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Preparation of Hemolysin for Detection of some Viral and Bacterial Antigens

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SUMMARY

Hemolysin (amboceptor) which is the most important constituent in complement fixation test (CFT) was prepared by inoculation of sheep RBCs S/C in Bosket rabbits in two concentrations (10% and 20%)

Blood samples were obtained weekly from inoculated rabbits to measure the titre of induced Hemolysin by CFT. It was found that Hemolysin titer was increased with the number of inoculation till the 7th inoculation then began to decrease with the 8th inoculation. However the titer of Hemolysin obtained from rabbits inoculated with 20% sheep RBCs was higher (1/5800) than that obtained from rabbits inoculated with 10% red cell suspension (1/4500). Similar results were reported to the imported Hemolysin. By using the prepared Hemolysin in CFT for diagnosis of FMD and Brucellosis it gave the same results as that obtained by imported one

INTRODUCTION

Foot and mouth disease (FMD) is still one of the most important infectious disease that could not neglected where it causes great economic losses among cloven hoofed animals including losses in the milk meat and draft potentials (Anon et,al.,1978). It is characterized by a high mortality in young animals. Several outbreaks of FMD were recorded in Egypt as reported by Moussa et. al., (1974); Daoud et. al., (1988); El-Nakashly et. al., (1996) and Farag et. al.,(2004 and 2005) where the causative agent was FMD virus type O while the last recorded outbreak was due to type A (Abd El-Rahman et. al., 2006).

Brucellosis (undulant fever, Mediterranean fever or Malta fever) is a zoonotic disease is transmitted by direct or indirect contact with infected animals or their products. It affects people and animals of all age groups and both sexes. In Egypt, control of brucellosis depends on test and slaughter

policy and vaccination of brucella free animals (OIE, 2008).

Specific diagnostic antiserum (Hemolysin, anti-sheep RBCs., amboceptor) is useful for specifically recognizing antigen-antibody complex which could be easy seen when precipitation occurs which makes the identification of the causative agent more easier.

Specific, rapid and sensitive serological test like complement fixation test (CFT) is required for detection of FMD virus, its typing and titration of its antibodies as well as for detection of Brucella organism and their antibodies (Moussa et. al., 1973 and Blasco et. al., 1994).

Complement fixation test (CFT) is the most widely used test for the serological confirmation of brucellosis in animals. As in cattle brucellosis, despite its complexity and the heterogeneity of the techniques used in different countries, there is agreement that this test is effective for the serological diagnosis of brucellosis in sheep and goats (Farina, 1985; MacMillan, 1990 and Alton, 1990). However, under field conditions, the sensitivity of CFT has been reported to be somewhat lower (88.6%) than those of the Rose Bengal RB (92.1%) and iELISA (100%) for diagnosing *B. melitensis* infection in sheep (Blasco et al, 1994-b and Nielsen et. al., 2000).

In a pan-American and European comparative study, the results on sensitivity for the different tests were: cELISA (76.0%), buffered plate agglutination test (77.5%), CFT (83.1%), iELISA (90.1%) and fluorescence polarization assay –FPA (91.5%)> While the sensitivity of RB is sufficient for the surveillance of free a areas at the flock level, RB and CF should be used together in infected flocks to obtain accurate individual sensitivity in test- and – slaughter programmers. Nielsen et, al., 2000.

According to the great importance of FMD and Brucella, accurate and rapid identification of the causative agent is an essential step in controlling such diseases. This purpose needs the availability of specific diagnostic antiserum like Hemolysin, so the present work was designed to prepare anti sheep RBCs in rabbits to be available as a local product on request which could be considered of diagnostic and economic value in detection of FMD virus and Brucella organism and its antibodies. And other causative agents.

MATERIALS AND METHODS

1- Animals:

1.1-Sheep:

Three one year old apparently healthy local breed sheep were used. They were free from external and internal parasites, negative to FMD virus (Type O and A) and brucella antigens and antibodies as they were screened by serum neutralization test (SNT) these

sheep were housed in separate isolates under hygienic measures. And used for preparation of sheep RBCs required for Hemolysin preparation.

1.2- Rabbits:

Nine Bosket rabbits each of about 2-3kg body weight were used for preparation of Hemolysin. These rabbits were housed under hygienic conditions in separate isolates receiving balanced ration and adequate water where they were divided into 3 groups (3 rabbits/ group). The first and second groups were used for haemlycin preparation while the third group was kept as control without inoculation.

