CONJUGATION OF *CLOSTRIDIUM CHAUVOEI* AND *CLOSTRIDIUM SEPTICUM* HYPERIMMUNE SERA WITH FLUORESCIN ISOTHIOCYANATE FOR SEROLOGICAL USES

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SUMMARY

Hyperimmune sera were prepared in sheep against Clostridium chauvoei (C.chauvoei) and Clostridium septicum (C.septicum) where their immunoglobulins were precipitated using saturated ammonium sulfate solution. The concentration of such immunoglobulins was adjusted to be 20mg/ml then conjugated with fluorescin isothiocyanate. The direct fluorescent antibody technique was carried out on culture smears of the 2 strains and muscle sections prepared from infected Guinea pigs by each strain separately. This technique was carried out in a homologous and a heterogeneous manner using the prepared conjugates. It was found that homologous conjugates showed more clear apple green reactions while heterogeneous conjugates resulted in barely visible reactions indicating that there was very low or even no cross reaction between Clostridium chauvoei (C.chauvoei) and Clostridium septicum (C.septicum) confirming the possibility of the use of fluorescent antibody technique to distinguish between them.

INTRODUCTION

The genus Clostridium includes many pathogenic species to man and animals. From these species *Clostridium chauvoei (C.chauvoei)* and *Clostridium septicum (C.septicum)* are two closely related species causing a variety of disease conditions in cattle and sheep as black leg; gas gangrene and umbilical infections in newly born animals *(Batty and Walker, 1963).*

Identification of pathogenic clostridia by cultural and biochemical tests is time and material consuming requiring long incubation period and various test media to distinguish the closely similar organisms *(Gadalla et.al., 1966).*

C.chauvoei and C.septicum found in a variety of pathological materials and their diagnosis in fresh or slightly decomposed muscles is indicative of a specific infection; but with advanced decomposition, their isolation becomes more difficult and other organisms could invade the lesions. So, rapid techniques are required to obtain rapid diagnosis and accurate differentiation between closely related species in direct smears from pathological specimens and even culture smears *(Creech and Jones, 1941).*

A more rapid, sensitive and highly specific technique is the fluorescent antibody technique depending mainly on antigen-antibody reaction in the presence of a fluorescent dye (fluorescin isothiocyanate "FITC") which irradiates with ultraviolet light emitting apple green fluorescence *(Tizard, 1996)*. Fluorescent antibody technique (FAT) depends on the presence of specific antisera conjugated with FITC. Many workers used FAT to differentiate between the different species of clostridia *(Geck and Szanto, 1961; Batty and Walker, 1963 and 1964; Martig, 1966 and Awad, 1982)*.

The present work was designed to prepare sheep hyperimmune sera against *Clostridium chauvoei* and *Clostridium septicum* conjugated with FITC in order to evaluate their use in FAT to diagnose and differentiate between these two closely related species, in addition to provide a local reagent of good quality and low cost instead of the expensive imported one which is not available on request.

MATERIALS AND METHODS

1-Sheep:

12 apparently healthy local breed sheep of about one year old were used for preparation of *Clostridia* antisera where 5 animals were used for *C.chauvoei* and another 5 animals for *C.septicum* antiserum while 2 animals were kept as control.

2- Guinea pigs:

15 healthy Guinea pigs of about 350 gm body weight were used in the experimental infection with clostridia organisms where 5 animals were infected with *C.chauvoei* and another 5 animals with *C.septicum* while 5 animals were kept without infection as control.

3- Clostridia strains:

Local *C.chauvoei* and *C.septicum* lyophilized strains isolated from cattle were supplied by the Department of Anaerobic Bacterial Vaccine Research; Veterinary Serum and Vaccine Research Institute; Abassia, Cairo and used for experimental infection of Guinea pigs and for antigen preparation to be used in preparation of hyperimmune antisera..

4- Smear samples:

Smears of *C.chauvoei* and *C.septicum* were prepared from the muscles of experimentally infected Guinea pigs (each animal was inoculated intramuscular with 0.5ml of 18 hours bacterial culture with 0.5ml of 5% calcium chloride). In addition frozen sections were prepared from the muscles of the same animals and examined according to *Batty and Walker (1963)*.

5-Preparation of hyperimmune sera:

Preparation of *C.chauvoei* and *C.septicum* hyper immune sera in sheep was carried out according to *Benedict (1967)*.

6- Estimation of serum proteins:

Serum total protein was estimated in the prepared hyper immune sera according to *Weichselbaum (1946)* while serum albumin and globulin were

estimated according to *Ness (1965)* using comercial kits (Biomerieux, France).

7-Precipitation of immunoglobulins in the prepared antisera:

Precipitation of immunoglobulins in the prepared sheep *C.chauvoei* and *C.septicum* hyper immune sera was carried out using saturated ammonium sulfate solution according to *Narin and Marrack (1964).* After estimation of the immunoglobulin, its concentration was adjusted to be 20mg/ ml in normal saline.

