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COMPARISON BETWEEN SOLID PHASE ELISA, DOT ELISA AND AGAR GEL PRECIPITATION TEST FOR DETECTION OF BURSAL DISEASE VIRAL ANTIGEN IN BURSAL HOMOGENATES

BY

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SUMMARY

Forty-four bursal samples were collected from naturally infected and normal broiler chicks. The samples were homogenized and the homogenates were tested by agar gel precipitation test (AGPT), solid-phase ELISA (by indirect double antibodies sandwitch and single antibody ELISA systems) and Dot ELISA for detection of infectious bursal disease virus (IBDv) antigen. The reacted positive samples showed 86.3,88.6 and 88.6 percentage in AGPT, solid-phase ELISA and Dot ELISA, respectively.

The findings suggest that the Dot ELISA is faster, more economic and easily applicable procedure than the other two techniques. The indirect double antibody sandwitch technique is more sensitive than the indirect single antibody EIISA system.

INTRODUCTION

Infectious bursal disease was first described by Cosgrove in 1962 (Cosgrove, 1962). The disease is widespread in chickens and is of great economic importance for both broilers and pullet growers (Hitchner, 1978). The disease has been shown to affect the chickens, primarily the bursa of Fabricius, BF (Cheville, 1967). The immunodepression effect induced by IBDV increases the incidence of diseases caused by other opportunistic pathogens and prevents young chickens from responding optimally to routinely used vaccines (Sharna, et al.,1 989). Therefore rapid and accurate diagnosis of IBD is considered very important tool for controlling and subsequently minimizing the economic losses caused by this disease..

In fact, diagnosis of any viral disease could be achieved by one or more of the following:- the isolation and identification of the virus, the detection of specific antigens and the demonstration of the development of specific antibodies (Buxton and Fraser, 1977). Since few years, the detection of IBDV or its antigen in bursal homogenates has been needing up to 5-6 days by AGPT and up to 14 days for virus isolation and identification (Fenner, et al., 1987). This long time required for diagnosis of IBD seems to be enough for spreading the infection to other flocks.

Solid-phase ELISA was considered as a rapid and accurate diagnostic technique for detecting IBDV-antigen (Snyder, et al., 1988a; Snyder, et al., 988b and Kumar and Rao, 1991). Dot-ELISA was used for detecting some viral antigens, other than IBDV, by many investigators (Benette and Yeoman, 1983; Hawekes, et al.; 1982).

The present study aimed to select the most sensitive more acurate and easily applicable diagnostic technique for rapid diagnosis of IBD comparing the results obtained by AGPt, solid-phase ELISA (indirect and double antibody techniques) and Dot-ELISA with each other.

MATERIAL AND METHODS

*** Vaccine:-**

Inactivated vaccine as water-oil emulsion produced by TAD pharmazeutisches Werk GMBh, D-2190 Cuxhaven 1, Germany. (batch No. 0904) was used for perparation of rabbit anti-IBDV hyperimmune serum.

*** Bursae :-**

Thirty-nine bursal samples were collected from chicks naturally infected with IBDV in Beni-Suef Governorate. Another five busal samples were taken from two-week old, nonvaccinated apparently healthy hubbard chicks and used as noninfected controls.

*** Sera :-**

1- Pre-immune normal rabbit serum was obtained from two apparently healthy two-month old rabbits and used as negative control serm.

2- Rabbit hyperimmune serum was prepared according to Hermann,et al., (1988). The serum was obtained by injecting rabbits, two-month old, with 2 ml of inactivated IBDV vaccine for each one as an initial dose. Three booster doses, 1 ml each, of the vaccine were given to each rabbit at 14, 21 and 28 days after the initial dose. After two weeks of the last inoculation the rabbits were bled and the serum was separated, inactiated at 5°C for 30 min, and then aliqouted in small sterile vials a kept of - 20 °C .

3- Chicken anti-IBD serum was obtained fr immune birds, previously immunized wi inactivated oil adjuuent IBDV vaccine.

4- Goat anti-rabbit and anti-chick peroxidase-labelled globulins were purchas from sigma chemical company, USA.

* Nitrocellulose membrane was obtained fro Sartorius company West Germany.

*** Preparation of bursal homogenates:-**

Each bursal sample was homogenized with a equal volume (W/V) of phosphate buffe saline. The homogenates were subjected t freezing and thawing for three successiv times, and then clarified by low-spee centrifugation (5000 r.p.m.) for 30 minutes The suppernatnat fluid was decanted in steril vials.

*** Agar gel precipitation test:**

It was carried out after Hirai and Shimakura (1972) with some modification in the diamete of cut wells. The diameter of the well was 8 mm instead of 30 mm and the distance between the central and peripheral wells was 2.5mm.

* Solid phase ELISA was applied according to Yolken (1980). The assay was applied by two systems, the first one called indirect-single antibody ELISA for assaying antigen. In this system, the bursal homogenate was coated on the ELISA plate overnight then rabbit anti-IBD hyperimmune serun was added, and the plate was incubated then washed and goat anti-rabbit O-globulin peroxidase conjugate was added.

