CONJUGATION OF IMMUNOGLOBULINS PREPARED AGAINST INFECTIOUS BURSAL DISEASE VIRUS IN DIFFERENT HOSTS WITH FLUORESCINE ISOTHIOCYANATE

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Abstract

Three different immunoglobulins were prepared against infectious bursal disease virus (IBDV) in different hosts (chicken, ducks and rabbits) and conjugated with fluorescine isothiocyanate (FITC).The efficacy of these preparations was evaluated through the scoring of IBDV concentration in different sites of infected specific pathogen free (SPF) embryonated chicken eggs with different strains of the virus (Bursa-Vac., D-78 and 228E) using the direct fluorescent antibody technique (FAT) in a comparison with the virus titration test. It was found that the prepared conjugates were able to detect the IBDV in the different sites of infected eggs when they were diluted up to 1: 10^5 giving strong; moderate or weak positive FAT according to the virus concentration in the tested site. There were no differences in the experimental results among the different used viral strains, so, the prepared IBDV immunoglobulins conjugated with FITC provide local products that facilitate and enhance a rapid and accurate diagnosis of IBD the thing which is required to achieve a successful control.

INTRODUCTION

Infectious bursal disease (IBD) is an acute highly contagious viral disease of young chicken caused by a virus of high resistance to disinfectants. So, once a poultry house became infected, the disease remains endemic in such house and new flocks placed in this place will be exposed to virus infection in early stage. IBD virus destroys the lymphocytes of the main organs of the immune system of chicken (Bursa of Fabricius), the thymus, spleen and caecal tonsils resulting in immunosuppression (McFeran *et al.*, 1980).

As it is known, poultry industry is the most highly developed segment of the world food production, and accordingly, rapid and accurate diagnosis of poultry disease is a main goal for veterinarian and poultry producers. One of the most specific, sensitive, accurate and rapid diagnostic methods is the fluorescent antibody technique (FAT) which is considered an ideal diagnostic tool for diagnosis of viral diseases depending on the detection of viral antigen at the sites of lesions (Pegenortel and Neurath, 1985). FAT is also widely used for detection of specific viral antigens in pathologic viral materials and infected cell cultures (Osman *et al.*, 1994); in embryonated eggs (Clark *et al.*, 1972) and tracheal cultures (Bhattacharfee *et al.*, 1994).

Among the detection and identification of IBDV in different affected organs, the direct FAT was proven to be an adjunct technique (McFeran, *et al.*, 1980).

The present work was designed to prepare specific immunoglobulins against IBDV conjugated with FITC in different hosts to be used as local preparations for diagnosis of IBDV instead of the imported reagents which are usually of high cost and not available on request.

MATERIALS AND METHODS

1-Infectious bursal diseae virus (IBDV) strains

Bursa Vac, D-78 and 228-E strains of IBDV were kindly supplied by U.S. Vet. Sanofi, Animal Health Inco Over Land Park, 1' 1's 66210.

These strains were propagated in SPF embryonated chicken eggs in order to detect their incidence in the different sites of infected eggs using the FAT in a comparison with virus titration test.

2-Cell culture adapted D-78 strain of IBDV

A VERO cell culture adapted D-78 strain (Afaf *et al.*,2000) was used for serum neutralization test.

3-Embryonated chicken eggs (ECE)

Specific pathogen free (SPF) 9-days ECE obtained from Nilo SPF eggs, Koom Oshiem, Fayoum, Egypt, were used for propagation of the different IBDV strains.

4-Propagation of IBDV in ECE

Each of the three mentioned strains of IBDV was propagated in 9-days old SPF-ECE according to Cessi and Nordelli (1970). The dead embryos with their fluids

were collected separately under aseptic conditions in addition to the liver of each embryo. The viral strains were titrated in SPF-ECE and the virus titers were expressed as embryo lethal dose (ELD_{50})/ml following the method of Reed and Muench (1938).

