CONJUGATION OF A PPR HYPER IMMUNE SERUM WITH FLUORESCINE ISOTHIOCYANATE FOR SEROLOGICAL USES.

BY

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ABSTRACT

In the present study; a PPR hyper immune serum was prepared in local breed of goats. It was found to have specific PPR neutralizing antibodies of a titer 1024;total protein, albumin and globulin ratios of 5.9±0.21, 2.87±0.18 and 3.07±11gm% respectively. The immune globulin in such serum was precipitated using ammonium sulphate, purified and conjugated with flourescine isothiocyanate. This prepared hyper immune serum was evaluated by applying the fluorescent antibody technique on VERO cells cultured slides infected with PPR virus using different dilutions of the conjugated serum. Positive fluorescent reaction was obtained up to a conjugate dilution of 1:10000 as the results obtained on the use of imported conjugate.So,it could be concluded that the locally prepared PPR hyper immune serum conjugated with fluorescine isothiocyanate could be used in serological tests and have the same value of the imported one which is not usually available and of high cost.

INTRODUCTION

Pest des Petits Ruminants (PPR) is a contagious viral disease of small ruminants characterized by pyrexia, catarrhal nasal and ocular discharges, necrotic stomatitis and an intestinal and lymphoid tissue

reactions (Appiah,1982).It was also known as pseudorinderpest of small ruminants, stomatitis of sheep and goat pneumoentritis complex.

Rapid and accurate diagnosis of fatal diseases like PPR is an essential step in disease control. So, many diagnostic methods were developed including virus isolation (Ronald,et.al.;1971); and virus identification using agar gel precipitation test (AGPT); virus neutralization test (VNT) and Fluorescent antibody technique (FAT) (Garagadannec and Lalanne,1942 ; Rossiter,at.al.,1985 and Mouaz, et.al.1995).

FAT was found to be sensitive as virus neutralization (Durojajye,1984). FAT could be used to detect PPR antigen in infected tissues (Liess,1963; Osman,et.al. ,1994 and Mouaz,et.al.1995). The application of FAT for the diagnosis of viral diseases was discussed by Regenortel and Neurath (1985).They stated that such diagnosis depends on the detection of viral antigen at the site of lesion or on the detection of early produced specific IgM.The later was considered to be one of the most sensitive rapid technique for the diagnosis of viral infections.

The main goal of the present study is to prepare a specific standardized PPR hyper immune serum conjugated with fluorescine isothiocyanate to be used as a diagnostic agent help to obtain rapid and confirmed results especially in emergency cases. The presence of such material locally could safe the time and cost where the imported conjugate is usually unavailable and supplied in small amounts of high price.

MATERIAL AND METHODS

1.1-Animals:

Seven local breed goats of about 10 months old were used in the present work. They were found to be health and free from rinderpest and PPR antibodies as screened by serum neutralization test. Five goats were used to prepare PPR hyper immune serum while the other 2 goats were kept as test control. All animals were fed balanced ration and kept under hygienic measures and subjected to daily clinical examination.

1.2.Mice:

Fifteen adult albino Swiss mice were used in the present study to prepare mice liver powder according to Narin and Marrack (1964). The liver powder was used to remove the non-specific fluorescence from the prepared conjugate.

2-Viruses:

2.1-PPR virus:

The local strain of PPR virus (Egypt-87) at its 20^{th} passage on VERO cells was used for vaccine preparation (Khodeir and Mouaz,1998) and for the preparation of PPR hyper immune serum and for its application in serum neutralization test (SNT).

2.2.Rinderpest virus:

The ROBK strain of rinderpest virus at its 99^{th} passage in calf kidney cell cultures and adapted to VERO cells (Osman,et.al. 1985) was used for SNT to screen the test animals before the application of the designed experiments.

3-Serum neutralization test (SNT):

Both qualitative and quantitative SNT were carried out using the micro-titer technique according to Rossiter,et.al.(1985) to screen the test animals before inoculation and to estimate the induced antibody titer in the prepared hyper immune serum.

4-Preparation of PPR hyper immune serum in goats:

It was prepared in 5 goats free from rinderpest and PPR antibodies according to Ihemelanade,et.al. (1985) and Mouaz,et.al. (1998).

5-Estimation of serum proteins:

Serum total protein was estimated in the prepared PPR hyper immune serum according to Weichselbaum (1946) while serum albumin was estimated according to Ness (1965). These tests were carried out using commercial kits of Biomerieux Laboratory reagents and products, Maroyl'France.

6-Precipitation of the immunoglobuin:

It was carried out using a saturated solution of ammonium sulphate according toNarin and Marrack (1964).The globulin concentration was detected and adjusted to be 20mg/ml using phosphate buffer solution.

