

# PERFORMANCE OF A LOCALLY PRODUCED FOWLPOX VACCINE UNDER LABORATORY AND FIELD CONDITIONS IN THE SUDAN

By

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## ABSTRACT

The present study was carried out to determine the potency and efficacy of a locally produced fowlpox vaccine (Beaudette strain) under laboratory and field conditions. Serum samples from various age groups of experimental and farm chickens vaccinated at the age of three months were collected. Agar gel immunodiffusion (AGID) and passive haemagglutination test (PHA) were employed to determine the antibody responses in sera of vaccinated birds. All the sera were found to contain antibodies against the virus using AGID.

Using PHA, gradual increase of the antibody titre was observed in farm chickens sera till 10 months of age and thereafter titres started to decline. There was no case of fowlpox disease in any of the farms. Potency tests showed that the minimum dose of the locally produced fowlpox vaccine required to protect 50% of the inoculated chickens against fowlpox (PD<sub>50</sub>) is 10<sup>3.3</sup> EID<sub>50</sub>/ml.

## المخلص

أجريت الدراسة لتحديد معايرة وكفاءة جذري الطيور (عترة/بوديت) المنتج محلياً تحت ظروف المعمل والحقل. جمعت عينات الأمصال من أعمار مختلفة لدجاج ملقح في عمر 3 أشهر.

تمت الاستفادة من الانتشار المناعي للهلام وتلازن الدم السالب لتحديد الاستجابة المناعية في أمصال الطيور الملقحة.

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نتائج تلازن الدم السالب أظهرت زيادة متدرجة في عيار الأجسام المضادة في دجاج الحقل حتى عمر 10 أشهر ومن ثم بدأ العيار في الهبوط. لم تسجل أي حالات مرضية بجذري الطيور في أي من المزارع، أثبت اختيار الكفاءة أن الحد الأدنى المطلوب من جرعة لقاح الجذري المنتج محلياً لحماية 50% من الدجاج الملقح هي  $10^{3-3}$  EID<sub>60</sub>/mL.

## INTRODUCTION

Fowlpox (FP) is a relatively slow spreading viral disease naturally affecting chickens and turkeys of all ages, sexes and breeds and experimentally any of the avian species (Tripathy and Cunningham, 1984; Tripathy, 1991). It is characterized by wart-like nodules on the skin and diphtheretic necrosis of the membranes lining the mouth and upper respiratory system (Tripathy, 1980; Tripathy, 1991). The first report of the disease in Sudan was in 1936 (Anon, 1936) and later was reported to be prevalent all over the country (Khogali, 1970; Elhoussein *et al.*, 1998).

The disease is caused by a virus which is a member of the genus *avipoxvirus*, subfamily *chorodopoxviridae* and the family Poxviridae (Tripathy, 1991). Variations in the antigenicity of various strains of the virus were previously confirmed by Mait *et al.* (1991).

Control of fowlpox depends mainly on vaccination. There are two major types of live virus vaccines used for immunization of birds against the disease, fowlpox virus vaccine (Cunningham, 1973) and pigeon pox virus vaccine (Tripathy and Cunningham, 1984). Total protection was reported to be attained only after vaccination of day old chicks and adult chickens with an oil adjuvanated fowlpox virus vaccine via the wing web method (Peleg *et al.*, 1993). Comparative evaluation of various fowl-pox vaccine strains for their efficacy to elicit the immune response in birds was previously studied (Baxi and Oberoi, 1999). Continuous monitoring of the performance of the vaccine used in the field is required to ensure its proper use under field conditions. In the present study the immunogenicity and protective potential of a locally produced fowlpox vaccine were targeted for evaluation.

## **MATERIALS AND METHODS**

**Embroynated Chicken Eggs:** Elevent to twelve (11-12) day- old fertile chicken eggs used in all experiments were obtained from the flock of the Central Veterinary Research Laboratory (CVRL) in Soba, Khartoum (Sudan).

**Fowlpox Vaccine:** Fowlpox vaccine containing the Beaudete strain of the virus was kindly supplied by the Department of Vaccine Production, CVRL. The vaccine virus was propagated on the chorioallantoic membrane (CAM) of 11-12 day-old embroynating chicken eggs.

**Antisera:** Hyperimmune and preimmune sera were used as positive and negative control respectively in the tests employed. They were obtained from the Department of Vaccine Production, Soba, CVRL

**Titration of the Fowlpox Virus Vaccine:** Briefly two vials of freeze-dried FPV vaccine were reconstituted in 2 ml of normal saline containing antibiotics. Ten fold serial dilutions of the vaccine were then made 0.1 ml of the reconstituted vaccine from each dilution starting from  $10^{-2}$  to  $10^{-4}$  was inoculated onto the CAMs of embroynating chicken eggs using 4 eggs for each dilution Four eggs were kept as uninoculated control. The eggs were then incubated at  $37^{\circ}\text{C}$  (Minbay and Kreier, 1973).

The inoculated eggs were candled daily to test the viability of the embryos. Embryos dying 24 hours post inoculation were discarded as non-specific deaths. Deaths that occurred after 24 hours were attributed to the growth of the virus (Minbay and Kreier, 1973).