5-Experimental hosts

Five of each of Hubbard chicks, local breed ducks and New-Zealand rabbits were used for preparation of hyper-immune sera against IBDV. Serum samples from these hosts were screened for IBDV antibodies before the application of the experimental work using serum neutralization test, and all of them were found to be free from such antibodies. Each animal species was kept separately under hygienic measures receiving balanced ration and adequate water.

6-Preparation of IBDV hyper immune sera

Three different hyper immune sera were prepared against IBDV in chicks, ducks and rabbits using 228-E strain according to Abd Elwanis *et al.* (2002).

7-Serum neutralization test (SNT)

To estimate the neutralizing antibodies of IBDV in the prepared hyper immune sera, the B-procedure of SNT was adopted according to Weisman and Hitchner (1978). The antibody titer was calculated as the reciprocal of serum dilution which neutralizes and inhibits the CPE of 100-200 $TCID_{50}$ of the virus.

8-Precipitation of immunoglobulins

The immunoglobulins in the prepared IBDV hyper immune sera were precipitated using saturated ammonium sulphate according to Narin and Marrack (1964). The globulin concentration was determined and adjusted to be 20mg/ml using phosphate buffer solution.

9-Conjugation of the obtained immunoglobulins with fluorescine isothiocyanate (FITC)

The final obtained immunoglobulins hyper immune sera, were conjugated with FITC according the method describedy by Narin (1969).

10- Evaluation of the prepared conjugates

The efficacy of chicken, duck and rabbit IBDV immunogolobulins conjugated with FITC was evaluated through scoring of the IBDV concentration in different sites

of the infected ECE with different strains of IBDV by the application of direct FAT in a comparison with virus titers .

The direct FAT was carried out on impression smears prepared from tissue homogenates of whole infected embryos and livers in addition to slides flooded with the embryonic fluids (allantoic and amniotic). FAT was carried out according to Soliman *et al.* (1989).

11-Scoring of IBDV concentration

A grading system of 0 to 4+ according to Bhattacharfee *et al.* (1994), was used to score the positive reaction of FAT (apple green fluorescence) among the tested IBDV using the prepared conjugates. It was concluded that 0 denotes negative reaction (no staining), while, 4+ indicate positive reaction (clear good staining) up to the entire examined sample.

RESULTS

Table 1. IBDV neutralizing antibody titers in the prepared hyper immune sera.

Tessted sere	IBDV neutralizing antibody titer*
Chicken	1024
Duck	256
Rabbit	128

*IBDV neutralizing antibody titer= the reciprocal of serum dilution which neutralized and inhibited the CPE of 100-200 $TCID_{50}$ of the virus.

Table 2. Titers of different strains of IBDV different sites of infected embryonated chicken eggs.

Virust sit	Titers of IBDV strains expressed as EID_{50}/ml		
	B. Vac	D-78	228-E
Allantioc fluid	5.66	5.30	6.00
Aminiotic fluid	3.50	3.20	30.0
Liver	2.60	2.80	20.0

Table 3. Scoring of the concentration of different IBDV strains by the direct FAT Using chicken anti-IBDV immunologbulin conjusated with FITC.

Tested sit of the virus	FAT reaction of IBDV strains		
	B-Vac	D-78	228-E
Allantioc fluid	+++	+++	+++
Amniotic fluid	+	+	+
Liver	+	+	+
Whole embryo Homogenate	++++	++++	++++

Table 4. Scoring of the concentration of different IBDV strains by the direct FAT using chicken anti-IBDV immunologbulin conjusated with FITC.

Tested sit of the virus	FAT reaction of IBDV strains		
	B-Vac	D-78	228-E
Allantioc fluid	+++	+++	+++
Amniotic fluid	+	+	+
Liver	+	+	+
Whole embryo Homogenate	++++	++++	++++

Table 5. Scoring of the concentration of different IBDV strains by the direct FAT using chicken anti-IBDV immunologbulin conjusated with FITC.