1.3-Guinea pigs:

Four Guinea pigs of about (350–400gm) were housed under hygienic conditions and used for preparation the complement according to Alton et. al.,(1988).

2-Antigens:

2.1-Brucella antigen:

A whole-cell antigen composed of heat-Killed *B. abortus* cells was prepared according to Alton et. al., (1988) and supplied by the Department of Serum and Antigen Research, Veterinary Serum and Vaccine Research Institute., cairo, Egypt.

2.2-FMD virus antigen:

FMD virus types O (O1/3/93) and A (A/Egypt/2006) were prepared in BHK cell culture according to Lefevre and Diallo (1990) and supplied from the Department of Foot and Mouth Disease Research, Veterinary Serum and Vaccine Research Institute., Cairo, Egypt.These antigens were used in CFT to estimate the prepared Hemolysin.

3- Hyper Immune Sera (HIS):

FMD type O and A antisera were prepared in Guinea pigs. Alton et. al.,(1988). And brucella antisera were supplied by the Department of Serum and Antigen Research, Veterinary Serum and Vaccine Research Institute, Cairo, Egypt, and used in the CFT.

4- Complement:

It was obtained from fresh normal Guinea pigs serum according to Alton et. al., (1988) and stored at -70°C in small quantities till used in CFT.

5- Sheep RBCs:

Sheep RBCs were withdraw aseptically with an equal volume of Alsever's solution and thoroughly mixed, washed twice with PBS to prepare 10 and 20% cell suspensions in PBS according to Alton et. al.,(1988).

6- Preparation of Hemolysin:

The first group of rabbits (3 rabbits) was inoculated by 10% suspension of sheep RBCs in a dose of 1ml/rabbit injected subcutaneously. The second group of rabbits was treated in the same manner using 20% RBCs suspension. Rabbit inoculation was repeated weekly up to eight weeks according to Moussa et. al., (1973) and Alton et. al., (1988) where serum samples were obtained from all rabbit groups every week post inoculation then all rabbits were scarified 10

days after the last injection. The sera of rabbits was inactivated in water bath at 56°C for 30 minutes. Equal volumes of the obtained Hemolysin and glycerin saline were mixed together and stored at -18°C till titrated with complement fixation test.

7-Hemolysin titration:

It was carried out using 2 fold dilutions starting from 1:100 up to 1:6400 according to Alton et. al.,(1988). The highest dilution which showed 100% haemolysis considers as one unit of Hemolysine titer. So, for example if such dilution is 1/2400 we use 4 unit from it (1/2400 x 4 = 1/600) to be used in the proper test.

8-Heamolytic system:

Heamolytic system is used as indicator in complement fixation test consisting of 4 minimum heamolytic doses of Hemolysin + equal volume of sheep RBCs 2% in PBS diluent mixed and synthesized at 37°C for 15 minutes Alton et. al.,(1988).

N.B: The Hemolysin reacts and combines with RBCs and lyses them in the presence of complement.

Scoring of reactions in the CFT consider in unit form.

0 = no. of RBCS cells remaining (complete lyses)

Traces = approx. 1 – 24% cells remaining.

1 = approx. 25% cells remaining (that is considered as one unit)

2 = approx. 50% cells remaining

3 = approx. 75% cells remaining

4 = approx. 100% cells remaining according Health Protection Agency (2009)

9-Complement titration (C.T):

It was carried out according to Alton et. al., (1988) where the complement was prepared in 10 folds dilution in veronal buffer. The highest dilution of complement which showed complete heamolysis (100%) consider one unit of complement.

10- Direct and indirect complement fixation tests:

These tests were carried out using FMD and brucella antigen and their specific antibodies according to **Alton et. al.,(1988).**

RESULTS AND DISCUSSION

As it is well known that FMD and Brucella represent the most important diseases of cloven hoofed animals and their control depends to a great extent on the rapid, accurate and sensitive specific diagnosis. Such purpose needs the availability of specific anti sheep RBCs (Hemolysin) which used in CFT.

The present study showed that the two methods were adapted for preparing the Hemolysin (amboceptor) from rabbits, it was found that the titre of hemolytic serum obtained from rabbits inoculated with 20% sheep red cell suspension was higher than that obtained from rabbits inoculated with 10 % red cell suspension.

The frequency of inoculation of rabbits for more than 7 injections led to decrease in the serum titer as shown in Table (1). These results agree with that obtained by Moussa et. al.,(1973).