* The second system is called " indirect double-antibody sandwitch ELISA technique" for assaying antigen. In this system, the EllSA plate was coated with rabbit anti-IBD hyperimmune serum diluted 1:1000 overnight (as capture antibodies) then bursal homogenate, diluted 1;10 was added and the plate was incubated and washed. Then, chicken anti-IBD serum was added followed by goat-antichicken O-globulin peroxidase-labelled was added.

* Dot-ELISA was applied according to Hawekes et al., (1982). In this test intracellulose membrane was used instead of ELISA plote. Bursal homogenates were dotted on the membrane (3-5u for each sampe). Then the membrane was tested as ELISA plote for developing the reaction.

RESULTS

Table (1) shows clearly that, the AGPT produced 38 positive samples out of 44 tested with a percentage of 86.3% whereas both of Dot-ELISA and solid-phase ELISA produced 39 positive samples out of 44 tested with a percentage positive 88.6%.

Table (2) shows the sensitivity between two systems used in solid-phase ELISA. The obtained results in this table shows clearly that, the indirect single antibody ELISA for assaying antigen is less sensitive than double antibody sandwitch ELISA for the same purpose exprssed by lower optical densities than the second technique. Samples which showed negative results in Dot-ELISA produced also negative result in both single and double indirect ELISA.

DISCUSSION

The obtained results in Table (1) showed clearly that , the AGPT produced 38 positive samples out of tested samples with a percentage 86.35 whereas the solid-phase ELISA and Dot-ELISA produced 39 positive samples with a percentage 88.6% for both techniques. This result indicates clearly that the AGPT is less sensitive than the solid-phase ELISA and Dot ELISA. For enhancing the development of precipitation lines between tested antigen and hyperimmune serum, cutted wells of

Table (1): Detection of infectious bursal disease virus antigen in bursal homogenates

No. of tested samples	AGPT			Dot- ELISA			Single Ab ELISA			Double Ab ELISA		
	Neg.	Post.	% post.	Neg.	Post.	% post.	Neg.	Post.	% post	Neg.	Post.	% post.
44	6	38	86.3	5	39	88.6	5	39	88.6	5	39	88.6

Table (2): The difference between indirect single and double antibody ELISA for assaying IBDV-antigen .

	Optical densities				
Sample No.	Indirect single antibody ELISA	Indirect double antibody ELISA	Sample No.	Indirect single antibody ELISA	Indirect double antibody ELISA
1	0.688	0.929	23	0.616	0.909
2	0.596	0.922	24	0.637	0.864
3	0.567	0.946	25	0.600	0.947
4	0.576	0.875	26	0.594	0.951
5	0.627	0.933	27	0.667	0.928
6	0.574	0.948	28	0.624	0.843
7	0.583	0.953	29	0.637	0.949
8	0.603	0.873	30	0.639	0.942
9	0.688	0.929	31	0.667	0.876
10	0.624	0.926	32	0.639	0.756
11	0.632	0.937	33	0.598	0.935
12	0.568	0.894	34	0.602	0.932
13	0.608	0.930	35	0.658	0.749
14	0.609	0.939	36	0.230	0.179 *
15	0.601	0.937	37	0.195	0.152 *
16	0.601	0.906	38	0.659	0.932
17	0.850	0.955	39	0.650	0.931 *
18	0.840	0.941	40	0.157	0.141
19	0.690	0.941	41	0.730	0.880
20	0.669	0.905	42	0.141	0.112 *
21	0.672	0.908	43	0.211	0.187 *
22	0.666	0.927	44	0.650	0.860

* Samples collected from apparently normal birds .

8mm diameter were used unstead of wells of 3 mm in diameter . On comparing of AGPT with solid-phase ELISA and Dot ELISA, we found that, the AGPT needed 6-12 hours after its modification, instead of 48-72 hours (Hirai and Shimakura, 1972; Ide, 1975 and Ulbrich and Zureck, 1977) whereas the other techniques needed only 3 hours for obtaining accurate result. Besides, the positivity of the AGPT can only appear when the serum and antigen were used undiluted and in large quantities (30-50u) in each well but in case of solid-phase ELISA the antigen was used after it had been diluted 1:10 and in Dot-ELISA the antigen was used undiluted in few amount not exceeding 3-5ul. The hyperimmune serum was used with a dilution of 1:100 in solid-phase ELISA and 1:20 in case of Dot ELISA.

This study clearly suggested a potential use of Dot ELISA for rapid and definitive diagnosis of IBD thereby eliminating the need for conventional virus isolation and identification. The Dot-ELISA is readily applicable in most diagnostic laboratories. It does not require special equipments and necessary reagents. Furthermore, this Dot ELISA does not need ELISA reader as in case of solid-phase ELISA and the blue dots developed in the NCM can be seen easily with the naked eye.

Table (2) shows clearly that, the indirect double antibody sanwitch ELISA is more sensitive than the indirect single antibody ELISA system as indicated by higher optical densities. The mean optical densities obtained in positive samples (39) by double antibody techniques was 0.91 whereas in single antibody technique was 0.615 with a difference 0.296.This indicates clearly that, the indirect double antibody sandwitch ELISA is more sensitive than the single antibody ELISA by 0.296 . The obtained result is in agreement with those obtained by Voller et al. (1978) and Yolken, (1980).

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