

Precipitating Antibodies in Response to Fowl pox Vaccine (Beaudette strain) Administered Through Three Different Routes and Comparison of the Sensitivity of AGPT and CIEP

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ملخص البحث

أعدت هذه الدراسة لقياس المناعة المكتسبة ضد لقاح جدري الطيور عترة بوديت (Beaudette strain) عند تطعيم الدجاج بثلاثة طرق هي وحذ كفاف الجناح، جراب الريش وإحداثا خدوش بالعرف. قيست المناعة المكتسبة بعد ثلاثة، سبعة وأحد عشر أسبوعاً باستخدام اختباري الترسيب المناعي في الأجار (AGPT) و الرحلان الكهربائي المعاكس (CIEP). وجد أن درجة المناعة المكتسبة في الطيور المطعمة عن طريق جراب الريش وكفاف الجناح و خدوش العرف هي 100% و 88.3% و 66.6% على الترتيب. ورغم اختلاف طرق التطعيم استطاعت كل الطيور المطعمة مقاومة الجرعة التي أعطيت لكل منها بعد اثني عشر أسبوعاً من التطعيم.

Summary

The immunological response of chickens to fowlpox vaccine (Beaudette strain) was studied, using three different routes of vaccination; wing web, feather follicle and scarification of the comb. The humoral antibody response was detected after three, seven and eleven weeks using Agar Gel Precipitation Test (AGPT) and Counter Immunoelectrophoresis (CIEP) Test. Seroconversion in the vaccinated birds was 100% when the vaccine was administered by feather follicle method compared with 88.3% and 66.6% for wing web and scarification of the comb, respectively. However, birds vaccinated by all methods withstood virulent challenge at 12 weeks post vaccination.

Introduction

Fowlpox is a contagious, and slowly spreading viral disease of chickens and turkeys worldwide. The disease is characterized by the formation of proliferative lesions and scabs on the skin and diphtheritic lesions in the upper parts of the digestive and respiratory tracts (Tripathy, 1991).

Vaccination against fowlpox is usually done in endemic areas or when an outbreak occurred in the last season (Mockett, 1990). Various methods of vaccination can be used, but there are two main

commonly used; the wing web and feather follicle (Mockett, 1990). A third method of vaccination, which has been used under experimental condition, was the oral route. This method has the advantage of mass administration, but two high doses of attenuated virus vaccine at 5 and 25 days of age have been recommended (Mockett, 1990).

Formerly, fowlpox vaccine was imported from Kenya before it was produced locally for the first time in the Sudan at the Central Veterinary Research Laboratories in 1972 (Ali *et al.*, 1983). Up to the present time no seromonitoring data on fowlpox antibody in vaccinated birds were published. The present work was designed to study the immunological response to the fowlpox vaccine (Beaudette strain) via three different routes; wing web, feather follicle and scarification of the comb.

Materials and Methods

Viruses:

Fowlpox vaccine:

The department of viral vaccine production, CVRL, Soba kindly supplied the Beaudette strain of Fowlpox vaccine (FPV). The virus was titrated using the chorioallantoic membrane (CAM) of 11-12 day-old embryonating chicken eggs (ECE). The average vaccine titre was 10^9 EID₅₀/ml based on presence of lesions on CAM.

Challenge virus:

The virus was readily isolated from scabs on combs and legs of naturally infected birds from a poultry farm in Shambat and inoculated onto the CAM of 11-12 day-old ECE. Five days post inoculation the CAMs of viable embryos with confluent pock lesions were harvested and homogenized. The identity of the virus was confirmed by means of Agar Gel Precipitation Test (AGPT) where clear precipitin lines resulted with known positive fowlpox antiserum. The isolated virus had a titre of 10^9 EID₅₀/ml and was used as a challenge virus (0.01ml/chick) throughout the experiment.

Vaccination experiment:

One hundred and twenty Bovans chickens at the age of eight weeks old and free from detectable FP antibodies as indicated by AGPT, were obtained from the Central Veterinary Research Laboratories (CVRL) farm at Soba. Birds were divided into four groups and vaccinated with FP vaccine (Beaudette strain) as follows: -

The first group (35 birds) was vaccinated with the undiluted FP vaccine (w/w) by wing web method using a bifurcated needle.

