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Evaluation of an ELISA for Detection of Antibodies to Sendai Virus in Swine Sera

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Abstract: An indirect enzyme-linked immunosorbent assay (ELISA) was developed for the detection of IgG serum antibodies to swine Sendai virus. To evaluate the preliminary potential of ELISA, sera from 10 healthy pigs and 34 sera from finishing pigs suffering from respiratory signs were tested. The results were compared with those obtained from the same sera by hemagglutination inhibition assay.

Key Words: Sendai virus, ELISA, IgY, swine sera

Introduction

Sendai virus (SV) is a member of the family Paramyxoviridae, genus Paramyxovirus. It causes respiratory tract disease in rodents (1). Respiratory diseases are of significant pathological importance in swine, in particular in piglets. Contact between rodents and pigs is common in swine-breeding farms, and SV participates in the etiology and pathogenesis of respiratory diseases in swine, resulting in enhanced clinical manifestations (2,3). It is therefore important to use the most sensitive test available in the diagnosis of SV infection. Currently, one of the most useful tests is hemagglutination inhibition (HI) reaction. However, pigs are contaminated with pneumotropic viruses that are in some respects very similar to the paramyxoviruses, especially in their hemagglutinating activity. Swine sera also contain nonspecific agglutinins and inhibitors that interfere with serological reactions. In this respect, enzyme-linked immunosorbent assay (ELISA) offers certain advantages over HI assay in the diagnosis of infection. The quality of any diagnostic test depends on the quality of its components, largely on antigen purity, the kind of antibodies and preparation of serum samples. The use of bird immunoglobulins, which are phylogenetically distant from those of mammalians, offers distinct superiority. The aim of this study was to develop an indirect ELISA using purified SV as an antigen and conjugated egg yolk immunoglobulins to detect porcine SV antibodies and then comparing the results with HI assay.

Materials and Methods

SV strain Fushimi was obtained from the Institute of Virology (Moscow, Russia). The seed stock was grown in the allantoic cavities of 10-day-old embryonated hen eggs from a 10^6 dilution of seed virus, and the allantoic fluid was harvested after incubation at 36 ºC for 72 h. The virus was then partially purified from these fluids by 2 methods. The first one involved differential sedimentation at 4000 x g for 15 min and 44,000 x g at 4 ^{O}C for 2 h on a 40% sucrose cushion. The virus pellet was suspended in PBS (0.015 M phosphate-buffered saline, pH 7.4), sonicated for 1 min in ice and titrated in hemagglutination (HA) assay with chicken erythrocytes. The second method (4) was virus adsorption-elution on chicken red blood cell ghosts prepared from chicken erythrocytes to avoid hemolysis of fresh erythrocytes. Blood was washed with PBS 3 times by centrifuging at 1000 x g for 10 min. Erythrocyte sediment was diluted with PBS at a 1:8 (vol/vol) ratio and 38% formaldehyde was added at a 1:4.5 (vol/vol) ratio while being gently stirred at room temperature for 3 h. The suspension was left at 4 ºC overnight and after the last washing at 700 x g for 2 min; the ghosts were suspended in PBS containing 0.5% sodium azide and stored at 4 ºC for 2 months. To

use, a portion was washed in PBS. One volume of prepared chicken red blood cell ghosts was added to 4 volumes of allantoic fluids containing 8000 HAU/ml. The resulting elution fluid was subjected to HA assay. SV antigens prepared by the 2 methods were compared for their suitability for ELISA. The control antigen was prepared in a similar manner from normal allantoic fluids by centrifugation at 5000 x g at 4 °C for 30 min and then used for background elimination in ELISA.

To avoid interference with HI reaction, sera were pretreated by heating, chicken erythrocytes and kaolin. The sera were inactivated over 30 min at 56 °C, incubated with chicken erythrocytes, followed by centrifugation at 1000 x g for 10 min; then 25% kaolin (Fluka) in PBS was added, and sera diluted to 1/10 was obtained after centrifugation at 1000 x g for 10 min.

HA and HI assays were performed in 96-well plates (V-shaped bottom, Nunc A/S, Roskilde, Denmark). The HA assay was used to identify and quantify the virus. Chicken erythrocytes (50 µl of 0.75% [v/v]) were added to test samples diluted in PBS. The reaction was read after 40 min incubation at 4 °C, the titers being the reciprocal of the highest virus dilution causing total hemagglutination. SV antibody titers were measured in HI assay by the method described previously (5).

