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Preparation and evaluation of lumpy skin disease hyperimmune serum coniugated with fluorescein

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Hyperimmune serum against lumpy skin disease virus (LSDV) was prepared by inoculation of the virus in successive doses into susceptible two calves with complete and incomplete Freund's adjuvant. Identity tests were applied by agar gel precipitation test (AGPT) and indirect fluorescent antibody technique (IFAT). Serum neutralization test (SNT) and ELISA tests were applied for titration and evaluation of the hyperimmune sera before conjugation with fluorescent. Total protein concentration of the prepared LSDV antisera was 0.8 g/dl. Separation of anti-LSDV immunoglobulins IgG were done using ammonium sulphate followed by conjugation with fluorescein isothiocyanate at pH 9.6. The anti-LSDV IgG conjugated fluorescein sterile and was used to detect LSDV in the MDBK cells and gave good results to dilution 1/20 while the reference conjugate to 1/30.

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INTRODUCTION

Lumpy skin disease is an infectious eruptive viral skin disease affecting cattle and caused by a Neethling strain of Capripox virus which is antigenically related to African sheep and goat pox viruses (**Methews, 1982 and Abd El-Razek, 2003**).

Fluorescent antibody test (FAT) was most widely employed in LSD and sheep-goat pox (SGP) diagnosis (**Davies and Otema, 1978, OIE, 1989, 1992**).

The isolation of LSDV from cattle in Egypt was confirmed by FAT by using anti-Capripox virus hyperimmune sera conjugated with FITC (**House et al., 1990**), which mentioned that FAT was rapid, accurate and sensitive serological technique for the diagnosis of pox viral diseases by detection of the intracellular viral antigen, in addition, FAT was used for rapid detection of the viral antigen in infected tissue culture.

The aim of this study is to prepare local, specific and economic conjugated antisera with FITC against LSD V for rapid diagnosis of this virus to safe time and cost

MATERIALS AND METHODS

Material:

1. Animals

1.1. Two calves of about 1 year old were used for the preparation of LSDV hyperimmune serum. They were screened and proved that they are free from antibodies against LSDV.

1.2. Five adult Albino Swiss mice were used for the preparation of liver powder according to **Nann and Marrack (1964)**. The liver powder was used to remove the non-specific fluorescence from the prepared conjugate.

2. Virus:

Lumpy skin disease virus (Ismailia strain) screened MDBK, was kindly supplied from FADDL through Dr. J. House, the virus isolated during outbreak of cattle in 1989, at Ismailia governorate, Egypt.

It was propagated on lamb testicle cells, then propagated on MDBK cells for 60 passages by **Aboul Saoud (1996)**. The virus has a titre of 6 $10g_{10}$ $TCID_{50}$/ml.

3. Fluorescein isothiocyanate:

It was obtained from Sigma, USA.

4. Dialysis bag:

It was obtained from Sigma, USA.

5. Ammonium sulphate:

It was obtained from Sigma, USA.

6. Conjugates:

6.1. Fluorescein-labeled affinity purified antibody to bovine IgG (H+L):

It was kindly supplied from FADDL through **Dr. J. House.** It was used as a reference positive control conjugate to evaluate the locally prepared one.

6.2. Anti-bovine IgG (whole molecule) peroxidase coniugate:

It was obtained from ICN biomedicals, INC California, USA and used in solid phase ELISA.

7. Lumpy skin disease virus antigen and antisera:

Standard purified reference LSDV antigens and antisera were kindly supplied from FADDL Plum Island, USA through Dr. J House.

8. Cell cultures (MDBK):

The cells were obtained from Ames, Iowa Laboratories, USA and were grown and maintained as described by **Manual of Methods for Virology (1984).**

Methods:

1. Preparation of LSDV hyperimmune serum:

LSDV hyperimmune sera were prepared according to **Puranchand et al. (1985).**

2. Evaluation of prepared LSDV hyperimmune Serum:

2.1 Purity Test:

In accordance with the United States Code of Federal Regulation (CFR) (1987). It was applied in Central Laboratory for Evaluation of Veterinary Biologics (CLEVB), Abbasia, Cairo.

2.2. Identity test:

The LSDV antigen was identified by IFA using reference LSDV antisera as described by **Davies and Otema (1978).**

2.2.1. Agar gel precipitation test (AGPT):

It was applied according to the method described by **Sharma and Dhanda (1972) and Abd El-Razek (1999).**

2.2.2. Serum neutralization test (SNT):

This was carried on microtitre plate 96-well using cell culture method according to **House et al. (1990)** to screen the test animals before inoculation and to estimate the induced antibody titre in the prepared hyperimmune serum.

2.2.3. Enzyme linked immunosorbent assay (ELISA):

Solid phase ELISA (indirect method) was done according to the method described by **Williams (1987).**

3. Precipitation of the immunoglobulin:

It was carried out by using a saturated solution of amunonimn sulphate (75%) according to **Narin and marrack (1964),** the globulin concentration was determined and adjusted to be 20 mg/ml in phosphate buffer solution (PBS).

3.1. Estimation of total protein:

Measurement of the amount of total protein concentration of LSDV antiserum, was done by calorimetric method using spectrum diagnostic total protein reagent and read absorbance of serum at 540 nm, , according to **Cannon et al. (1974) and Tietz (1994).**

Calculation:

$$\text{Serum protein concentration (g/dl)} = \frac{A\ \text{specimen}}{A\ \text{Standard}} \times 6$$

4. Conjugation of the prepared immunoglobulins with FITC:

It was carried out according to **Narin (1969).** The immunoglobulins were adjusted to be at least 20 mg/ml PBS, then diluted in equal volume of chilled carbonate bicarbonate buffer pH 9.6. 1 mg of FITC powder was dissolved in 1 ml cold carbonate-bicarbonate buffer. 100 µl of diluted FITC were mixed with 100 mg immunoglobulins in PBS and left for 24 hours at 4 °C for complete conjugation, then pH was adjusted to 9 with sodium hydroxide solution. The non-specific FITC was removed by using 1 % mice liver powder and dialyzed against several changes of PBS of pH 7.4 at 4°C.