Five days post inoculation the shell over the artificial air sac was removed with forceps. The CAMs were removed after the contents of the eggs were discarded, and examined for the presence of pock lesions (Minbay and Kreier, 1973).

**Field Serum Samples:** A total of one hundred and eighty serum samlpes from various poultry farms in Khartoum state were collected at least one month following vaccination of birds using Beaudette strain vaccine. These birds were only vaccinated once. Collected serum samples were inactivated at  $56^{\circ}\text{C}$  for 30 minutes then stored at  $-20^{\circ}\text{C}$  till used.

**Preparation of Fowlpox Antigen:** The fowlpox CAM antigen for use in AGPT was prepared using sodium deoxycholate (SDC) as described by Tamador (1998).

**Agar Gel Precipitation Test (AGPT):** The immunodiffusion test in 1% agarose was carried out as described by Tripathy (1996). The agar plates were incubated for 24 hours at room temperature in a humid chamber and examined for the presence of the precipitation bands in a dark room using indirect light.

**Passive Haemagglutination (PHA) Test:** Fowlpox sensitized sheep RBCs were prepared as described by Tripathy *et al* (1970). The test was carried out using 2 fold serial dilutions of test sera in round bottom microtitre plates as described by Whiteman and Heterick (1965). Results were expressed as  $\log_2$  titers of the highest dilution that resulted in agglutination.

**Challenge Virus:** The challenge virus was isolated from a field outbreak and proved to be highly virulent (Safaa, 2001).

**Experimental Vaccination and Challenge of Chickens:** Two groups of 3 months old Hisex chickens (25 bird each) were either vaccinated once (group 1) or received a second dose of the vaccine when 4 months old (group 2). Ten birds from each of group 1 and 2, using the challenge virus (0.1ml inoculum/bird) were challenged at 6 month of age. Five unvaccinated birds were used as control.

**Determination of Protective Dose 50 (PD<sub>50</sub>) of the Vaccine:** Three vials of the freeze dried fowlpox vaccine were reconstituted each in 1 ml of glycerine buffer and the contents of the three reconstituted vials were mixed together. The vaccine mixture was then diluted so that the following dilutions were obtained:  $10^{-0.3}$ ,  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$  and each dilution was used to vaccinate groups of birds (0.1ml/bird). All birds were challenged with field strain 3 weeks after vaccination

## RESULTS

**Antibody Detection Using AGPT:** All sera obtained from the experimental birds (group 1 and 2) produced positive reaction after 24 hours of incubation. All field sera were also positive.

**Determination of Antibody Titre Using PHA Test:** RBCs agglutination was observed after 2-4 hours of incubation. Agglutination occurred at different levels of serum dilution. The mean antibody titre of those

vaccinated twice ( $\log_2 6.9$ ) was slightly higher than those vaccinated once ( $\log_2 6.7$ ), when measured at 8 months of age however, the overall antibody mean titre in sera obtained from the farms ( $\log_2 5.38$ ) was higher than the titre of the experimental animals ( $\log_2 5.23$ ), (Tables 1, 2 and 4).

**Table 1: PHA Titre in Farm Samples (15 Samples for Each Age Group)**

Age of Birds (Month)	Abs Titre Mean	Age of Birds (Month)	Abs Titre Mean
4	3.9	10	6.3
5	6.4	11	5.0
6	6.6	12	6.6
7	3.8	13	5.1
8	6.5	14	3.7
9	5.1	15	3.2

**Experimental Vaccination and Challenge of Birds:** The PHA titres of the birds in this experiment before and after challenge are shown in (Table 2). Except for transient drop in egg production no other signs of FP were observed in vaccinated birds.

**Table 2: Mean PHA Titre in Challenged and Unchallenged Chickens**

Age of chicken (month)	Vaccinated once (10birds)		Vaccinated twice (10 birds)	
	BC *	AC **	BC *	AC **
8	6.7	6.9	6.9	7.1

\* Before challenge, \*\* After challenge

**Determination of Protective Dose<sub>50</sub> of Fow Pox Virus Vaccine:** As a result of challenge of birds, some birds developed typical Fowlpox lesions at the site of scarification 7-10 days post challenge (Table 3).

**Table 3: Response to Virulent Fowlpox Virus Following Challenge of Vaccinated Birds Using Comb Scarification**

Vaccine dilution	No. of birds	No. of birds with lesions	No. of birds without lesions	% of Birds without lesions	Ratio of birds without lesions
$10^{-3}$	5	1	4	80	0.8
$10^{-1}$	5	3	2	40	0.4
$10^{-2}$	5	4	1	20	0.2
$10^{-3}$	5	5	1	20	0.2
$10^{-4}$	5	5	0.0	0.0	0.0

Lesions developed in all six control birds, The PD<sub>50</sub> of fowl pox virus vaccine ( $10^{3.3}$ ) was calculated using Karber method.