Yolk antibodies (IgY) from hens' egg yolk anti-swine IgG extracted with poly(ethylene glycol) (6) were conjugated in a ratio of 2:1 (w/w) with horseradish peroxidase (Sigma) (5 mg/ml) by the method of periodate oxidation (7). The ELISA was carried out on Nunc-Immuno™ Maxi Sorp™ (Lot. No. 442,404) 96-well polystyrene plates in a reaction volume of 100 µl. The plates were coated by adding SV antigen (6 HAU/well) diluted in the coating buffer (0.05 M carbonate-bicarbonate buffer, pH 9.0) and incubating at 37 °C for 2 h. The plates were washed 4 times with PBS containing 0.05% Tween 20 (PBS-T) between each of the following incubations (at 37 °C for 1 h) with antigen, blocking solution, sera and conjugate. The coated plates were blocked with 2% bovine serum albumine (BSA) in the coating buffer. Sera for testing were diluted with PBS-T to a final dilution of 1/50. The conjugate was diluted to 1/2000 with PBS-T. The peroxidase conjugate was visualized with 0.5 mg/ml o-phenylenediamine (Sigma) in 0.5 M citrate-phosphate buffer pH 5.0 with 0.003% H_2O_2. The enzyme-substrate reaction was stopped after 15 min by adding 50 µl of 2.0 M H_2SO_4. The absorbance was measured spectrophotometrically at 492 nm using a Titertek Multiskan microtiter plate reader (Flow Laboratories, UK).

Ten sera of healthy pigs and 34 sera on 4 farms in different regions of Lithuania from finishing pigs suffering from respiratory symptoms, in addition to pooled known negative sera of pigs with no serological response (10 replicates) and pooled sera with positive response (12 replicates), were used as negative and positive controls on each plate and were obtained from the Lithuanian National Veterinary Laboratory. The optical density (OD) of the antigen reaction minus the OD of the reaction of the control antigen prepared from normal allantoic fluid (the mean OD of 20 control antigen reactions was 0.080 ± 0.015) was considered the ELISA value. Antibodies to the porcine reproductive and respiratory syndrome virus were tested by ELISA commercial test IDEX S.A., Cergy, Pontoise Cedex, France.

Results

Ten sera of healthy pigs and 34 sera on 4 farms in different regions of Lithuania from finishing pigs suffering from respiratory symptoms were tested in HI and ELISA assays for the detection of SV-specific antibodies. The sera testing positive in HI assay had titers ranging from 1:8 to 1:256. The cut-off determined for ELISA was higher than (0.20 ± 0.05) for positive sera, and sera that reacted lower than the positive sera (0.20 ± 0.05) were considered as negative.

To avoid interference with HI reaction 34 sera were pretreated by heating, chicken erythrocytes and kaolin. As the result, the titers were reduced about 2-fold and 4 positive sera with low titers (1:8) showed negative results after pretreatment (Table). The same unpretreated and pretreated 34 sera were tested by ELISA for the detection of SV-specific IgG serum antibodies. The differences in ELISA between pretreated and unpretreated sera were negligible. ELISA reduced the number of positive sera to 14, but a serum from farm No. 2 that was negative in HI reaction was weakly positive (0.24 ± 0.02) in ELISA. Titration of this sample showed it to be positive. Serum from farm No. 4 was positive in both HI tests, but negative in ELISA. This serum tested for antibodies to porcine reproductive and respiratory syndrome virus by the ELISA commercial test (5) was strongly positive.

Table. Detection of antibodies to SV by HI assay and ELISA in swine sera in various Lithuanian regions.

Farm	No. of sera tested	HI		HI*		ELISA	
		Positive	Negative	Positive	Negative	Positive	Negative
1	14	9	5	9	5	8	6
2	11	4	7	2	9	3	8
3	7	6	1	4	3	3	4
4	2	2	-	2	-	-	2
Total	34	21	13	17	17	14	20
%	100	61.8	38.2	50	50	41.2	58.8

*- HI reaction with sera pretreated by kaolin

Discussion

SV antigens prepared by 2 methods were compared for their suitability for ELISA. There was not noticeable difference in antigen-antibody reaction, except that the effect of sorption on microtiter plates was 2 times higher for antigens prepared by the adsorption-elution method than for that prepared by differential sedimentation. It is possible that the virus adsorption-elution method is milder than centrifugation at high speed and excludes sonication, which may influence antigen sorption capacity.

Swine sera contain nonspecific agglutinins and inhibitors that interfere with serological reactions. Pretreatment with kaolin in addition to heating and incubation with chicken erythrocytes prevents nonspecific reactions due to adsorbtion of nonspecific agglutinins and inhibitors to kaolin. The titers of antibodies in HI reaction with kaolin-pretreated sera were reduced and prevented false positive results. ELISA does not require such pretreatment.

The use of polyclonal immunoglobulins from immunized hens for preparing conjugates has advantages over the use of mammalian immunoglobulins in that IgY neither binds to the Fc-receptors of the mammalian complement (8) nor does it interfere with rheumatoid factors (9). Additionally, the production and purification of IgY are more economical and simpler than with mammalian immunoglobulins.

The developed ELISA for the detection of SV-specific serum antibodies by preliminary study may be more specific than HI assay. ELISA also offers certain advantages over HI assay in respect of standardization and day-to-day variables, such as erythrocyte sensitivity fluctuations and antigen titer variations.

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