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 fluorescein 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: 10⁵ 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 (Bhattacharjee 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 disease 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 Cesti 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 (ELD₉₀)/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 TCID₉₀ 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 fluorescein isothiocyanate (FITC)

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

10- Evaluation of the prepared conjugates

The efficacy of chicken, duck and rabbit IBDV immunoglobulins 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 Bhattacharjee 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.

<table>
<thead>
<tr>
<th>Tested sera</th>
<th>IBDV neutralizing antibody titer*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td>1024</td>
</tr>
<tr>
<td>Duck</td>
<td>256</td>
</tr>
<tr>
<td>Rabbit</td>
<td>128</td>
</tr>
</tbody>
</table>

*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.

<table>
<thead>
<tr>
<th>Virus site</th>
<th>Titers of IBDV strains expressed as EID_{50}/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B. Vac</td>
</tr>
<tr>
<td>Allantoic fluid</td>
<td>5.66</td>
</tr>
<tr>
<td>Amniotic fluid</td>
<td>3.50</td>
</tr>
<tr>
<td>Liver</td>
<td>2.60</td>
</tr>
</tbody>
</table>
Table 3. Scoring of the concentration of different IBDV strains by the direct FAT using chicken anti-IBDV immunoglobulin conjugated with FITC.

<table>
<thead>
<tr>
<th>Tested site of the virus</th>
<th>FAT reaction of IBDV strains</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>B-Vac</td>
</tr>
<tr>
<td>Allantoic fluid</td>
<td>++++</td>
</tr>
<tr>
<td>Amniotic fluid</td>
<td>+</td>
</tr>
<tr>
<td>Liver</td>
<td>+</td>
</tr>
<tr>
<td>Whole embryo Homogenate</td>
<td>+++++</td>
</tr>
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Table 4. Scoring of the concentration of different IBDV strains by the direct FAT using chicken anti-IBDV immunoglobulin conjugated with FITC.

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<td>+</td>
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<tr>
<td>Liver</td>
<td>+</td>
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Table 5. Scoring of the concentration of different IBDV strains by the direct FAT using chicken anti-IBDV immunoglobulin conjugated with FITC.

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<tr>
<td>Liver</td>
<td>+</td>
</tr>
<tr>
<td>Whole embryo Homogenate</td>
<td>+++++</td>
</tr>
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</table>
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 (10^7.5, 10^7.2 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 (10^4.66, 10^5.3 and 10^6 EID_{50}/ml) than those recorded for the amniotic fluid (10^5.5, 10^5.2 and 10^5.3 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 (10^6.6, 10^7.8 and 10^8.2 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 embryonated 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 allantoic fluid through serial passages in embryonated eggs. Also, Hitchner (1970) used the isolate 2512 obtained from Winterfield in its 46th 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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precipitation test for detecting serological response to infectious bursal disease

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 جلوبولينات مناعية محضرة ضد فيروس مرض التهاب

غدة فصريش المعدة في عوامل مختلفة بمادة الفلوريسين ايسوثيوبونات

نبيل علي عبد الوهيس، محمد حسن خضير

مختبر حماية الأنسج والالتهابات البيطرية - مركز الفيروز الزراعية - وزارة الزراعة - جنوب جزيرة - مصر.

تم تحضير ثلاثة أنواع من السائل المناعي على الفيروز ضد فيروس التهاب غدة
فصريش المعدة في ثلاثة عوامل مختلفة (دجاج-البطة-الأسود) ثم تم فصل الجلوبولين من هذه
السائل باستخدام محلول ملحي من محلل الأمينوم وكذلك واقي وقي وقي مستوى الجلوبولينات
المترسبة ثم إجرائها بمادة الفلوريسين ايسوثيوبونات.

تم اختبار كفاءة هذه المستحضرات بإجراء اختبار الفيروز الفلوريسيني المناعي لقياس
درجة تركيز فيروس التهاب غدة فصريش المعدة في مواد مختلفة من بيض دجاج خالي من
العوامل المهدئة مخصص ومحصن بعد حقله بثلاث عوامل من الفيروز في البرمطم، 1989.

وقد أوضحت التجربة العملية ان الجلوبولينات المقتربة والمحضرة في هذا العمل ذات
كفاءة عالية وها الفرد على كشف وجود الفيروز حتى بعد تخفيفها الى 1:100000 ويبعد
استخدامها كمواد مشخصة لممرض السبأر بعد نظرها المستورداً على البرمطم والغير متوفراً في
وقت الحاجة فيه.