Immunogenicity of Newcastle Disease Vaccine in Southern Ground-hornbill (Bucorvus leadbeateri)

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Abstract

The southern ground-hornbill (*Bucorvus leadbeate*ri; hereafter SGH) is endangered in South Africa, Namibia, and Swaziland. