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Short communication

Cross Reactivities of Rabbit Anti-Chicken Horse Radish Peroxidase Conjugate with Sera of Some other Avian Species in ELISA System

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ABSTRACT
The cross reactivities of rabbit anti chicken horse radish peroxidase (conjugate) was tested with sera of Chicken, Ducks, Geese, Guinea fowl, Hawks, Pigeons and Turkeys in indirect enzyme linked immunosorbent assay (ELISA) technique. Sera from mammalian species (Bat, Equine and swine) were used as negative controls. The conjugate was coated on wells of ELISA micro titer plate and sera from the avian and mammalian species added. Reactivity was detected with OPD. There were losses of reactivities when sera were diluted as from 1\78125. Chicken and Turkey sera reacted with the conjugate without loss of reactivities when either normal rabbit serum (NRS) or bovine serum albumin (BSA) was used as blocking agent. Sera of other avian species and mammals did not react with the conjugate. It is concluded that rabbit anti chicken Horse radish peroxidase could be used to detect antibodies in chickens as well as Turkey and that BSA and NRS could be used as blocking agent without loss of reactivities.
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INTRODUCTION

The enzyme linked immunosorbent assay (ELISA) is one of immunological techniques that employ labeled reagents in testing scheme for amplification of an immunological reaction. The development of ELISA was first reported in 1972 (Engvall and Perlmann 1972). In avian medicine, ELISA has been used for detection of antibodies to various diseases in chicken sera (Marquardt *et al* 1980; Snyder *et al* 1983; Biggs and Skeels 1984; Adeniran and Oyejide 1995; Owoade *et al,* 2006). Although there have been many configurations of ELISA that were developed for chicken, the indirect ELISA which uses chicken antiglobulin labeled enzyme for detection of chicken globulin is the most commonly used. Chicken antiglobulin labeled enzyme conjugate is commercially available for use in diagnosis and detection of antibodies to various chicken diseases. It is also a component of most commercial chicken ELISA kits. There have been instances when antibodies to various disease agents need to be detected from sera of other common avian species such as Ducks, Guinea fowl, Pigeons Turkeys and wild birds. In addition, other less common avian species could require use of indirect ELISA for disease surveillance. Non-chicken enzyme conjugate are presently rare to come by, or not available commercially. This study investigate the cross reactivities of rabbit anti-chicken horse radish peroxidase (sigma) with sera of some other avian species. The aim is to determine its suitability for the detection of antibodies in other avian species in an ELISA system.

MATERIALS AND METHODS

Sera samples

Blood samples (from which sera were obtained) from each avian species(Chicken, Ducks, Guinea fowls, Pigeons, Turkeys) and mammal (Equine) were obtained by jugular vein puncture while blood samples from Cattle egrets, Goose, Hawks and Bats (Pteropus poliocephalus) were obtained by decapitations. The blood was allowed to clot and the resultant sera collected after centrifugation. Sera samples were collected from 6 chicken (as positive control), 5 swine, 5 equine and 24 bats as negative control. Thirty eight Ducks, 9 Geese, 64 Guinea fowl, 2 Hawks, 69 Pigeons and 10 Turkey sera that were available were tested.

In order to test for reactivity of each of the sera with the rabbit component of the conjugate, each serum was blocked with Bovine serum albumin (BSA) and normal rabbit serum (NRS) separately.

Optimization of reagents.

Phosphate buffered solution containing 0.05% Tween 20 was used as dilution and washing buffers. The optimal conjugate dilution has been determined in a previous experiment as 1/2000 (Adeniran and Oyejide 1995). The optimal chicken serum dilution was determined to be approximately (1/100) by reacting 1/2000 dilution of conjugate with its serial dilutions (Figure 1).

For each of the test procedures wells of ELISA plate were coated with 100ul of diluted serum. Coated wells were blocked with either 100ul PBS-Tw20-1%BSA or PBS-Tw20-1%NRS for 1hr at room temperature and washed with washing buffer (0.05%Tw20 in PBS) three times with 5 minutes soaking. One hundred micro liter of diluted conjugate was added, incubated at room temperature (29°C) for 1hr and then washed three times with washing buffer. Thereafter 100ul of OPD (sigma fast) was added and incubated for 15minutes at room temperature. Finally the reaction was stopped with 100ul of stop solution (1M $H_2S0_{4)}$. Optical density (OD) reading was obtained at 492 using Sunrise® (touchsceen model) ELISA plate reader.

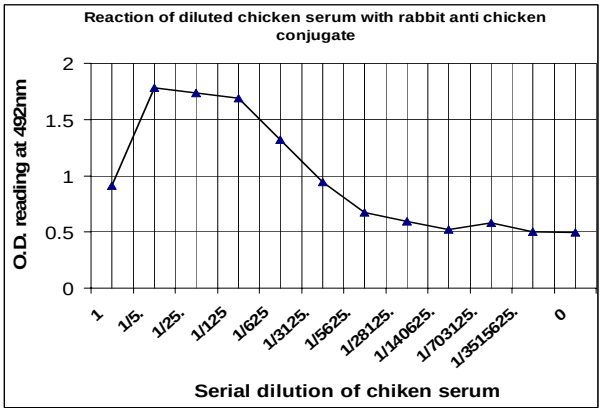

Figure 1:
Determination of Optimum dilution of chicken serum.

RESULTS

The mean of OD reading for each animal and avian species were calculated from raw O.D. for each of the blocking medium (BSA and NRS) and presented in Table 1 and Figure 2. The mean optical density reading for the chicken sera (positive) control are 2.6258 and 2.2180 for BSA and NRS blocking agent respectively. The mean O.D. for the negative controls are 0.0963 and 0.0998(bats), 0.0735 and 0.0900(equine), 0.1898 and 0.2265(swine) for BSA and NRS as blocking agents respectively. Optical density readings for avian species are 0.1755 and 0.1943 (cattle egret), 2.6258 and 2.218(chicken), 0.5543 and 0.5248(ducks), 0.4958 and 0.6150(G. fowl), 0.5615 and 0.5998(geese), 0.4045 and 0.4495(hawks), 0.3910 and 0.4653(pigeons), 2.1478 and 2.1535(turkeys) for BSA and NRS as blocking agents respectively (Table 1). Serial dilutions of serum of each species were also made and reacted with the conjugate, the result is presented in Figure 3.

Table 1:
Comparison of BSA and NRS

Species	BSA	NRS	Mean
Hawk	0.4045	0.4495	**0.427**
Goose	0.5615	0.5998	**0.581**
Duck	0.5543	0.5248	**0.54**
G.fowl	0.4958	0.615	**0.555**
Pigeon	0.391	0.4653	**0.428**
Equine	0.0735	0.09	**0.082**
Bat	0.0963	0.0998	**0.098**
C.egret	0.1755	0.1943	**0.185**
Swine	0.1898	0.2265	**0.208**
Turkey	2.1478	2.1535	**2.151**
Chicken	2.6258	2.218	**2.422**
Coating buffer	0	0.101	**0.051**

sera. Mean O.D. value of 2.151 for turkey is clearly higher than the negative control sera (mean O.D. are 0.082 for equine, Bat 0.098, and swine 0.208) and close to O.D. value for Chicken (2.422) this showed cross reactivity with anti-chicken conjugate. This implies that the rabbit anti-chicken conjugate is suitable for the detection of antibodies in turkey sera in ELISA system.

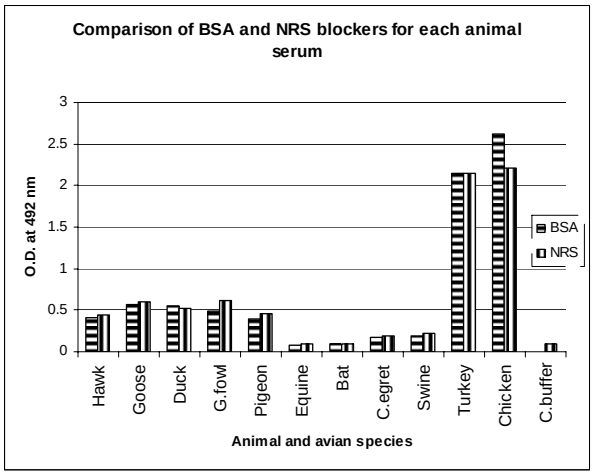

Figure 2:
Comparison of BSA and NRS

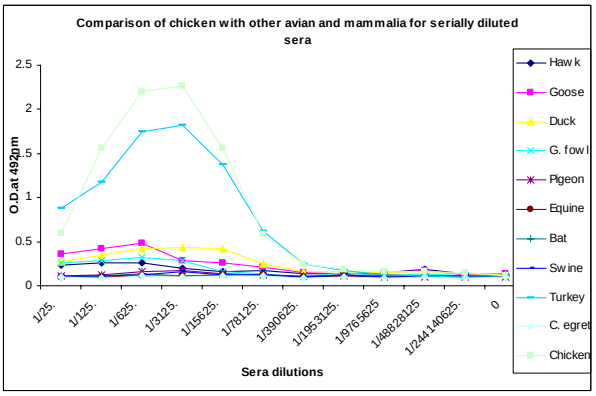

Figure 3:
O.D. readings of serially diluted sera.

DISCUSSION

The mean O.D. 2.422 for chicken as positive control clearly differentiate from the mean O.D. 0.082 for equine, Bat 0.098, and swine 0.208, the negative controls. This confirms absence of cross reactivity between anti-chicken conjugate and mammalian

The O.D. value for cattle egret (0.185), duck (0.540), goose (0.581), hawk (0.427) and pigeon(0.428) are close to the negative controls O.D. for equine(0.082), Bat (0.098), and swine (0.208) but far less than O.D. for chicken sera (2.422). This shows that the sera of these avian species do not cross react with the rabbit anti-

chicken conjugate and therefore not suitable for detection of antibodies in their sera. However, the lack of cross reactivities between chicken serum and Duck, Goose, Hawk, and Pigeon makes the serum of each of these avian species suitable in an antigen capture ELISA system for antigen detection among other uses in chicken immunology.

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