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IMMUNE RESPONSE OF *C. PERFRINGENS* TYPE A VACCINE IN CALVES

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ABSTRACT

Two monovalent vaccines were prepared from the highly toxigenic isolates of *C. perfringens* type A inducing enterotoxaemia in young calves. Vaccination of pregnant cattle with this vaccine is of much importance for protection of young calves from sudden death. In this study four pregnant cattle were vaccinated with two doses of each vaccine monovalent vaccine (one adjuvanted with montanide oil ISA 206 and the other adjuvanted with aluminum hydroxide gel, the first dose 2 ml s/c following with 2 ml (second dose) with three weeks interval. Blood samples were collected from each group and from their newly born calves one week after birth. The antitoxin values expressed in international units (IU). All pooled serum was determined by serum neutralization test and ELISA. It was found that C. perfringens type A vaccine either adjuvanted with gel or montanide oil gave high antibody titer in pregnant dams. The maternal immunity for calves reached 11 weeks in two vaccines. From these results, it can be concluding that maternal immunity affords good degree of protection of newly born calves against enterotoxaemia.

Keywords: *C. perfringens* type A, montanide oil ISA 206, pregnant cattle, calves, maternal immunity, enterotoxaemia

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1. INTRODUCTION

Calves enterotoxaemia is one of the serious problems facing calves within the first months of age, the importance of this problem emerges from the fact that calves play a major role in our future animals' wealth as a source of animal protein which is necessary to fulfill with the requirement of the rapidly increasing human population **(Awad 1980)**.

Enterotoxaemia caused by *C. perfringens* type A is probably the important cause of sudden death in young calves. Enterotoxaemia morbidity rate is variable; however, in general, it does not exceed 10% of the herd but its lethality is high and usually kills 100% of the affected animals **(Miyashiro et al. 2007)**.

Vaccination against clostridial diseases have been practiced for many years in cattle **(Stokka et al. 1994)**The effectiveness of immunization depends on several factors as type of vaccine and the adjuvant used **(Chirase et al. 2001)**.

So, this study was designed to prepare a vaccine from the highly toxigenic isolates of *C. perfringens* type A for protection of young calves from enterotoxaemia using two different adjuvant (aluminum hydroxide gel and montanide oil) and evaluation the immune response of pregnant cattle and their newly born calves to each vaccine.

2. MATERIALS AND METHODS

2.1. Vaccine preparation:

The vaccine was prepared from the highly toxigenic isolates of *C. perfringens* type A according to methods of **(Gadalla et al. 1974)** and inactivated by formaline in 0.5% concentration (**Gadalla et al. 1969)**. Inactivated cultures and toxoids were separated and concentrated using ultra filtration system (Millipore corporation USA) and merthiolate was added as preservative as 1% percentage. This inactivated culture and toxoid was divided into two parts.The first one was adjuvanted with montanide oil ISA206 in equal parts of an aqueous and oil phase according to **(Barnett et al.1996)**, the second one was adjuvanted with aluminium hydroxide gel added as concentration of 20% according to **(El-Sehamy et al. 2004)**. These vaccines were tested for purity and safety in laboratory animals according to (**British Veterinary Pharmacopoeia 2007)**.

2.2. Vaccination schedules

Two groups of pregnant cattle (each group consists of four pregnant cattle) were vaccinated with two doses of each vaccine (monovalent vaccine prepared from the highly toxigenic isolates of *C. perfringens* type A), the first dose 2ml S/C following with 2ml (second dose) with three weeks interval. Vaccination time was at the last period of pregnancy of cattle. Three weeks after the second dose blood samples were collected from each group of pregnant cattle before parturition and from their newly born calves one week after birth and for eleven week after birth. The antitoxin values expressed in international units (IU); all pooled serum was determined by serum neutralization test according to (**Gadalla et al. 1971**).

2.3. Experimental design

Collected sera from all groups of pregnant cattle and their born calves assayed for antitoxin values expressed in IU/ ml using SNT (serum neutralization test) and ELISA according to **(Mattar et al. 2002)**.

3. RESULTS

As shown in table (1) blood samples collected from pregnant cattle before partuition, evaluated by SNT showing high antitoxin titer reached 30 I.U./ ml in group of pregnant cattle vaccinated with monovalent vaccine adjuvanted with montanide oil ISA 206 while group of pregnant cattle vaccinated with monovalent vaccine adjuvanted with aluminium hydroxide gel antitoxin titer reached 40 I.U./ ml.