5. Evaluation of the prepared immunoglobulins with FITC:

5.1. Purity test:

The prepared IgG with FITC was tested against bacterial, fungal, mycoplasma and extraneous virus contaminants.

5.2. Fluorescent antibody technique (FAT):

It was carried out according to **Davies and Otema (1978),** preparation of infected and non-

infected control cover slips cultured with MDBK cells, different dilutions of conjugation were used up to 1/50.

RESULTS AND DISCUSSION

This work was designed to prepare hyperimmune sera against LSDV conjugated with fluorescein isothiocyanate to be used for virus diagnosis and titration in a trial to provide a specific local reagent to easily detection of LSDV to save time and costs.

The results of AGPT to prepared LSDV hyperimmune sera were found to contain specific LSDV precipitating antibodies. These results were in agreement with these obtained by **Sharma and Dhand (1969, 1971) and Sharma et ale (1987).**

The prepared LSDV hyperimmune sera was found to contain specific LSDV neutralizing antibodies of a titre 32 and 400 for previous viruses respectively as detected by SNT and ELISA. These results agreed with those obtained by **Martin et ale (1975), Sharma et al.(1987), OIE (1989) and Agag et al. (1992)**

The total protein in the prepared LSDV antisera was 0.8 g/dl and this amount of proteins was satisfactory to use in conjugation with fluorescein. These results were in agreement with **Anderson et al. (1975) and Nowotony (1979).** The total protein of the negative sera was less than 0.8g/dl. So, it was clear that the globulin as the immune protein forming the antibodies appeared to be higher than negative sera of the non-inoculated animals. These results are in agreement with those obtained by **Kataria and Sharma (1993).** The titre of conjugated LSDV. IgG antibodies reached 1:32 which of good titre to use for conjugation **(Nowotony, 1979).**

The conjugated anti-LSDV hyperimmune senun demonstrated typical fluorescence staining reaction (apple green fluorescein up to 1 :20).

Negative control cells showed dull green staining (negative reaction) when reacted with both conjugated at different dilution. Moreover, detection of LSD viral antigen intracytoplasmic of infected cells were also recorded by **Davies and Otema (1978), House et al. (1990) and OIE (1992).**

From this result, we can say that we could prepare anti-LSDV IgG conjugated with fluorescein of low price, rapid (within 1 hour), good

titre and good quality and quantity in pure form to be used for diagnosis of LSD infection and for identity evaluation of produced vaccine.

Table (1): Evaluation of prepared LSDV hyperimmune sera before conjugation

Purity Test	Identify Test		Titration before conjugation		Total Concentration
	AGPT	IFAT	SNT (NA)	ELISA	
Sterile*[1]	+*2	+	32*[3]	400*[4]	0.8 g/dl

*[1] Sterile: Free from contaminants (Bacterial, mycoplasma, fungus and extraneous viruses)
*[2] +: Indirect presence of antibodies against LSDV
*[3]: Titre of neutralizing antibody after successive inoculation
*[4] S/P: ELISA titre of indirect method. Cut-off about 128
*[5] g/l: gram per deciliter
AGPT: Agar Gel Precipitation Test
IFAT: Indirect Fluorescent Antibody Technique
SNT: Senun Neutralization Test, NA: Neutralizing Antibody
ELISA: Enzyme Linked Immunosorbent Assay

Table (2): iteation of prepared anti-LSDV-(IgG) fluorescein onjugate and reference anti-bovine IgG fluorescein conjugate

Conjugate dilution	Prepared anti-LSDV lgG conjugate with FITC	Reference anti-bovine lgG fluorescein conjugate	Control *[3]
1/2	+++*[1]	+++	-
1/5	+++	+++	-
1/10	++	+++	-
1/15	+*[2]	++	-
1/20	-	++	-
1/30	-	+	-
1/40	-	-	-
1/50	-	-	

*[1] Strong positive reaction
*[2] Weak positive reaction
*[3] Non-specific negative non-infected cells

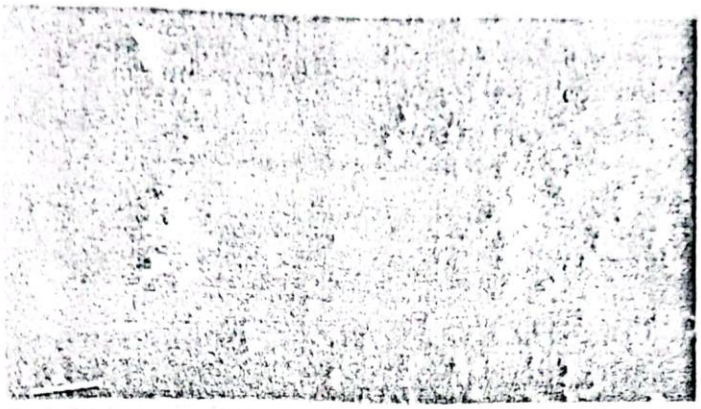

Photo (1): MDBK cells infected with LSDV and stained with anti- LSDV conjugated fluorescein, notice that apple green fluorescence

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