Table 4: PHA Titre in Experimentally Vaccinated Chickens and Farm Samples

Experimentally vaccinated		Farm samples	
Age of chickens (month)	Mean log <sub>2</sub>	Age of chickens (month)	Mean log <sub>2</sub>
4	1.91	4	3.9
5	4.1	5	6.4
6	6.1	6	6.6
7	6.1	7	3.8
8	6.7	8	6.5
9	6.5	9	5.1
overall	5.38	Overall	5.23

**Determination of the Egg Infective Dose<sub>50</sub> (EID<sub>50</sub>) of FP Vaccine:** Embryos inoculated with various dilutions of FP vaccine were examined for pock lesions, 5 days post inoculation. The virus titre in the vaccine was 10<sup>8.7</sup> EID<sub>50</sub>/ml as determined by Karber method (1936).

## DISCUSSION

The present study was carried out to determine the efficacy of a locally produced fowl pox virus vaccine “Beaudette strain” under field conditions in the Sudan and whether or not a booster dose is needed. This was accomplished by observing vaccinated birds in the field for fowl pox disease outbreaks and by measuring antibody responses in these birds and finally by virus challenge of experimentally vaccinated birds. Furthermore, the PD<sub>50</sub> of the vaccine preparation used was also determined.

It was also observed in the present study that purification of fowl pox antigen using 2% sodium deoxycholate (SDC) gave clear precipitation lines in AGPT. This is in agreement with Tamador (1998) who observed similar findings.

The use of agar gel immunodiffusion test (AGID) showed that vaccination of birds using locally produced fowl pox vaccine provoked antibody response in all sera from experimentally vaccinated chickens (those vaccinated once and those vaccinated twice) from the age of four months to nine months, and sera from farms (vaccinated once) from the age of four months to the age of fifteen months. There were no fowl pox lesions observed following

challenge of the experimentally vaccinated chickens at the age of 6 months, but there was a transient decrease in egg production on both groups. The results of AGID and PHA obtained supported by the absence of disease development in challenged birds and in the farms from which the sera were collected suggest that the locally produced fowl pox virus vaccine was potent, and one dose of the vaccine at age of three months was enough for total protection. The passive haemagglutination test (PHA) showed that the titre of antibody response of the birds that received two vaccine doses was slightly higher than that of those vaccinated once, and the antibody titre in both increased gradually with increased age. This may suggest virus persistence in the bird tissues amplifying the antigenic mass of the virus.

In the field sera the antibody titre increased gradually till the age of ten months (the highest titre) and then started to decline. Surprisingly the titre in farm samples was higher than the titre of experimental chickens. This, in spite of the fact that we used feather follicle route while the farmers used the wing web route. This increase in the antibody titre in the field may be due to exposure to natural infection. This may be supported by the observation that the antibody titre in the experimentally vaccinated chickens increased eight weeks post challenge with virulent virus in both groups of birds that had been vaccinated once or twice.

Tripathy *et al* (1970) stated that passive haemagglutination test detects antibodies in sera of chickens by the 3rd week after inoculation and for at least 15 weeks. In this study passive haemagglutination test detected antibody against fowl pox virus in the sera of the chickens up to 12 months post vaccination.

Titration of the locally produced fowl pox virus vaccine was accomplished to determine the amount of virus required to infect 50% of the chick embryo (embryo infective dose  $EID_{50}$ ). The  $EID_{50}$  was determined by the presence of the pock lesion on the CAM and showed that the vaccine preparation used contained  $10^{8.5} EID_{50}/ml$ . In previous reports it was established that the suitable potency for an attenuated live fowl pox virus vaccine is likely to be in the region of  $10^5 EID_{50} /ml$  (Tripathy, 1996). This result indicates that this vaccine is highly potent.

In previous reports, it was observed that a booster injection 8

and 14 weeks after primary vaccination increased the titre gradually with increased age. The highest antibody titre was observed 15 weeks post inoculation and remained constant up to 18 weeks (Siddique *et al.*, 1997). In this study we found that a booster dose 4 weeks post initial vaccination increased the titre gradually but the maximum titre in those vaccinated once and those given a booster dose was attained by the end of 12 weeks post vaccination and remained approximately constant till 24 weeks post vaccination. However, in sera from farms the titre undulated with the maximum ( $\log_2 6.8$ ) titre being observed at 28 weeks post vaccination. Rise in a passive haemagglutination titre, which we observed 4 weeks post challenge, also agreed with Baxi and Oberoi (1999), who found that the antibody titre increased 7 days post challenged.

Immunity test ( $PD_{50}$ ) is generally used to determine the immunogenicity of fowl poxvirus vaccine. For satisfactory immunization, at least 80% of the controls should have lesions of fowl pox and at least 80% of the vaccinated birds should not have lesions of fowl pox (Cunningham, 1973). In the present study, 100% of control birds developed lesions following challenge of birds while 80% of the vaccinated birds developed no lesions of fowl pox. The  $PD_{50}$  was found to be  $10^{3.3}$ , thus the minimum dose titre required to vaccinate birds is estimated around  $10^{5.2}$  EID<sub>50</sub>/bird.

Result of  $PD_{50}$  ( $10^{3.3}$ ) suggests that instead of using one vial per 100 birds as recommended by the manufacturer, the same vial can be used for 1000 birds. In this case, maternal immunity needs further research so as to know the optimal time for vaccination.

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