Earlier detection of infectious laryngotracheitis virus by development of Dot-ELISA in commercial layer farms

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Abstract

A rapid and sensitive dot-ELISA was developed for the rapid detection of antibodies against infectious laryngotracheitis (ILTV) virusin a single serum sample. Sera samples from 213 layer chicken were screened for antibodies to field ILTV and a total of 196 serum samples were found positive for ILTV. The sensitivity, specificity and accuracy of the test were calculated. The dot-ELISA test was found to be highly sensitive, specific with a K value of 0.99 and hence could be very useful for serological diagnosis ofILTV from isolates and could be used as a penside test in the field itself.

Key words: Diagnosis, Dot-Enzyme linked immune assay ELISA,Infectious laryngotracheitis virus, Penside test.

INTRODUCTION

Infectious laryngotracheitis (ILT) is a non-neoplastic, clinical, acute, highly contagious and severe upper respiratory tract infection of chickens, resulting in huge economic losses to the poultry industry (Ou and Giambrone, 2012).ILT is a significant respiratory disease of chickens in many countries and causes reduced egg production and predisposes to other respiratory pathogens (Guy and Garcia, 2008).In the recent past, Namakkal, the second largest egg bowl of the nation, experienced the outbreak of Infectious laryngotracheitis due to intensified poultry production especially in layers.Higher death rates were noticed due to severe outbreak of this infectious diseases questioning the profit of poultry farmers. As the conventional methods of diagnosis of ILT are time consuming, rapid methods are the priority and need of the hour. Hence, less expensive on spot Dot-ELISA was developed for early deduction of ILTV from field out breaks in layers.

MATERIALS AND METHODS

Test serum samples

A total of 213 serum samples were collected from the layer flocks located around NamakkalDistrict of Tamil Nadu state, India, and these serum samples were inactivated at 56°C for 30 minutes and stored at -80°Cuntil further screening for Dot-ELISA. Clinically affected layer birds of 34 farms were visited and the characteristic signs of ILT were observed and recorded.

Dot-ELISA for virus identification

The Infectious laryngotracheitis virus (ILTV) from 34 field samples suspected for ILT were detected as per the method described by Subramaniam *et al* (2010) with minor modifications.Rabbit hyper immune serum were previously prepared and used for detection of ILT antigens in Dot-ELISA Test. Optimum concentrations of serum samples and conjugate were arrived by preliminary checker board titration. The reference ILTV at 1µl volume was spotted onto nitrocellulose membrance (NCM) and incubated at 37° C for I h in a humid chamber. One hour after incubation, the NCM was washed three times with wash buffer (PBST) and incubated for 60 min in blocking solution (7.5% skim milk powder). The membranes were again washed three times with PBST and then incubated at 37 °C for 45 m with positive

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serum (1:500, 1:1000 and 1:2000) in serum dilution buffer. The NCM was incubated with serum and washed three times in PBST and incubated with anti-rabbit igG HRPO conjugatye (Sigma, USA) diluted 1:5000 in conjugate diluents. The NCM were exposed to the substrate (5mg of diaminbobenzidine (DAB) (Sigma, USA) in 10 ml of distilled water and 10µl of 2% hydrogen peroxide) for 10 min in dark. The enzymatic reaction was stopped by washing the NCM in tap water.

Statistical analysis

The sensitivity, specificity and accuracy of dot ELISA test as described by Venkatesh(2006) was calculated

Sensitivity: a / (a+c)

Specificity: d / (b+d)

Accuracy: a+d / (a+b+c+d)

The results obtained from the tests were analyzed for the percentage of agreement withthe use of Kappa statistics. The kappa statistics is a decimal measure of agreement between two tests, especially inthe absence of a standard and is defined as kappa or K.K = (a+d - P) / 1- P, where P= (a+b) (a+c) + (c+d) (b+d) andP is the probability, a: is the number of samples positive by both *i.e.*, test to be compared and gold standard test, b: is the number of samples positive by standard test whereas negative by test to be compared, c: is the number of samples negative by standard test and positive by test to be compared and d: is the number of samples negative by both.

Guidelines for interpretation of K values are as follows:

K value < 0.1 indicates poor agreement

K value between 0.1 and 0.2 indicates slight agreement

K value between 0.21 and 0.4 indicates fair agreement

K value between 0.41 and 0.6 indicates moderate agreement

K value between 0.61 and 0.8 indicates substantial agreement

K value> 0.81 indicates perfect agreement

RESULTS

The characteristic clinical signs such as nasal discharge, swollen and closed eyelids (sticky) and conjunctivitis,severe ocular discharge with crust formation and narrowing of eye commissures (almond shape eye).In the development of Dot-ELISA, the optimized conjugate dilution of ILTV from field samples were found to be 1:5000. The optimized conjugate dilution of the identification of isolate was 1:5000. The ideal incubation time for the substrate was found to be 10 min. The brown colour was well developed in positive control and no colour development in negative control membranes. Using the checker board titration, the optimum dilution of serum for detection of ILTV from field samples were found to be 1:500 which is in accordance with the findings of Botus*et. al.*(2010).

The kappa (K) value for ILTV isolates was 0.99 which indicated that they were in perfect agreement as per Subramaniyam*et.al.*(2010).

Okhubo *et. al.* (1988) reported that the ELISA systems have been developed for detection and quantitation of ILTV specific antibodies using purified field strain of virus as whole antigen and the same has been reproduced and developed as a penside diagnostic test for detection of ILTV field strains.

The conventional serological tests are time consuming and cumbersome and a penside test is highly suitable for this purpose. Hence, the dot-ELISA was developed for screening ILTV.

Using the checker board titration, the optimum dilutions of serum was standardized at 1:5000 and optimized anti-rabbit Ig G-HRPO conjugate dilution was 1:5000 for detection of ILTV from field samples. Similarity Lu (2003) developed and standardized dot-ELISA to detect avian influenza directly from clinical and field samples.

In comparison to plate ELISA, the developed dot-ELISA can be performed without the sophisticated equipment like ELISA reader and the dot-ELISA can be performed at farm level with minimum reagents. The farm personnel can perform the test as per the protocol and read the results without much effort. This field based diagnostic kit will be highly useful as a penside diagnostic test for identification of this dreadful ILT for farmers and veterinarians.

SUMMARY

Dot-ELISA was standardized for screening ILTV isolates.The test was standardized by optimizing serum dilution as 1: 500 and the optimized conjugate dilution of the identification of isolate was found to be 1:5000. The development of brown colour was confirmed as positive. This can be effectively used for screening this economically important disease in the field.

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