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Protecting Livestock – Improving Human Lives

Immune response in chickens to Fowl Pox Virus Vaccine and Newcastle Disease Vaccine co-administered by non-invasive routes

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Background

Newcastle Disease and Fowl Pox are viral infections of poultry. Outbreaks can have a tremendous impact on backyard chickens in developing countries where these birds are a significant source of protein. Vaccination of Fowl Pox in chicken is often accomplished by wing web puncture with a double-needle. This study assessed a non-invasive route of administration of a Fowl Pox vaccine via feather follicles in co-administration with a Newcastle Disease vaccine via eye drop. This is important, because in many developing countries para-veterinarians and community animal health workers are not legally allowed to administer parenteral products. Co-administration is beneficial in terms of reducing costs and the number of separate interventions.

Objectives

- **To measure antibody responses to Fowl Pox virus and Newcastle Disease virus in vaccinated chickens.**
- **To assess administration site reactions following the feather follicle route of vaccination against Fowl Pox.**

Materials and Methods

This study was randomised, partially blinded, parallel group designed. Eighty SPF chickens were allocated to four groups (n=20/group) and acclimatised for 14 days before the study was conducted over a period of 22 days. Each group shared the same airspace. The animal number followed sample size calculations for non-inferiority assessment between Newcastle Disease vaccination alone and its co-administration with a Fowl Pox vaccine. Blood samples were collected on Days 0, 14, and 21. Administration site reactions (= takes) were observed daily. **A take is a fowl pox lesion that consists of a swelling of the skin or a scab at the site where the vaccine was applied and its presence is evidence of successful immunisation.**

On Day 0 birds were vaccinated. A commercial prototype, live attenuated Newcastle Disease vaccine containing the I-2 strain (Avivac™ND-HS, Deltamune, South Africa) was administered intraocular at EID50 $10^{6.5}$ per 0.02mL dose into the left eye. A Newcastle Disease vaccine containing the I-2 strain was selected, because this strain is widely used in Africa.

A commercial, live attenuated Fowl Pox vaccine (Diftosec™, Merial, South Africa) containing strain DCEP 25 was administered at $CCID_{50}$ $10^{2.8}$ per dose of approx. 0.017mL by brush into open feather follicles of the left thigh.

Antibody titres to Fowl Pox virus were tested by ELISA according to OIE Manual. Antibody titres to Newcastle Disease virus were tested by haemaglutination inhibition (HI) test according to OIE standard procedures. Geometric mean HI titre values on Day 21 were assessed for non-inferiority based on a margin of 2 points on the log2 scale.

Table 1: **Study Design**

Group	No. of Birds	Test Vaccines	Dose Volume	Route of Vaccination	Age at Vaccination	Day of Vaccination	Study Completion
1	20	FP	0.017mL*	Feather follicle	5.5 weeks	Day 0	Day 21
2	20	ND	0.02mL	Eye drop	5.5 weeks	Day 0	Day 21
3	20	ND + FP	0.02mL + 0.017mL* (concurrently)	Eye drop + Feather follicle	5.5 weeks	Day 0	Day 21
4	20	ND + FP	0.02mL + 0.017mL* (concurrently)	Eye drop + Feather follicle	2.5 weeks	Day 0	Day 21

FP: Fowl Pox (**Diftosec™**, **Merial**); ND: Newcastle Disease (**Avivac™ND-HS**, **Deltamune**);
* Dose volume estimated based on total volume used.

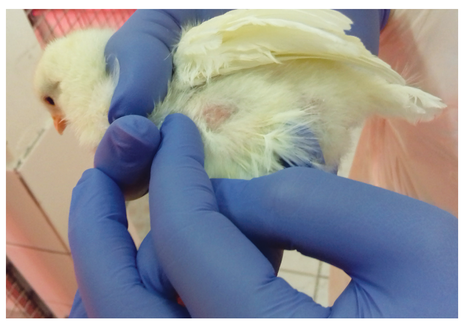


Figure 1
Application of the feather follicle route

(A) A group of adjacent feathers was plucked from an animal's left thigh and (B) a vaccine-dipped commercially available pigeon pox vaccine brush was rubbed with an upward motion inside the opening of freshly emptied, exposed holes of the feather follicles.

Results

On day four post vaccination four of the 2.5-week-old birds (Group 4) showed small circular lesions with mild swelling at the site of vaccination. One week after vaccination, 78% of all birds had taken, ranging from 70% (Group 3) to 90% (Group 4). Around the same time, takes became scabby and started to disappear. Over all groups, the percentage of birds with takes increased to a maximum of 80% by Day 10 post vaccination (70% (Group 3) – 95% (Group 4)). Eleven days post vaccination the total number of birds with takes had declined to 60%. Three weeks post vaccination all takes and scabs had disappeared.

Table 2: ***In vitro*** **monitoring of chicken immune response to vaccination**

	GM Antibody Titres					
Group	Newcastle Disease: ND-HI (log2)			Fowl Pox: S/P ELISA		
	Day 0	Day 14	Day 21*	Day 0	Day 14	Day 21*
1		not ND vaccinated		0.09	0.21	0.31[a]
2	0.40	8.35	8.13[a]		not FP vaccinated	
3	0.10	8.43	8.23[a]	0.07	0.20	0.34[a]
4	0.00	7.97	8.15[a]	0.06	0.15	0.17[b]

ND-HI: Newcastle Disease Haemagglutination Inhibition Test. HI antibody titres were regarded as positive if there was inhibition at a serum dilution of 1/16 (4 log2 when expressed as reciprocal) or more. S/P: Sample-to-Positive ratio; GM: Geometric Mean. *Different superscripts show significant differences at $p \leq 0.05$.

Figure 2 **Immune response to Fowl Pox vaccination by feather follicle route**

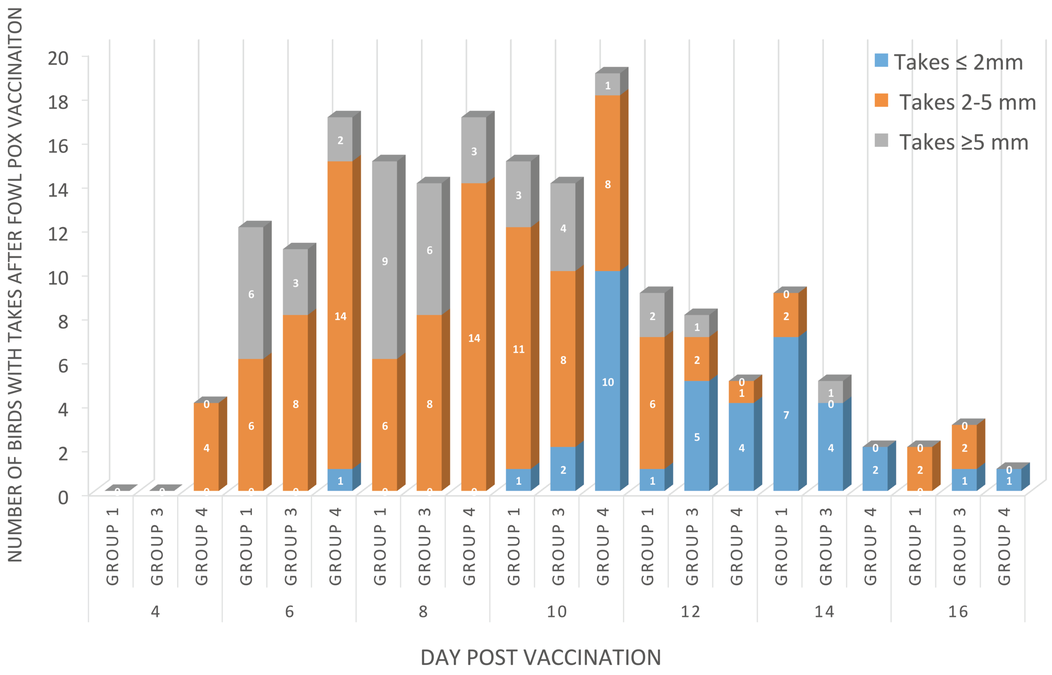
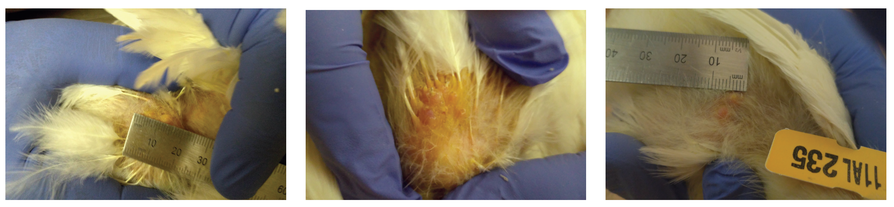


Figure 3
Takes after vaccination

(A) a Group 1 chicken on Day 6; (B) a Group 3 chicken on Day 8; (C) a Group 4 chicken on Day 5



Conclusion

Under field conditions Fowl Pox vaccine can in principle be administered by the feather follicle route at the time of Newcastle Disease vaccination.

1. Immunisation after Fowl Pox vaccination via feather follicles was shown by the development of takes. Published data confirms that birds that develop takes after Fowl Pox vaccination via wing web have good protection levels against challenge virus (El-Mahdy, 2014). However, this study indicates that the age of a chicken might have an effect on its immune response to Fowl Pox vaccination. Additional data are needed to assess if a booster vaccination might be required for chickens when vaccinated before 5-weeks-old.
2. Good immunity levels to Newcastle Disease vaccination was shown by ND-HI serology, which is clearly related to the immune status of the bird.

References

El-Mahdy, S.S., Mikheal, C. (2014). Efficacy of fowl pox vaccines against Egyptian isolated strain during 2012. *Veterinary World*, 7(9):656-660.

OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2016, Chapter 2.3.14, Newcastle Disease.

OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2016, Chapter 2.3.10, Fowl Pox.

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