From the Table (2) it was also found that there was slightly difference between the efficacies of the locally prepared Hemolysin (1/4500, 1/5800) and the imported one (1/3200, 1/4500) .

The demonstrated results in Tables (3 and 4) revealed that the prepared Hemolysin was able to detect FMD and Brucella antigens and antibodies through the application of direct and indirect CFT confirming its validity. Regarding the use of CFT to such purposes; CFT was found to be valuable in determination of the antigenic relationship between different strains of FMD virus isolated from separated geographical foci in Egypt and from different species of animals at varying year intervals to detect the selected isolate used for preparation of the vaccine as reported by Samira El-kelany (1982). CFT was carried out directly for measurement of the antigenicity of FMD virus prior to preparation of FMD vaccine, and indirectly for evaluation the prepared vaccine by measuring the produced antibodies according by Manal AboEl-yazed (1990). In addition, CFT was used for typing of isolated FMD virus according by Abd El-Rahman (2006).

Also Brucella antigen and antibodies were determined and estimated using direct and indirect CFT as stated by Blasco et. al.,(1994a) and Sutherland (1985). The prepared Hemolysin could be considered of diagnostic benefit for FMD and Brucella as a local preparation available on request, also it is cheaper than the imported one.

Table (1): Titer of the prepared Hemolysin

Concentration of inoculated sheep RBCs	Hemolysin titer/ weeks post rabbit inoculation							
	1WPI*	2WPI	3WPI	4WPI	5WPI	6WPI	7WPI	8WPI
10%	0	1/800	1/1600	1/2000	1/2400	1/3000	1/4500	1/3800
20%	0	1/1200	1/2400	1/2900	1/3400	1/4200	1/5800	1/5400

*WPI= week post inoculation

Table (2): Comparative titration between the prepared and imported Hemolysin

Titrated Hemolysin	Hemolysin end titer
Induced by 10% RBCs	1/4500
Induced by 20% RBCs	1/5800
Imported lot No. 2302158	1/4500
Imported lot No. 1302175	1/3200

Table (3): Titration of FM D and Brucella antigens by direct CFT using the prepared Hemolysin

Dilution of tested antigens	Hemolysin dilution	
	1/4500	1/5800
FMD virus antigen diluted from 1/2 - 1/32	+	+
Brucella antigen diluted from 1/2 to 1/32	+	+

Table (4): Titration of antisera against FM D and Brucella microorgainsm by indirect CFT using the prepared Hemolysin

Dilution tested hyper immune sera	Hemolysin dilution	
	1/4500	1/5800
FMD hyper immune serum diluted from 1/2 to 1/256	+	+
Brucella hyper immune serum diluted from 1/2 to 1/512	+	+

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تحضير مادة الهيموليزين للكشف عن بعض الأنتيجينات الفيروسية و البكتيرية

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معهـــد بحـــوث الأمصـــال واللقاحـــات البيطريـــة العباسيـــة- القاهـــرة

طبقا لاعتبار الهيموليسين مكونا أساسيا فى اختبار المكمل المثبت فقد تم خلال هذا العمـل تحضـير يليسين كمستحضر محلى وذلك بحقن مجموعتين من الأرانب البوسكات حيث تم حقن المجموعة الأولى لق كرات الدم الحمراء للأغنام بتركيز ١٠% وحقنت المجموعة الثانية بتركيز ٢٠% من نفس الكـرات مراء و تركت مجموعة ثالثة من الأرانب دون حقن كضابط للتجربة. وبمقارنـة عياريـة الهيموليسـين حضر مع مثيله المستورد وجد أن الهيموليسين المحضر باستخدام ١٠% من معلق كرات الدم الحمراء ذو يارية ١/ ٤٥٠٠ بينما كانت عيارية المستحضر باستخدام ٢٠% من نفس المعلـق ١/٥٨٠٠ فـى كانـت يارية المستحضر المستورد (لوط رقم ٢٣٠٢١٥٨و اللوط رقـم١٣٢٠٢١٧٥) ١/٤٥٠٠و١/٣٢٠٠ علـى توالى. وبإجراء اختبارى المكمل المثبت المباشر والغير مباشر وجد أن المستحضر قـادر علـى معـايرة انتيجينات والأجسام المضادة لكل من الحمى القلاعية والبروسيللا الأمر الذى يؤكد نجاح توفير منتج محلى د الحاجة إليه فى أي وقت وبسعر أقل من المستورد.