Tested sit of the virus	FAT reaction of IBDV strains		
	B-Vac	D-78	228-E
Allantioc fluid	+++	+++	+++
Amniotic fluid	+	+	+
Liver	+	+	+
Whole embryo Homogenate	++++	++++	++++

DISCUSSION

The efficiency of any control measure and, in particular, any eradication scheme depends to a large extent on the sensitivity of the used technique employed for the diagnosis of the disease to be controlled.

Rapid and accurate diagnosis of IBDV is an essential requirement to reach a valuable control and eradication of such disease which faces poultry industry causing non-neglected economic losses.

The use of immunofluorescence technique to test infected organs, embryos and cell cultures is valuable for early detection and identification of IBDV (Lukert, 1986).

The obtained results tabulated in Table 1 revealed that the prepared hyper immune sera contained specific IBDV neutralizing antibodies with titers of 1024, 256 and 128 for chicken, duck and rabbit serum, respectively.

The results of virus titration demonstrated in Table 2, and the results of the direct FAT using the three prepared conjugates and scoring the virus concentration in the different tested sites (Tables 3,4&5), showed that the recorded virus titers came in a parallel manner. The highest titers of IBDV ($10^{7.8}$, $10^{7.5}$ and $10^{7.4}$ EID_{50}/ml) were recorded for the whole embryo homogenate infected with B.Vac, D-78 and 228-E strains, respectively, recording maximum scores of virus concentration (4+) as shown in Photo 1, while, other sites (allantoamniotic fluid and liver) showed lower virus titers, and accordingly, lower virus concentration scores by FAT. These findings come in agreement with those obtained by Hitchner (1970) who stated that IBDV replicates with higher titers in the embryo than allantoamniotic fluid.

In the same respect, the allantoic fluid showed higher titers of IBDV ($10^{5.66}$, $10^{5.3}$ and 10^{6} EID_{50}/ml) than those recorded for the amniotic fluid ($10^{3.5}$, $10^{3.2}$ and $10^{3.3}$ EID_{50}/ml) for B.Vac, D-78 and 228-E strains, respectively. Meanwhile, the virus concentration scores using FAT were 3+ (photo-2) and 2+ (photo-3) in order for the two fluids.

Lowest IBDV titers ($10^{2.6}$, $10^{2.8}$ and $10^{2.2}$ EID_{50}/ml) were recorded in the liver for the three strains, respectively, and the results of virus concentration scoring by the FAT using the prepared conjugates, confirm the results of virus titration where the recorded scores were mainly +. These results appear to be agreed with those of Muller *et al.* (1979) who referred these findings to the fact that IBDV at first reaches the liver then distributes to the other sites.

In addition, identification of IBDV using the direct FAT applied on infected embryos and organs had proven to be an adjunct to the isolation and identification of IBDV as stated by (McFeran *et al.*, 1980).

It was also noticed that the IBDV concentration in the different sites of emryonated eggs, as recorded by the FAT using the prepared immunoglobulins, was varied depending on the virulence of the inoculated virus strain. Tables 3, 4 and 5 demonstrate that the hot 228-E strain has the highest titer and virus score, specially in the allantoic fluid compared to the other stains (B.Vac and D-78). These observations agree with what reported previously by Winterfield (1969) who obtained increased IBDV concentration in the alantoic fluid through serial passages in embryonated eggs. Also, Hitchner (1970) used the isolate 2512 obtained from Winterfield in its 46^{th} passage to perform a growth curve study.

The prepared anti-IBDV immunoglobulins conjugating with FITC were found to be valuable resulted in positive FAT reactions even when diluted up to $1:10^5$. So, it could be concluded that such preparations are satisfactory efficient to be used for the identification of IBDV in different embryonic specimens with regard to the antibody titer in the immunoglobulin which indicated that chicken immunoglobulin was the preferable one followed by duck and lastly by rabbit immunoglobulin.

Furthermore, the use of such local preparations provides good reagents of low cost available at any time of request, and accordingly, aid to perform accurate and rapid diagnosis.

