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Quantitative indirect ELISA for pseudorabies antibody detection

Ratree Wongwatcharadumrong

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Abstract

A quantitative indirect ELISA was developed for use to evaluate pseudorabies (PR) virus infection status of swine herds, herd response to vaccination, and for screening individual pigs for PR virus infection. A total of 568 sera, collected from 15 herds distributed throughout central Thailand, were used in the study. Results indicate that ELISA titers of 48 and 76 units or greater represent virus neutralization titers greater than 4 and 16. Values of these magnitudes are suggestive of effective immune response to vaccination and active infection in vaccinated herds respectively. The sensitivity and percent agreement of the quantitative ELISA was 96.7 and 88.4% respectively. The small difference in agreement between the two tests was due primarily to VN negative sera that reacted positively in the ELISA. The high level of sensitivity of the quantitative ELISA also makes it suitable for use as a screening assay.

Key words : Pseudorabies, quantitative indirect ELISA, virus neutralization test.

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* Virology Unit, Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand.

Introduction

Pseudorabies (PR) has been epidemic in Thailand since 1977 (Sunyasootcharee *et al.*, 1978). The disease is routinely controlled by the use of a wide variety of commercially available vaccines to prevent losses due to clinical disease. The effectiveness of individual vaccination programs is evaluated by measuring post vaccination antibody titers. The conventional virus neutralization (VN) test has been routinely used for this purpose. However this assay is time-consuming and expensive to perform. The indrect ELISA (Durham *et al.*, 1985 ; Afshar *et al.*, 1987 ; Wongwatcharadumrong and Moreno-Lopez, 1990) has been shown to be more sensitive and more economical to perform than the VN test for screening purposes. However the screening ELISA is not quantitative and cannot be used to evaluate the response of herds to vaccination nor to evaluate herd response to wild type virus infection. The current study describes the modification of the screening ELISA to permit the expression of antibody titers in terms of a quantitative ELISA unit.

Materials and methods

Sera

Standard positive control (SPC) serum was prepared by pooling 20 sera collected from known PR virus infected pigs with VN titers of at least 128. Standard negative control (SNC) serum was prepared by pooling sera collected from 56 known negative weanling pigs. Test sera were collected from 568 pigs distributed among 15 herds in central Thailand. Standard sera were aliquoted and all sera were stored at -20 °C until used.

Cells and virus

The virus used in the present study was the locally isolated CB-1 strain as described in a previous report (Wongwatcharadumrong and Moreno-Lopez, 1990). The PK-15 cell line was used to propagate the virus in serum-free Eagle's minimum essential medium (EMEM).

Virus neutralization test

The VN test was conducted as described in a previous report (Wongwatcharadumrong and Moreno-lopez, 1990).

Buffers and reagents

ELISA dilution buffer (EDB), pH 7.4, consisted of 0.15M NaCl, 0.001M EDTA, 0.05M Tris and 0.05% Tween 20 and contained 0.05% bovine serum albumin (BSA). Conjugate* was affinity purified goat anti-swine IgG conjugated to horseradish peroxidase. Substrate* was tetramethylbenzidene (TMB) and hydrogen peroxide. Washing solution was 0.0001M Na_2HPO_4 with 0.01%Tween 20.

Preparation of antigens for indirect ELISA

The viral and control antigens were prepared by extracting proteins from virus infected and virus-free PK-15 cells respectively with TE buffer (0.05M Tris, 0.025M EDTA pH 7.4) containing 0.5% Triton X-100**. The details of the procedure was described in a previous report (Wongwatcharadumrong and Moreno-Lopez, 1990).

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* Kirkegaard and Perry Laboratory (KPL), My, U.S.A.

** Sigma Chemicals.

Determination of a standard ELISA reference curve

The SPC serum was serially diluted two-fold in EDB beginning at 1:50 and terminating at 1:256,000. The highest dilution of SPC serum that produced an optical density (OD) greater than that which was produced by a 1:50 dilution of SNC serum was given an arbitrary value of 2 ELISA units. Each consecutive lower dilution of the SPC serum was given a relative value two-fold greater than the preceeding dilution so that the lowest dilution of the SPC serum represented 128 ELISA units or greater. The curvilinear relationship between OD and ELISA units was found to be best represented (R^2 = 0.98) by the equation :

$$y = B_0 * e^{B_1 * X}$$

where y = ELISA units, B_0 = X intercept, B_1 = slope and X = OD.

Each of these values was determined for individual plates using the above formula which permitted the calculation of ELISA units for individual test sera.

Test procedure

Test plates were prepared by adding 50 ul of optimum dilutions of test and control antigens to duplicate alternate rows of 96 well flat bottom polystyrene plates.* This format facilitated the determination of net OD values. The plates were then allowed to sit overnight at 4 °C to permit antigen adsorption. All antigen plates were washed 3 times and stored at 4 °C until used but never for longer than one week.

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* Libro, Flow laboratories.

The ELISA was conducted similar to that which was described previously (Wongwatcharadumrong and Moreno-Lopez, 1990). Essentially, each plate contained 16 test sera which were diluted 1:50 in EDB. Fifty ul of each diluted serum was added in duplicate to test and control antigen wells. Duplicate 50 ul volumes of EDB, 1:50 diluted SNC serum and 6 appropriate dilutions of SPC serum, which were chosen to cover the range from 2 to 512 ELISA units, were also added to the plate. The plates were then incubated at room temperature for 60 minutes and washed as before. The affinity purified goat anti-swine IgG* diluted to 1:3000 in EDB was added to all wells and the plates were reincubated at room temperature for 30 minutes and washed as before. Fifty ul of substrate was added to each well. The reaction was stopped after 15 minutes by adding 25 ul of 1 M H_2SO_4. The optical densities were determined at 450 nm using a Titertek ELISA plate reader. Net OD's were determined by subtracting the mean OD control antigen from the mean OD viral antigen.

The minimum positive threshold OD value was established by testing 30 sera collected from known PR virus free pigs. The mean ELISA reaction was 1.53 units and the upper 95% confidence limit was 2.03 units. Consequently, a positive threshold value of 5.0 units was selected. This value was never exceeded when field sera, which were VN and latex agglutination test negative, were tested by the ELISA.

Characterization of the relationship between ELISA units and VN titers

Test sera from 15 individual herds were separately grouped according to their VN titers. The mean ELISA titer in units and the 95% confidence limits were calculated for each serum group in each herd.

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* KPL

Results

A comparison of the antibody titers determined by the VN test and the ELISA is summarized in Table 1. There is an apparent direct correlation between the two assays. The mean ELISA titers and their corresponding upper 95% confidence limits increase with the VN titers. The mean coefficient of variation for all sample means between herds was 22.1% and ranged from 14 to 29%.

Table 1. Comparison of serum antibody titers by the virus neutralization test and the indirect ELISA

VN titer (Log 2)	ELISA titer in units + SE	Upper 95% confidence limit	Number of Samplemeans
neg	11.7 + 2.7	14.4	14
1	17.2 + 4.8	22.0	10
2	40.1 + 7.1	47.2	10
3	48.3 + 12.0	60.3	12
4	68.6 + 10.4	79.0	10
5	106.4 + 31.1	137.5	11
6	170.4 + 31.2	201.6	12
7	148.6 + 35.4	184.0	10
8	141.1 + 22.5	163.6	6
9	192.9 + 53.2	246.1	7

The mean relative sensitivity and percent agreement of the quantitative ELISA with respect to the VN test when titers of 2 and greater were considered positive were 96.7 and 88.4 respectively.

Discussion

Earlier this laboratory developed and indirect ELISA which utilized antigen derived from a local virus strain. The test was proved to be very effective for screening individual pigs within herds and was more economical and provided results sooner than the VN test (Wongwatcharadumrong and Moreno-Lopez, 1990). However, the screening ELISA is unable to provide quantitative results. Consequently it cannot be used to evaluate the effectiveness of vaccination programs or the status of PR virus infections in swine herds.