7-Conjugation of the prepared immunoglobulin with flourescine isothiocyanate:

It was carried out according to Narin (1969).

8-Imported PPR antiserum conjugated with fluorescin isothiocyanate:

PPR antiserum conjugated with fluorescin isothiocyanate (of goat origin) was supplied by USDA_Aphis-FADDAI,U.S.A. It was used as a reference to evaluate comparatively the locally prepared one.

9-Fluorescent antibody technique (FAT):

FAT was carried out according to Soliman,et.al. (1989) to test and evaluate the prepared conjugated PPR hyper immune serum in a comparison with the imported one.It was applied on cover slips cultured with VERO cells and infected with PPR virus. The conjugates were diluted up to 10^5.

RESULTS AND DISCUSSION

The present work was designed for preparation of a hyper immune serum against PPR conjugated with floulrescin isothiocyanate to be used in serological tests in a trial to provide a specific local reagent for diagnosis of PPR saving time and costs.

The prepared PPR hyper immune serum was found to contain specific PPR neutralizing antibodies of a titer 1024 as detected by SNT. Such results were obtained by pApiah (1982); Diallo,et.al.(1989) and Mouaz,et.al.(1998) obtained similar results and showed that the hyper immune serum prepared in goats is better than that prepared in rabbits.

Among serum proteins it was found that the total protein in the prepared serum (5.32±0.21gm%) was higher than the negative serum control (5.14±0.22gm%). The albumin was decreased from 2.07±0.18 gm% in the control serum to 1.79±0.32 gm% while the serum globulin was increased from 3.07±0.11 gm% to 3.53±0.10 gm% (Table-1).It is clear that the globulin as the immune protein forming the antibodies appeared to be higher than albumin in the hyper immune serum. These results come in agreement with those of Ayoub,et.al.(1964); Kataria and Sharma (1993) and Hanan (1998).

Fluorescent microscopy of the stained cell culture infected cover slips, revealed that the prepared PPR hyper immune serum and conjugated with the fluorescin isothiocyanate, give positive reactions (apple green fluorescent) were detected up to a dilution of $1:10^4$ similar to those obtained on the use of the imported serum conjugate (Table-2).

Many authors detected PPR antigen in infected specimens and cell cultures like Liess (1963); Osman,et.al.(1994) and Mouaz,et.al.(1995).

FAT has gained a wide acceptance in virology especially in detection of viral antigens in animal tissues (Goldman,1968).

From the results obtained in the present work, it can be concluded that the locally prepared PPR hyper immune serum conjugated with fluorescin isothiocyanate could be used successfully in the detection of PPR antigen in infected tissues saving time and coast and providing rapid accurate results.

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Table (1): Estimation of PPR hyper immune serum parameters

Tested serum	PPR antibody titer*	Total serum protein gm%	Serum albumin gm%	Serum globulin gm%
PPR hyper immune serum	1024	5.32±0.21	1.79±0.32	3.53±0.10
Negative serum control	0	5.14±0.22	2.07±0.18	3.07±0.11

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

Table (2): Titration of PPR hyper immune serum conjugated with fluorescin isothiocyanate

Conjugate dilution	Reactions with	
	Local prepared conjugate	Imported conjugate
1:10	+ve	+ve
$1:10^2$	+ve	+ve
$1:10^3$	+ve	+ve
$1:10^4$	+ve	+ve
$1:10^5$	-ve	-ve