Birds of the second group (35 birds) were vaccinated using the feather follicle method by plucking feathers of the major feather tracts on the bird's back and the undiluted vaccine was brushed onto the open follicles.

The third group (35 birds) was vaccinated with the undiluted vaccine by scarification of the comb.

The fourth group (15 birds) was left as unvaccinated contact control. Each group was housed in a separate cage and all cages were present in a large poultry house.

Serum collection:

Blood from the wing vein or from the heart was collected from the experimental birds before vaccination and at 3, 7 and 11 weeks post vaccination (PV) and incubated overnight at room temperature.

The sera were separated and clarified by centrifugation at 2000 rpm for 10 minutes and stored at -20°C till tested.

Challenge experiment:

Twelve weeks PV, all vaccinated and non-vaccinated birds were challenged with FP virus (field isolate) by scarification of comb and wattles. Following challenge all birds were observed daily for clinical signs.

Serological tests:

Preparation of fowlpox antigen:

Two vials of freeze-dried FP vaccine were reconstituted in normal saline containing antibiotics, ten fold serial dilutions were then made covering the range 10^{-1} to 10^{-3} . 0.1ml of 10^{-3} dilution of the virus were inoculated onto the CAMs of 11-12 day-old embryonating chicken eggs. Five days PV the infected CAMs were examined and CAMs with confluent pock lesions were collected and homogenized in a laboratory emulsifier (Silverson Machine Ltd) The homogenized material was sonicated with sonifier B-12 (Branson Sonic Power Company). Amount of 2% sodium deoxycholate (SDC) was added to it at a ratio of 1:1. The treated antigen was centrifuged at 2000 rpm for 10 minutes, the supernatant was collected and used as an antigen in AGPT and CIEP test.

Agar Gel Precipitation Test (AGPT):

The test was performed as described by Tripathy (1996). The medium was composed of 1gm agarose in 100 ml of 8% NaCl solution and 0.2 µl of sodium azide (0.01%). AGPT was performed using 30 µl of known positive FP antigen, test sera and a known positive control FP antiserum.

Counter immunoelectrophoresis (CIEP) test:

The test was performed as described by Das *et al.* (1990). The CIEP medium was composed of 1 gm agarose dissolved in 75ml Tris buffer and 25ml DDW. Using either tris buffer (0.2 M, pH, 7.4) or acetate buffer (0.1M, pH 5.5.), the slide was incubated in the buffer tank at a constant current (10 MA per slide). After one hour of incubation the slide was examined for the presence of precipitin lines.

Results

Agar Gel Precipitation test (AGPT):

Three weeks PV, the average seroconversion level was 100% when the vaccine was administered via feather follicle compared with 88.3 and 66.6% for wing web and scarification of comb, respectively (fig. 1). The control group showed seroconversion level of 60% but these antibodies disappeared after 7 weeks.

The seroconversion level of the birds vaccinated by feather follicle route remained constant (100%) up to 11 weeks PV, while that of wing web declined from 70% (7 weeks) to 66.6% (11 week PV). The seroconversion produced by vaccination via scarification of combs was 60% during 7 and 11 weeks PV.

Counter immunoelectrophoresis (CIEP):

Using acetate buffer, the seroconversion level was 100% up to 11 weeks for feather follicle group. When the vaccine was administered by the wing web method seroconversion level dropped from 90% at three weeks PV to 80% and 66.6% at seven and eleven weeks PV, respectively. As for scarification of comb, the seroconversion level was 66.6% at three weeks, increased to 80% at seven weeks PV and dropped to 60% at eleven weeks. The control group showed seroconversion level of 60% and 20% at three and seven weeks respectively and antibodies were not detected at eleven weeks PV as Fig 2. When tris buffer was used in CIEP test the seroconversion level in all vaccinated birds was very low Fig (3).