The quantitative test developed in the current study is similar to that which was described by Spencer and Burgess (1984) and Bock *et al.*, (1986) and provides the capability to quantitate PR virus specific antibody levels. This ability is demonstrated in Table 1. The mean ELISA titer of sera progressively increases as the VN titer increases. Past experience in this laboratory with the VN test indicates that vaccinated herds with mean VN titers of 16 and greater are infected with virus (Wongwatcharadumrong, 1992), while titers of 4 and greater are indicative of a good vaccination response (Platt, 1984). Consequently mean herd ELISA titers of 48 and 76 units or greater (see Table1) can be assummed, with a relatively high degree of confidence, to represent an effective herd immune response to vaccination and active PR virus infection in vaccinated herds respectively.

The quantitative test described in this study, however, cannot be used to predict equivalent VN titers of individual sera because the degree of variation is too large. For example the coefficient of variation of mean ELISA values between herds for sera grouped by VN titers was 22.1% and ranged from 14 to 29%. In contrast the coefficient of variation of mean ELISA values within herds for sera grouped by VN titers was 58% and ranged from 22 to 88%.

The sensitivity of the quantitative test described above was lower than that which was previously reported for the PR screening test used in this laboratory (Wongwatcharadumrong and Moreno-Lopez, 1990). Consequently additional refinement and evaluation of the quantitative ELISA needs to be done before it can be routinely used as a screening assay.

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การวัดระดับภูมิคุ้มกันต่อโรคพิษสุนัขบ้าเทียมโดยวิธีอีไลซ่า

ราตรี วงษ์วัชรดำรง *

บทคัดย่อ

การวัดระดับภูมิคุ้มกันต่อโรคพิษสุนัขบ้าเทียมโดยวิธี ELISA แบบ indirect แสดงค่าเป็น ELISA unit โดยคำนวณเปรียบเทียบกับซีรั่มมาตรฐาน เมื่อทดสอบซีรั่มจำนวน 568 ตัวอย่าง จากฟาร์มสุกร 15 แห่ง ในเขตภาคกลางของประเทศไทยพบว่า ผลการศึกษาสามารถนำไปใช้ในการประเมินสภาพการติดเชื้อโรคพิษสุนัขบ้าเทียมในฟาร์มตลอดจนลักษณะการตอบสนองทางอิมมูนต่อการฉีดวัคซีน รวมทั้งการตรวจคัดสุกรเป็นรายตัว เมื่อเปรียบเทียบระดับ ELISA unit กับระดับภูมิคุ้มกันที่ได้จาก virus neutralization (VN) test พบว่าระดับภูมิคุ้มกัน ELISA ที่มีค่าเท่ากับหรือมากกว่า 48 และ 78 units จะแสดงถึงระดับ ภูมิคุ้มกันจาก VN test เท่ากับหรือมากกว่า 4 และ 16 ซึ่งเป็นระดับที่บ่งชี้ถึงการตอบสนองที่ดีต่อการฉีดวัคซีนและการติดเชื้อไวรัสตามธรรมชาติตามลำดับ เมื่อศึกษาความไวของวิธี ELISA และความสอดคล้องกับ VN test พบว่ามีค่าเท่ากับ 96.7 และ 88.4% ตามลำดับ ความไม่สอดคล้องส่วนใหญ่เกิดจากการที่วิธี ELISA สามารถตรวจพบภูมิคุ้มกันในกลุ่มซีรั่มที่ให้ผลลบโดย VN test ซึ่งแสดงถึงความไวของวิธี ELISA ที่เหมาะสมในการใช้ตรวจคัดสุกรที่มีการตอบสนองทางภูมิคุ้มกันเป็นรายตัว

คำสำคัญ : โรคพิษสุนัขบ้าเทียม, ควอนทิเททิฟ อินไดเร็ก อีไลซ่า, ไวรัส นิวทรัลไลเซชั่น เทสต์

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* หน่วยไวรัสวิทยา ภาควิชาพยาธิวิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย