SEROPREVALENCE OF NEWCASTLE DISEASE INFECTION IN POULTRY BY ENZYME LINKED IMMUNOSORBENT ASSAY AND AGAR GEL IMMUNODIFFUSION IN RANCHI (JHARKHAND) INDIA

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Received: 22.08.2016
Accepted: 12.11.2016

ABSTRACT

The objective of this study was to study ND seroprevalence from suspected cases using enzyme linked immunosorbent assay (ELISA) and agar gel immunodiffusion test (AGID) in poultry in and around Ranchi and also to evaluate AGID with ELISA. In the present study, ELISA and AGID based ND seroprevalence study of 92 suspected sera samples in the year 2014-15 had revealed 63.04% and 51.09%, respectively. A total of 92 suspected poultry samples of central and western plateau agro-climatic zone in and around Ranchi district, Jharkhand during 2014-2015 were tested for the estimation of diagnostic sensitivity (Dsn) and diagnostic specificity (Dsp). The Dsn and Dsp were found to be 51.72% and 50.00%, respectively.

Key words: Newcastle disease, AGID, seroprevalence, ELISA, Dsn, Dsp

Introduction

Newcastle Disease (ND) is an acute infectious viral disease of domestic poultry which belongs to order Mononegavirales, family Paramyxoviridae and subfamily Paramyxovirinae (de Leeuw and Peeters, 1999). The most susceptible avian species are chickens with respiratory signs, nervous system impairment, gastrointestinal and reproductive problems (Nanthakumar *et al*., 2000).

For ND diagnosis, virus neutralization (VN) (Rubin and Franklin, 1957), single-phase complex plaque technique (Khadzhiev, 1984), haemmagglutination (HA) and haemmagglutination inhibition (HI) test (Haque *et al*., 2010), ELISA (Madsen *et al*., 2013), duplex-RT-PCR (Shirvan and Mardani, 2014) are commonly used frequently.

The present work has been planned to monitor ND seroprevalence using ELISA and AGID in poultry sera in Ranchi, Jharkhand and to get a comparative assessment of efficacy of the test.

Materials and Methods

The blood samples were collected from 92 chickens aged 2-6 wk from suspected cases in and around Ranchi between January 2014 and April 2015.

(A) ELISA: ELISA was carried out by Newcastle disease virus antibody test kit, (Affinitech Ltd.). Test was considered negative or positive if produced NDV ELISA unit was less than 15 or more than 15, respectively.

(B) AGID: This technique was based on the ability of antibodies to form precipitation lines specifically with the antigen. Free diffusion of both the antigen and antibody takes place in agarose gel resulting in precipitation lines, which were visible to the naked eye. The procedure was followed as per Ouchterlony (1948) with some modification. Sensitivity and specificity of AGID were calculated, considering ELISA as reference test as per Lalkhen and McCluskey (2008). Statistical analysis between ELISA and AGID was done as per Snedecor and Cohran (2004).

Results and Discussion

ND seroprevalence was reported on the basis of ELISA and AGID as 63.04% and 51.09%, respectively (Table 1, Fig. 1 and Fig. 2). However, it was also reported by several workers from India and abroad even in the apparently healthy birds (Mai *et al.*, 2014 and Geetha, 2014). In the present survey, ND seroprevalence has been reported on the basis of ELISA as 63.04% while the prevalence rate of disease was also reported through ELISA by Musako and Abolnik (2012) and by Sharma *et al*. (2015) as 73.3% in Nigeria and 66.3% in West Indies, respectively. Seroprevalence by AGID was recorded as 51.09% in the present work which was in confirmation with 52.94% by Roy and Venugopalan (1997), 64.10% by Mazengia *et al*. (2010) in Ethiopia and 41.8% by Egbal *et al*. (2012).

The relative sensitivity and specificity of AGID compared to ELISA were calculated as 51.72% and 50.00%. Taking ELISA as a Gold Standard test, the sensitivity and specificity of the AGID assay were found to be low. Detail of true positive, false negative, true negative and false positive are depicted in Table 2. On the basis of Chi-square (X^2) test, ELISA and AGID were found non significant (Table 3).

Acknowledgements

The authors are thankful to the Vice Chancellor and Dean, Ranchi Veterinary College, B.A.U., Kanke, Ranchi, Jharkhand for providing necessary facilities to carry out the proposed work.

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Table 1: Overall place-wise seroprevalence of ND infection in poultry using ELISA and AGID

S.No.	Place	SAMPLES	ELISA (%)	AGID (%)
1.	RVC, Kanke	10	8(80)	6(60)
2.	Hochar	5	2(40)	4(80)
3.	Husir	7	4(57.14)	4(57.14)
4.	Pataratoli	5	4(80)	4(80)
5.	Chirondi	5	2(40)	2(40)
6.	Simartoli	4	3(75)	3(75)
7.	Boria	7	4(57.14)	3(42.86)
8.	Sangrampur	5	4(80)	2(40)
9.	Arsanday	7	3(42.86)	3(42.86)
10.	Milatcolony	4	2(50)	0(0)
11.	Chuditola	4	2(50)	3(75)
12.	Bhitha	2	1(50)	0(0)
13.	Mahuatoli	3	2(66.67)	1(33.33)
14.	Chandave	5	4(80)	2(40)
15.	Kumharia	7	5(71.43)	3(42.86)
16.	Bariatu	4	3(75)	2(50)
17.	Kokar	3	2(66.67)	2(66.67)
18.	Chutia	5	3(60)	3(60)
Total		92	58 (63.04)	47(51.09)

Table 2: Relative performance of AGID to ELISA for N**D**

Test	**ELISA (reference test)**			
	Results	**Positive**	**Negative**	**Total results**
AGID	Positive	30 (TP)	17 (FP)	47(AGID +ve sample)
	Negative	28 (FN)	17 (TN)	45 (AGID -ve sample)
	Total results	58 **(ELISA +ve sample)**	34 **(ELISA -ve sample)**	**92 (Total sample)**

Table 3: Statistics for ELISA and AGID for ND

Result/Technique	**ELISA**	**AGID**	**TOTAL**	X^2
POSITIVE	58	47	105	
NEGATIVE	34	45	79	2.68^{NS}
TOTAL	92	92	184	

Fig.1: ELISA conducted on poultry sera sample for Newcastle disease

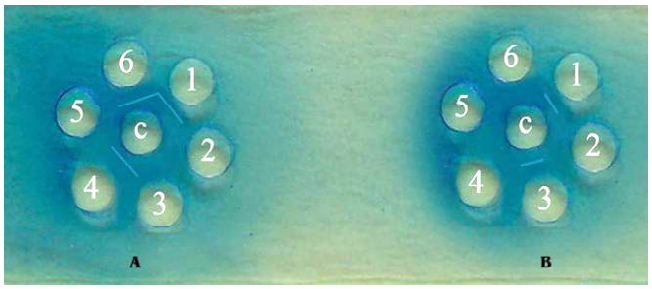

Fig.2: AGID stained slide for Newcastle disease (Coomassie brilliant blue)

A.C. F1 Newcastle disease antigen
1. Positive control
2. Negative control
3. Test sample 1 (-ve)
4. Test sample 2 (+ve)
5. Test sample 3 (-ve)
6. Test sample 4 (+ve)

B.C.- Lasota Newcastle disease antigen
1. Positive control
2. Negative control
3. Test sample 1 (-ve)
4. Test sample 2 (+ve)
5. Test sample 3 (-ve)
6. Test sample 4 (+ve)

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