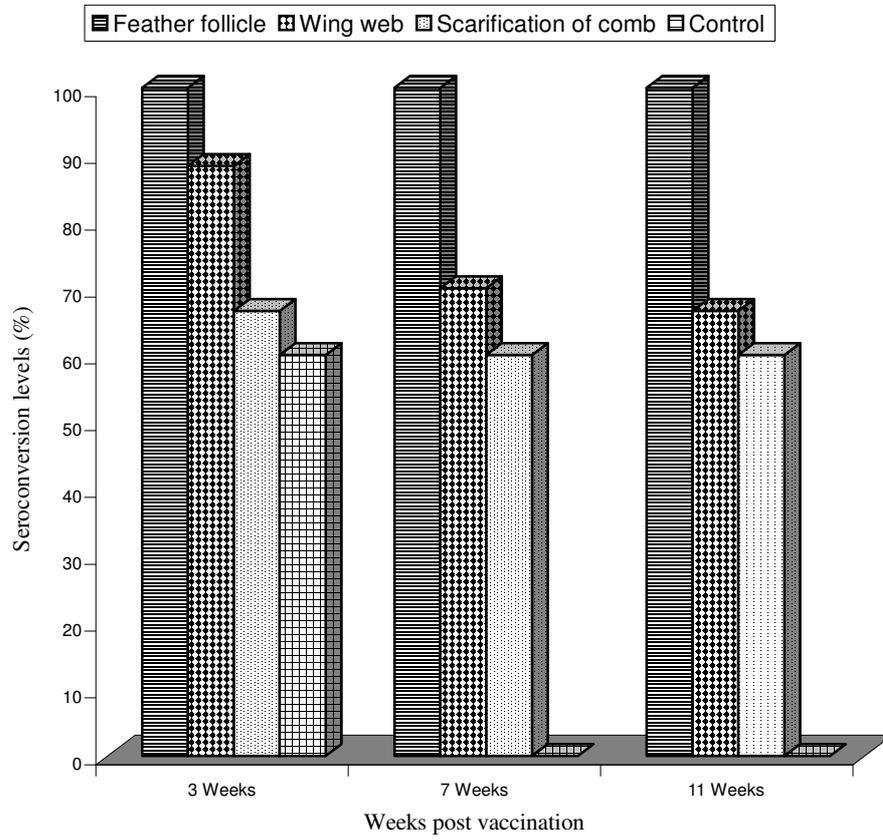


Fig 1. The seroconversion results of fowl pox vaccine as measured by AGPT

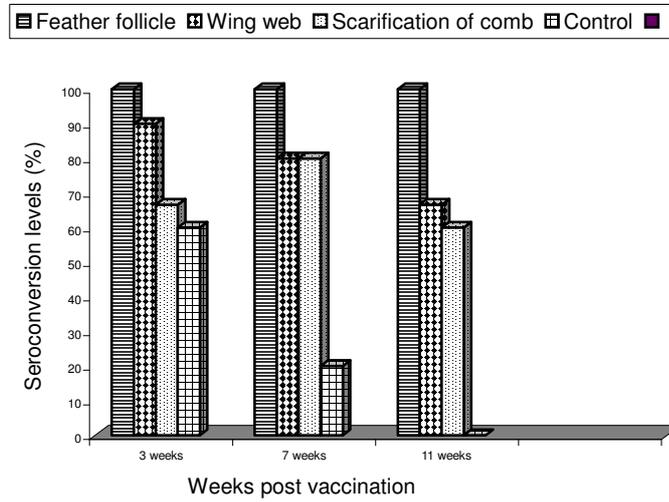
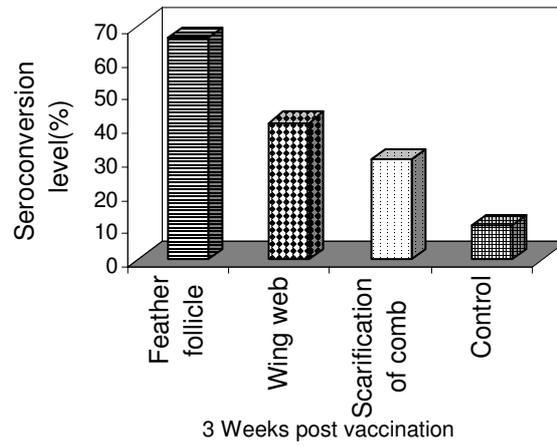


Fig 2. The seroconversion results of fowl pox vaccine as measured by CIEP using acetate buffer

Fig 3: Results of the seroconversion of Fowl Pox vaccine measured by CIEP using Tris buffer



Challenge experiment:

Twenty-one days post challenge (PC), no obvious clinical signs were detected among all vaccinated birds. As for the control group, typical clinical signs of fowlpox (scabs on comb, wattles and legs) were observed seven days PC.

