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ORIGINAL ARTICLE

Protective antibody response following oral vaccination of feral pigeons (*Columba livia*) with Newcastle disease vaccine (strain I-2) coated on oiled rice

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Abstract The novel vaccination technique for feral pigeons was developed in the present study. Multi-age feral pigeons were vaccinated orally with Newcastle disease (ND) strain I-2 vaccine coated on oiled rice. The results showed that 14 days after vaccination 40% of pigeons seroconverted with HI GMT of ≥ 3 $\log_2$ whereas 28 days after vaccination the seroconversion rate of these birds reached 100%. Moreover, all vaccinated pigeons survived the challenge of virulent *Newcastle disease virus* (NDV). The findings from the present study indicated that the use of ND (strain I-2) vaccine in feral pigeons is feasible and resulted into the production of protective antibody response. Thus ND I-2 vaccine may prevent the spread of NDV to other birds particularly chickens. Furthermore the use of oral vaccine in feral multi-age pigeons overcomes the difficulty of catching these birds for individual vaccination.

Keywords *Columba livia* · Feral pigeons · Newcastle disease · Oral vaccine · Strain I-2

Abbreviations

EID_{50}	median embryo infectious dose
GMT	Geometric mean titre
HA	haemagglutination
HI	haemagglutination-inhibition
NDV	*Newcastle disease virus*

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Introduction

Newcastle disease (ND) is the most important disease affecting a wide range of bird species including pigeons (Alexander 1997). Newcastle disease can be a serious problem in pigeons. Pigeons can be infected with *Newcastle disease virus* (NDV), and when infected, they show clinical signs, such as depression, diarrhea, nervous signs, inability to fly and sudden death (Biancifiori and Fioroni 1983). Like infected chickens and turkeys, all ages are susceptible and can experience very high mortality. For instance Mangat et al. (1988) reported 60% mortality of racing pigeons in ND field outbreaks.

Previous studies by Alexander et al. (1984); Roy et al. (2000); Kapczynski et al. (2006); Onapa et al. (2006) and Carrasco et al. (2008) showed that NDV can be introduced to the free range chicken flocks through contamination of feed and water by droppings of ND infected pigeons. Because of their flying ability, pigeons have potential to disseminate NDV over a wider area among different bird species in naïve flocks and result into major epidemics (Alexander 1995; Alexander, 1997; Toro et al. 2005). Therefore the control of ND in pigeons is important due to the eminent risk posed by these birds in spreading ND.

Vaccination is the most effective method of controlling ND in poultry (Meulemans 1988). Conventional vaccination techniques are not easily applied to pigeons, because these birds are free-range flyers. Catching these birds for individual vaccination is somewhat difficult. Novel vaccination techniques for pigeons are required. Oral vaccination of pigeons using food will be a suitable approach because there will be no need to catch these birds individually.

No study has been done so far to use food for delivery of ND vaccine (strain I-2) to feral pigeons. Food-based ND vaccines have been used for vaccination of free range chickens (Spradbrow 1992; Samuel et al. 1993). Recently, Wambura (2009) successfully used ND vaccine (strain I-2) coated on oiled rice to vaccinate chickens. Similar techniques can be applied to control ND in feral pigeons. It was therefore the objective of the present study to establish a vaccination regime for pigeons and evaluate the efficacy of I-2 ND vaccine in these birds.

Materials and methods

Source of the virus for vaccine production

I-2 vaccine was produced locally at Sokoine University of Agriculture (SUA). The virus was obtained from a freeze-dried master seed of strain I-2 of NDV. This was propagated in embryonated eggs to produce the vaccine. The strain I-2 has no commercial ownership and is produced and supplied by the John Francis Virus Laboratory at the University of Queensland, Australia (Spradbrow and Copland 1996).

Source of eggs and propagation of strain I-2

All eggs used in this study were obtained from a hatchery at the Department of Veterinary Microbiology, SUA where the laying chicken flock tested negative to NDV antibody. Eggs were bought when they were 9-day-old after incubation and were used the following day. The propagation of I-2 vaccine was done as described by Spradbrow et al. (1995) and Alexander (1998). Briefly the eggs were incubated for 96 hours after inoculation of the I-2 virus followed by harvesting of the allantoic fluid. The allantoic fluid was tested for haemagglutination and clarified by centrifugation.

The preparation of rice

White long grain rice (Kyela) was obtained at normal retail outlets at Morogoro Central Market. Rice (10grams) was mixed with 1mL of blended vegetable oil (Sunola®) before mixing with the vaccine. The mixture was left for about 3 hours to let it thoroughly soak.

Rice mixed with oil was separately coated with the vaccine by shaking in a bottle while the vaccine was dripped onto it from a syringe. 10gram rice were mixed with 1mL of vaccine ($10^{7.5}$ EID_{50}/0.1mL) which made a nominal dose (Wambura et al. 2007). The mixture was prepared in the evening of the previous day ready for use in the next morning.

Source of birds and management

Pigeons of different ages were bought from different poultry keepers in Morogoro municipality. The birds were screened for NDV antibody and those which were found to be seronegative were included in the study. The birds were given chicken mash and water ad libitum during the day only.

Pigeons were kept on floor with deep litter during the day, however during the night they roosted in their individual lofts. This simulated the village condition where pigeons leave early in the morning for scavenging of food and return to their lofts late in the evening. Before the vaccination trial begun pigeons were housed for 14 days in animal isolation unit in order to be adapted to captivity.

Experimental design

Thirty (30) multi-age pigeons were randomly divided into two groups of 15 birds each. Group 1 pigeons were vaccinated orally with ND vaccine (strain I-2) coated on oiled rice whereas pigeons in group 2 were not vaccinated and were kept in a separate room to serve as a control.