Table 1: Values of mean antitoxin titer against *C. perfringens* type A alpha toxin in serum of pregnant cattle vaccinated with the prepared vaccines no. (1), (2) by serum neutralization test three weeks post vaccination:

Type of vaccine	Mean antitoxin titer I.U./ ml
Group (1) pregnant cattle vaccinated with vaccine no. (1)	30
Group (2) pregnant cattle vaccinated with vaccine no. (2)	40

Vaccine no.(1): monovalent *C.perfringens* type A montanide oil adjuvant vaccine.
Vaccine no.(2):monovalent *C. perfringens* type A aluminium hydroxide gel vaccine

Table 2: Mean *C. perfringens* alpha antitoxic values in serum of calves born to pregnant cattle vaccinated with vaccines no. (1), (2) by serum neutralization test:

Age of calves (week)	Calves borned to pregnant cattle vaccinated with vaccine no. (1)	Calves borned to pregnant cattle vaccinated with vaccine no. (2)
One	20	30
Two	15	25
Three	15	15
Four	10	10
Five	5	5
Six	5	5
Seven	3	3
Eight	2	2
Nine	1	1
Ten	1	1
Eleven	0.5	0.5

Table (3): Alpha antitoxin titer of *C. perfringens* type A in two groups of pregnant cattle measured by ELISA:

No. of samples	No. 1	No. 2	No. 3	No. 4	Mean per unit
pregnant cattle vaccinated with vaccine No. 1	41.08	39.5	36.48	30.67	36.82
pregnant cattle vaccinated with vaccine No. 2	56.88	50.82	43.05	41.89	48.16

Table (2) recorded that young calves borned to cattle vaccinated with monovalent vaccine adjuvanted with montanide oil ISA 206 showing high antitoxin reached 20,15,15,10,5,5,3,2,1,1,0.5 I.U./ml at weeks 1,2,3,4,5,6,7,8,9,10,11respectively .While young calves borne to cattle vaccinated with monovalent vaccine adjuvanted with aluminum hydroxide gel showing high antitoxin reached to 30,25,15,10,5,5,3,2,1,1,0.5 I.U./ml at weeks 1,2,3,4,5,6,7,8,9,10,11 respectively.

Table (4) Antitoxin titer of monovalent *C. perfringens* type A vaccine adjuvanted with montanide oil ISA 206 in young calves measured by ELISA:

Calves age	No. of calves				
	1	2	3	4	Mean
1*	24.59	24.18	36.08	34.86	29.92
2	17.97	20.94	15.67	25.40	19.99
3	15	15.81	14.45	----	15.08
4	13.78	13.37	13.64	-----	13.59
5	6.21	5	-----	---------	5.6
6	5.13	3.51	6.35	---------	4.9
7	3.24	2.97	---	---	3.10
8	2.97	2.29	--------	--------	2.6
9	1.62	2.29	----------	--------	1.95
10	1.48	1.89	------	-------	1.68
11	0.94	1.08	------	------	1.01

*Calves age/week

Table (5) Antitoxin titer of monovalent *C. perfringens* type A vaccine adjuvanted with aluminium hydroxide gel in young calves measured by ELISA:

Calves age	No. of calves				
	1	2	3	4	Mean
1*	41.08	39.05	36.48	30.67	36.82
2	31.80	34.32	30.67	----	32.26
3	28.10	26.62	25.40	-----	26.70
4	14.45	16.35	-----	-----	15.04
5	6.40	5.81	7.83	--------	6.68
6	5	5.13	---	---	5.06
7	2.70	2.83	----	----------	2.76
8	2.16	2.02	-------	----------	2.09
9	1.21	1.48	-----	-----	1.34
10	1.5	1.15	---	----	1.32
11	1.08	1	-----	------	1.04

*Calves age/week

Table (3,4) recorded that young calves borne to cattle vaccinated with monovalent vaccine adjuvanted with montanide oil ISA206 showing high antitoxin reached 29.92, 19.99, 15.08, 13.59, 5.6, 4.9, 3.10, 2.6, 1.95, 1.68, 1.01. I.U./ml at weeks 1,2,3,4,5,6,7,8,9,10,11 respectively. While young calves borne to

cattle vaccinated with monovalent vaccine adjuvanted with aluminum hydroxide gel showing high antitoxin reached to 36.82, 32.26, 26.70, 15.04, 6.68, 5.06, 2.76, 2.09, 1.34, 1.32, 1.04 IU/ml at weeks 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 respectively.

4. DISCUSSION

In the present study, a toxoid vaccine was prepared from the highly toxigenic isolates of *C. perfringens* type A which is the most causative organism that inducing enterotoxaemia in young calves. The immunogenic responses of these vaccines in pregnant cattle and their newly born calves were evaluated as shown in the tables. From results monovalent vaccine either adjuvanted with gel or montanide oil gave high antibody titer in pregnant dams as shown in table (1). The maternal immunity for calves reached 11 weeks in two vaccines as shown in table (2, 3).