Discussion

Conventionally FP vaccines were administered by the wing web method, however, trials of vaccination by other routes were reported (Hartwigk and Krasselt; 1959, Mockett, 1990; Sarma and Sharma, 1989). In this study AGPT and CIEP detected precipitating antibodies after vaccination of susceptible chickens with FP vaccine using different routes. The seroconversion level in the vaccinated birds was 100% when the vaccine was administered by F/F method compared with 88.3% and 66.6% when the vaccine was administered by wing web and scarification of the comb, respectively.

These findings indicate that vaccination by feather follicle method is the best and are in agreement with those of Hartwigk and Krasselt (1959) and Sarma and Sharma (1989). Although these authors tried feather follicles on the thigh not those on the back, yet the outcome was the same. The higher seroconversion level obtained after feather follicle administration of FP vaccine may be due to the multiple injuries made by feathers plucking which allowed the birds to receive more antigens.

The unvaccinated control birds showed low level of seroconversion 4 weeks PV as measured by AGPT and CIEP, which disappeared more quickly. Moreover, CIEP test showed lower levels of seroconversion (20%) at seven weeks PV, which disappeared again after eleven weeks. This finding might have occurred due to transmission of the vaccinal strain from vaccinated birds to the control group since they were housed in the same premises.

The AGPT was previously used to measure the immunity conferred by FP vaccine (Tripathy, 1996). However, this test failed to detect such low levels of antibodies as those of the control group, although Tripathy (1991; 1996) has recommended it for the diagnosis of FP disease. On the other hands, the counter immunoelectrophoresis (CIEP) was used for detection of antibodies to FP (Das *et al.*, 1990).

This study shows that CIEP test is more sensitive than AGPT since it did detect low levels of antibodies to FP vaccine seven weeks PV in

the control group when acetate buffer (0.1M, pH 5.5.) was used. But when tris buffer (0.2M, pH 7.4) was used it showed low level of seroconversion in birds of all the groups; 66.6% for feather follicle, 41% for wing web; 30% for scarification of the comb and 10% for the control group. Consequently, acetate buffer is preferable to tris buffer in CIEP test for FP antigen.

In conclusions, production of good immunity levels by administration of FP vaccine (Beaudette strain) via wing web, feather follicle and scarification of the comb was confirmed by means of AGPT, CIEP and challenge experiments. It is also indicated that acetate buffer is superior to tris buffer in detection of fowlpox antibodies.

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References

- Ali, B.H., Adlan, A.M., Salim, M. O., (1983) Vaccines Production in the Sudan. In: Proc. Ann. Conf. Anim. Resour. 15-20 Oct. 1983, Khartoum -Sudan
- Das, S.K. Maiti, N.K. and Sharma, S.N. (1990). *Ind. J. Anim. Sc.*, **60**(10):
- Hartwigk, H. and Krasselt, M. (1959). *Tierkeik*, **11**: 227-236.
- Sarma, D.K. and Sharma, S.N. (1989). *Trop. Anim. Hlth. Prod.*, **21**(2): 107-108.
- Mockett, A.A. (1990). Fowlpox and avian poxes. In: Poultry Disease, 3rd edn. By Jordan, F.T.W. EIBS, Bailliere, Tindall, London.
- Tripathy, D.N. (1991). Avian poxes. In: Diseases of poultry, 9th edn., Clanek, B.W., Barnes, H.J.; Beard, C.W.; Reid, W.M. and Yoder, Jr. H.W. eds., Iowa State Univ. Press. Iowa, USA.
- Tripathy, D.N. (1996). Fowlpox. In: Manual of standards for diagnostic tests and vaccines, office international De epizootic (OIE). Edited by the OIE standards commission and adopted

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by the international committee of the OIE, 8th. Edn, pp.
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