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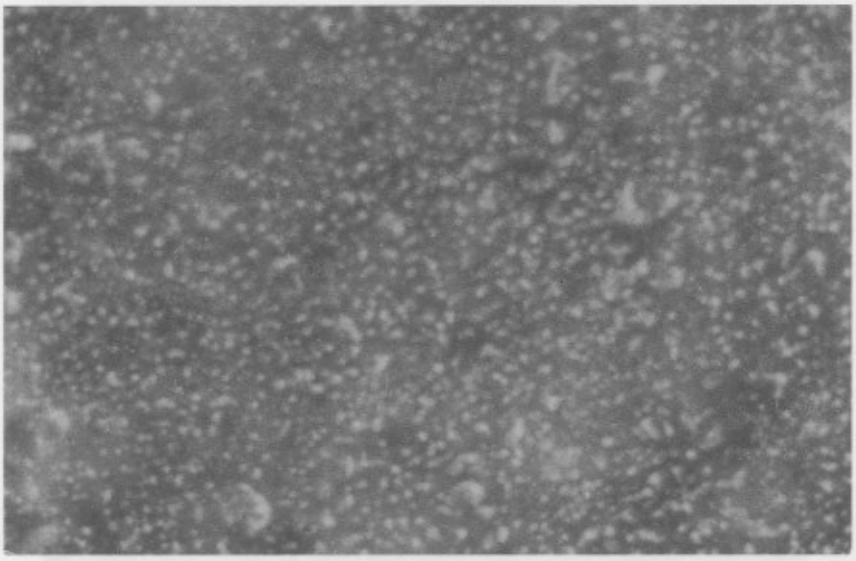

Photo. 1. High score (4+) of IBDV in the whole embryo homogenate as detected by the direct FAT. Showing clear apple green reaction.

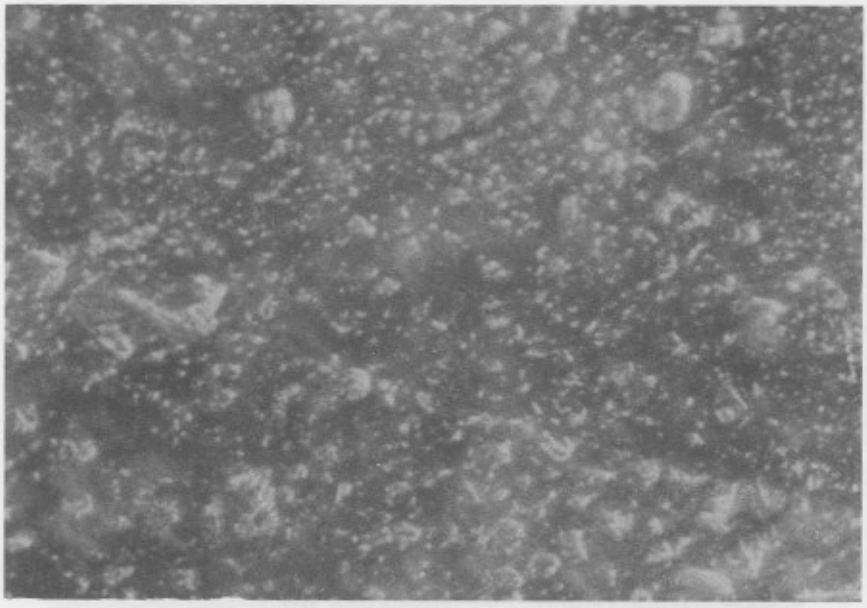

Photo. 2. Moderate score (3+) of IBDV in the allantoic fluid and liver as detected by the direct FAT.