Treatment Our results agree with (Ikbal et al. 1979) who concluded that vaccination of the adult cattle is important as the response of suckling calves from vaccination was very poor while that of the adult animals was of good level, It was found that calves sera born from immune mothers contained level of antibody sufficient to protect the calves for many months after birth. So the best method for protection the calves in young age are to vaccinate the mother 2 weeks before calving. The usage of montanide oil ISA 206 and aluminum hydroxide gel was very baneful in clostridial vaccines and these were confirmed by (El- Meneisy et al.2005) who used montanide oil ISA206 and potassium aluminum sulphate as adjuvants for clostridial vaccines and found that montanide oil was more potent and gave higher level of antibody than potassium aluminum sulphate vaccine . Also (Abdalla et al. 2011) found that the immune response of cattle to clostridial vaccine adjuvanted with potassium aluminum sulphate was less than that adjuvanted with aluminum hydroxide gel for Clostridium perfringens type A, it achieved antibody level more than the minimum protective level as the minimum protective level for C. perfringens type A is 0.1 I.U. /ml. (Weipers et al. 1964). (Verma, 1986) discussed that aluminum hydroxide gel gave better immunity and longer duration compared with alum precipitated adjuvant. (Mario, 1969) also reported that aluminum hydroxide gel gave satisfactory protection. The results revealed the importance of vaccination with *C. perfringens* type A vaccine for the protection of young calves from sudden death due to enterotoxaemia and also revealed the importance of maternal immunity as the best method for protecting the calves in young age is to vaccinate the mothers 2 weeks before calving.

It could be concluded that, Vaccination of pregnant cattle with monovalent vaccine is more efficient and protective for calves. Maternal immunity affords good degree of protection of newly born calves against enterotoxaemia.

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الإستجابة المناعية للقاح ميكروب الكلوستريديم بيرفرنجينز النوع أ فى العجول الصغيرة.

نورا محمد خلف[1] ، محمد حسنين عبيد[2] ، السيد مصطفي جليلة[2] ، عبد المنعم محمد مصطفي [2]، علاء عبد الفتاح محمد المنيسي [1]

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الملخص العربي

تم تحضير لقاح احادى من الالفا توكسيد من ميكروب الكلوستريديم بيرفرنجينز من النوع أ الذى يسبب التسمم المعوي الكلوستريدي فى العجول الصغيرة الذى يسبب الموت المفاجئ بدون أي أعراض سابقة لهذا فإن تحصين الامهات العشار بهذا التحصين لة بالغ الاهمية لحماية العجول الصغيرة من الموت المفاجئ. تم تقسيم الامهات الحوامل الى مجموعتين المجموعة الاولى تم تحصينها باللقاح الاحادى من ميكروب الكلوستريديم بيرفرنجينز النوع أ المحسن بزيت المونتانيد والمجموعة الثانية تم تحصينها باللقاح الاحادى من ميكروب الكلوستريديم بيرفرنجينز النوع أ المحسن بالجل.تم حقن كل مجموعة جرعتين الجرعه الاولي 2 سم تحت الجلد والجرعة الثانية 2 سم تحت الجلد وتليها بثلاث اسابيع وتمت متابعة الاجسام المضادة فى الامهات والعجول الصغيرة الناتجة من كل مجموعة على حدة بعد الجرعة الثانية بثلاث اسابيع باستخدام اختبار المصل التعادلى فى الفئران واختبار الاليزا. من نتائج الاختبارات السابقة وجد ان أستخدام زيت المونتانيد أى أس ايه 206و الجل كمحفزات مناعيه في اللقاح الأحادي يعطى نتائج متقاربة ولكن يفضل استخدام الجل للامهات الحوامل حيث يعطى نتيجة عالية وسريعة ومن ثم تصل فى المناعة الامية اعلي واسرع.ايضا تحصين الامهات الحوامل باللقاح الأحادي من الكلوستريديم بيرفرنجينز النوع أ يعتبر أكثر كفاءة وأعلى حماية للعجول الصغيرة حيث أنه بالقياس المناعي يعطى أعلى نسبة مع أطول مدة للمناعة. مما سبق نستخلص أن المناعة الأمية تعطى حماية كافية للعجول الصغيرة للحماية من مرض التسمم المعوي الكلوستريدي.

. **(مجلة بنها للعلوم الطبية البيطرية: عدد 26(2):137- 142, يونيو 2014)**