The pigeons in Group 1 were given the vaccine where each pigeon was estimated to eat 10g of vaccine coated rice (nominal dose). Thus 150g of vaccine coated rice were spread on a mat and the 15 pigeons were allowed to pick up until the rice was over. The vaccine was given to pigeons early in the morning before they were given any food. The pigeons were observed two times per day throughout the duration of the experiment to check for any abnormal behaviour.

Measurements of antibody response

Blood sample collection

Blood samples were collected aseptically from brachial vein of each pigeon on days 0, 7, 14, 21, 28 and 35 days after vaccination. The blood was allowed to clot to extract serum. Thereafter serum samples were stored frozen at -20ºC and subsequently used for a serological test.

Serological test

The haemagglutination-inhibition (HI) test was used with 4HA units of I-2 strain of NDV. The results were recorded as $\log_2 X$ of the reciprocal of the highest dilution which showed inhibition. The procedure for HI test used was as described previously by Allan and Gough (1974). Geometric mean titres were calculated in each experimental group.

Table 1 Antibody response in pigeons vaccinated orally with I-2 coated on oiled rice

Group	Antibody response at days after vaccination					
	0 [b]	7	14	21	28	35
Vaccinated[a]	<1[c]	<1	2.0	3.3	3.6	4.3
Unvaccinated	<1	<1	<1	<1	<1	<1

[a] = Nominal dose per pigeon (EID_{50})

[b] = Geometric mean titre (GMT)

[c] = Number of pigeons (n=15)

Challenge trial

Thirty five (35) days after vaccination, all pigeons in both groups were challenged with NDV virulent strain (10^3 CLD_{50}) which is pigeon origin had mean death time (MDT) for chicken embryo of 48h and intracerebral pathogenic index for day-old-chicks of 1.8 by inoculation into the nasal sinus. After challenge, the pigeons were examined daily for clinical signs consistent with ND. After the challenge trial was completed surviving pigeons were disposed off by euthanasia.

Results

Acceptability of oiled rice coated with ND vaccine

The pigeons in vaccinated group ate the oiled rice coated with ND (I-2) vaccine within 30 minutes without leftovers.

Antibody response and seroconversion

Table 1 indicates the NDV antibody response in pigeons vaccinated orally with I-2 vaccine coated on oiled rice. Moreover seroconversion results summarized in Table 2 showed that 14 days after vaccination 40% of pigeons seroconverted with HI GMT of ≥ 3 $\log_2$ whereas 28 days after vaccination the seroconversion rate of these birds reached 100%. All unvaccinated pigeons did not seroconvert as they were negative to NDV antibody throughout the study period.

Table 2 Seroconversion rates in pigeons vaccinated orally with I-2 coated on oiled rice

Group	Seroconversion percentage (%) with cut-off of HI titre ≥ 3 $\log_2$ at days after vaccination[a]					
	0	7	14	21	28	35
Vaccinated	0 (0/15)	0 (0/15)	40 (6/15)	53 (8/15)	100 (15/15)	100 (15/15)
Unvaccinated	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)

[a] The cut-off titre of ≥ 3 $\log_2$ is considered protective against field challenge by virulent NDV

Challenge trial

The results showed that 100% of all vaccinated pigeons (n=15) survived the challenge of virulent NDV and no signs of ND were observed. However, all unvaccinated pigeons (n=15) succumbed to the challenge and died of the signs consistent with ND (diarrhoea, nervous signs inability to fly and finally death within 3 days).

Discussion

Newcastle disease is an important disease which is a threat to the development of poultry industry if not effectively controlled. Therefore appropriate control strategies should be instituted. It has been difficult to establish vaccination regimes for free-range birds as most of the vaccination techniques are conventional and designed for use in domestic birds (Bailey et al. 1998). It was therefore the aim of the present study to develop the vaccination regime for feral pigeons.

Development of novel vaccination techniques against ND in free range wild birds which might act as carriers of NDV is important undertaking in the fight against this disease especially in chickens. The vaccination regime in feral pigeons if implemented effectively will avoid the circulation and spread of NDV to domestic birds (Carrasco et al. 2009).

The results from the present study revealed that ND (strain I-2) vaccine coated on oiled rice is safe and efficacious when used in feral pigeons. These results are in agreement with the findings from the study by Carrasco et al. (2009). Similar results have also been reported in chickens when ND strain I-2 vaccine coated on oiled rice was administered orally (Wambura 2009). Production of antibody titre of $\geq$ 23 or 2log3 by vaccinated chickens is considered to be protective in chickens against field challenge of NDV (Bensink and Spradbrow, 1999). This phenomenon could also apply to pigeons as previous study by Duchatel et al. (1992) showed that pigeons were protected against NDV virulent challenge with HI titre not exceeding 2log3.

Kapczynski and King (2005) suggested that current vaccination techniques should aim at producing a protective immunity with minimum antagonistic response in the birds. It is noteworthy that no adverse effects were observed in pigeons used in the present study.

The findings from this study indicated that the use of ND (strain I-2) vaccine in feral pigeons is feasible and resulted into the production of protective antibody response. Thus ND I-2 vaccine may prevent the spread of NDV to other birds particularly chickens. Moreover use of oral vaccine in feral multi-age pigeons overcomes the difficulty of catching these birds for individual vaccination. The pigeons could be vaccinated early in the morning before they leave for scavenging for food.

If the current vaccination technique is optimised it may be useful for vaccination of feral pigeons and minimise the risk of transmission of NDV from these birds to domestic poultry.

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