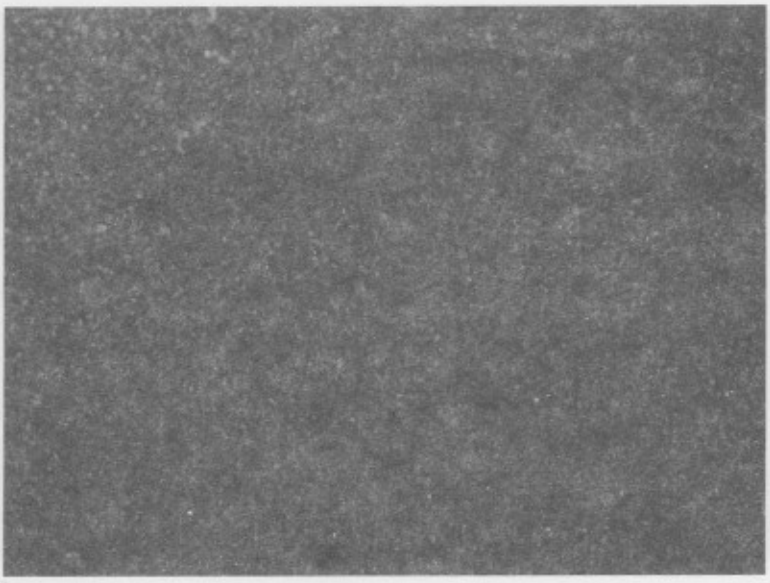

Photo. 3. Less moderate score (2+) of IBDV in the allantoic fluid and liver as detected by the direct FAT.

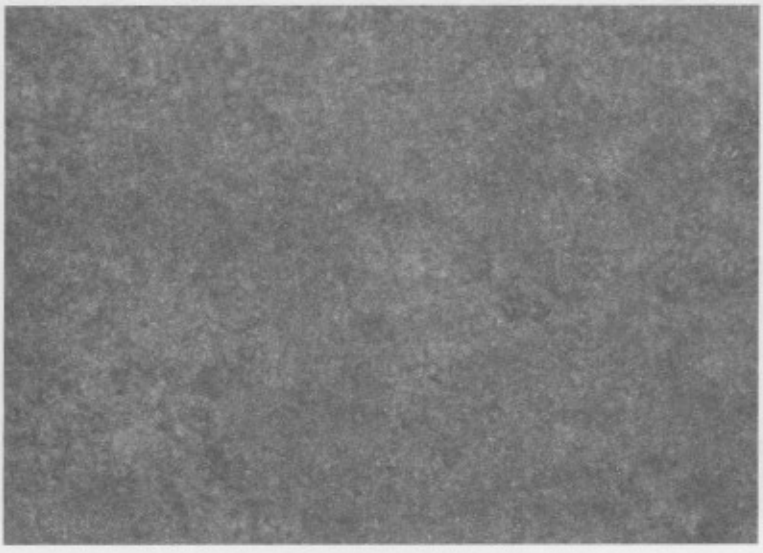

Photo. 4. Negative FAT (No fluorescent reaction).

اقتران جلوبيولينات مناعية محضرة ضد فيروس مرض التهاب غدة فابريشس المعدى فى عوائل مختلفة بمادة الفلوريسين ايسوثيوسيونات

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تم تحضير ثلاثة انواع من المصل المناعى عالى العيارية ضد فيروس التهاب غدة فابريشس المعدى فى ثلاثة عوائل مختلفة (الدجاج–البط–الارانب) ثم تم فصل الجلوبيولين من هذه الامصال باستخدام محلول مشبع من سلفات الامونيوم وبعد تنقية وقياس مستوى الجلوبيولينات المترسبة تم اقرانها بمادة الفلوريسين ايسوثيوسيونات.

تم اختبار كفاءة هذه المستحضرات باجراء اختبار الوميض الفلوريسنتى المناعى لقياس درجة تركيز فيروس التهاب غدة فابريشس المعدى فى مواضع مختلفة من بيض دجاج خالى من المسببات المرضية مخصب ومحضن بعد حقنه بثلاث عترات من الفيروس هى البرسا فاك ، د٧٨، E٢٢٨ . وقد اوضحت التجارب العملية ان الجلوبيولينات المقترنة والمحضرة فى هذا العمل ذات كفاءة عالية ولها القدرة على كشف وجود الفيروس حتى عند تخفيفها الى ١٠٠٠٠٠ : ١ وبهذا يمكن استخدامها كمواد مشخصة لمرض الجمبورو بدلا من نظيرها المستورد عالى الثمن والغير متوافر فى وقت الحاجة اليه.