8-Conjugation of the precipitated immune globulins with FITC:

Conjugation of the prepared *C.chauvoei* and *C.septicum* immune globulins with FITC was done followed the method described by *Narin (1969).*

9- Fluorescent antibody technique (FAT);

Direct FAT was carried out on smears and frozen sections of infected Guinea pig muscles using the prepared conjugates to evaluate them according to *Soliman et al. (1989).*

RESULTS AND DISCUSSION

C.chauvoei and *C.septicum* are two closely related species and both of them are pleomorphic and exhibit forms of great similarity. The biochemical reactions of the two organisms are also similar, and although they usually differ in the fermentation of sucrose and salicin, anomalous strains do occur. The only certain method of discrimination has been done by protection test in animals, but even here an antiserum to *C.septicum* will sometimes give protection against challenge with *C.chauvoei (Batty and Walker, 1963).* A rapid method of differentiating closely related species in direct smears from pathological specimens would have many advantages. The use of fluorescin labeled antibodies appears to offer such a method *(Creech and Jones, 1941).*

The present work indicated that the application of direct fluorescent antibody technique on culture smears of *C.chauvoei* and *C.septicum* and section of infected Guinea pig muscles using the prepared conjugates in both homologous and heterogeneous manners. The results showed that *C.chauvoei* and *C.septicum* stained with their homologous antisera fluoresced brightly and when stained with heterolgous antisera; they were barely visible (Photo 1&2). The muscle sections revealed the same behavior when stained in the same manners. These findings agree with what reported by *Batty and Walker (1963)* who concluded that it is possible to discriminate between *C.chauvoei* and *C.septicum* with fluorescent labeled antibodies and added that this was true not only in smears from cultures, but also in tissue sections and smears from pathological lesions. No more available data that discuss the results of the present work could be found. However, it could be concluded that fluorescent antibody technique provides a rapid method for the detection of *C.chauvoei* and *C.septicum* and differentiation between them.

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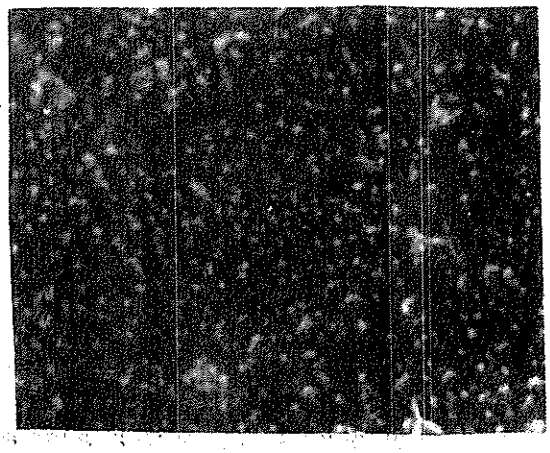

Photo (1): C.chauvoei and C.septicum stained with their nomologous antisera showing bright flurescent (100X).

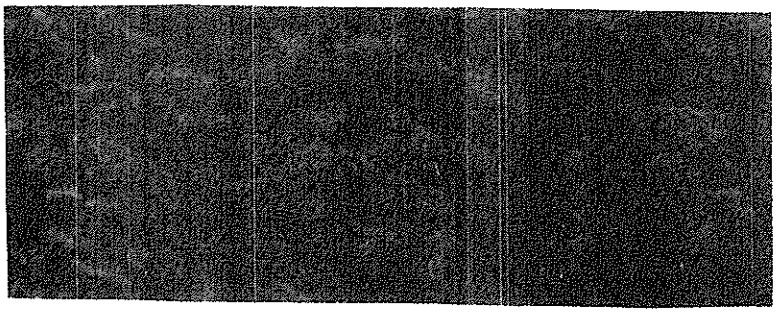

Photo (2): C.chauvoei and C septicum stained with their heterogenous antisera showing barely visible fluorescence (100X).

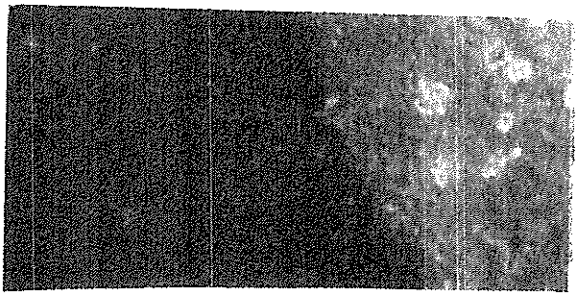

Photo (3): Negative fluorescent antibody technique (100X).

الملخص العربي

إقران مصل عالى العيارية ضد الكلوستريديم شوفياى والكلوستريديم سيبتيكم بالفلوريسينإيسوثيوسينات للاستخدام فى التجارب السيرولوجية

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تم تحضير مصلين عاليا العيارية فى الأرنب ضد كل من الكلوستريديم شوفياى والكلوستريديم سيبتيكم ثم تم ترسيب الجلوبيولين المناعى فى كل منهما باستخدام محلول مشبع من سلفات الأمونيوم وتم ضبط تركيزه ليكون 20مليجرام/مل فى محلول الملح الفسيولوجى حيث تم اقرانه بالفلوريسين إيسوثيوسينات0 وقد أجرى أختبار الوميض الفلوريسنتى المناعى المباشر على مسحات تم تحضيرها من مزارع -24 ساعة من كلا الميكروبين علاوة على شرائح نسيجية تم تحضيرها من عضلات لأرانب هندى تمت عدواها بكل ميكروب على حدة0 وقد أظهرت نتائج الأختبار أنه عند استخدام كل مقترن نوعى مع الميكروب المقابل له تكون شدة التفاعل أقوى وأوضح منها فى حالة أستخدام المقترن الغير نوعى الأمر الذى يمكن القول معه أن أختبار الوميض الفلوريسنتى المناعى يمكن استخدامه فى استبيان وجود أى من الكلوستريديم شوفياى والكلوستريديم سيبتيكم والتفريق بينهما كما أن المقترنين المحضرين خلال هذا العمل يعدان بمثابة المنتج المحلى الذى يساعد على الوصول إلى تشخيص سريع ورخيص التكلفة دون إنتظار لوقت قد يطول لوصول نظيره المستورد.