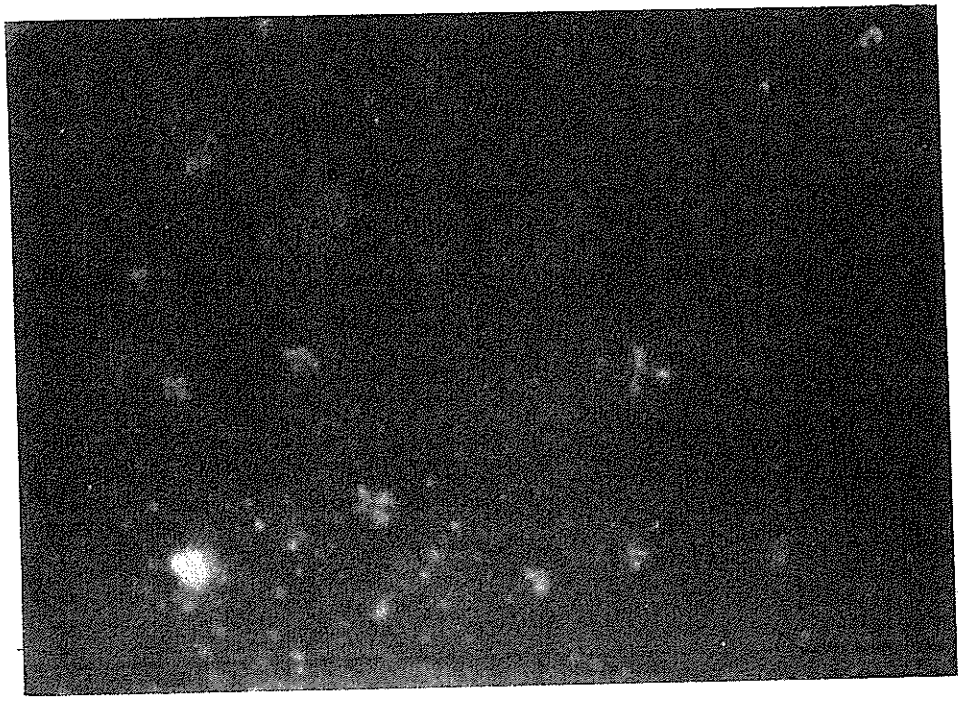

Fig.(1): Positive fluorescent antibody reaction in vero cell culture infected with PPR virus (Apple green fluorescent)

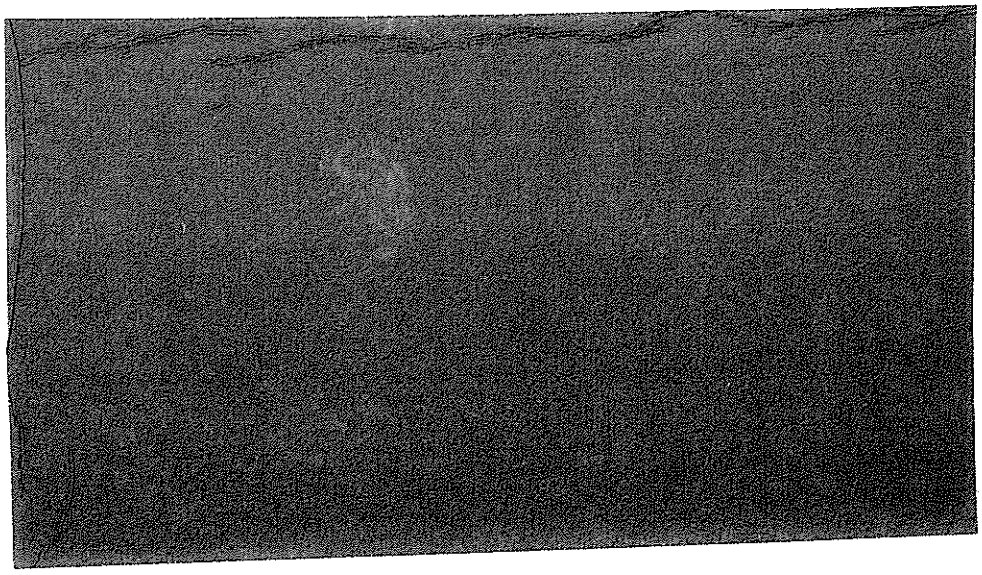

Fig.(2): Negative fluorescent antibody reaction

الملخص العربى

تحميل مصل عالى العيارية لطاعون المجترات الصغيرة بالفلوريسين أيسوثيوسيونات للأستخدام فى التجارب السيرولوجية

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فى هذه الدراسة تم تحضير مصل عالى العيارية لطاعون المجترات الصغيرة فى المعز البلدى. وبتقييم هذا المصل وجد أنه يحتوى على أجسام مناعية معادلة لطاعون المجترات الصغيرة بمعيار ١٠٢٤ وبروتين كلى وألبيومين وجلوبيولين بنسب٣٢ر٥±٢١ر. و٧٩ر١±٣٢ر. و٥٣ر٣±١٠ر. جرام% على التوالى.كما تم ترسيبب الجلوبيولين باستخدام سلفات الأمونيوم ثم تحميله بمادة الفلوريسين أيسوثيوسيونات ثم تم تقييم هذا المقترن بإجراء أختبار الوميض المناعى على شرائح نسيجية من خلايا كلى القرد الأخضر الأفريقى معدية بفيروس طاعون المجترات الصغيرة وباستخدام تخفيفات من المقترن المحضر حيث تبين أنه يعطى نتائج إيجابية حتى تخفيف ١٠٠٠٠:١ مماثلا بذلك نظيره المستورد. وعلى ذلك يمكن القول بأن المصل المقترن المحضر محليا يمكن استخدامه بدلا من المستورد الذى لا يتوافر بسهولة فى أغلب الأحيان بالإضافة إلى إرتفاع ثمن الكميات القليلة منه.