization. Another problem sometimes encountered with serum neutralization is cell toxicity. Serum must be handled carefully to prevent toxicity. This is sometimes difficult for the field veterinarian. The six toxic serums shown in Table 2 could not be run by serum neutralization. Since gel diffusion does not require cell culture, toxicity does not disrupt the procedure. The animal that was found to have pseudorabies antibody by AGID could not be tested using the conventional serum neutralization test, and thus would not have been identified as a potential carrier of the pseudorabies virus.

Finally, serum neutralization is a very time consuming technique, requiring 6 to 8 technician hours to test a herd of 50 hogs. In contrast, one technician can set up 50 AGID samples in approximately one hour. Both tests require 48 hours incubation time and about the same skill and time is needed for interpretation and recording. When an AGID antigen for pseudorabies becomes commercially available, hog producers should be able to receive more accurate results at a lower cost.

Table 1. Comparison of Serum Neutralization and Agar-Gel Immunodiffusion for the Detection of Pseudorabies Antibody, Nebraska Samples.

Serum Neutralization		Agar-gel Immunodiffusion	
Negative[1]	Positive[2]	Negative[3]	Positive[4]
6	15	5	16

[1]No viral inhibition at a final serum dilution of 1:4
[2]Viral inhibition at a final serum dilution of 1:4 or greater
[3]No precipitation between serum and viral antigen
[4]Visible precipitation between serum and viral antigen

Table 2. A Comparison of North Dakota Diagnostic Cases Examined for Pseudorabies Antibody by Serum Neutralization and Agar-Gel Immunodiffusion

Sample Condition	No. of Samples	Serum Neutralization		Agar-Gel Immunodiffusion	
		Negative	Positive	Negative	Positive
Uncontaminated samples	264	259	5	259	5
Toxic samples	6	NA[1]	NA[1]	5	1
Questionable samples	2	2[2]	0[2]	2	0

[1]Results could not be obtained by serum neutralization because of toxicity.
[2]Results obtained after samples were rerun using undiluted serum.

References

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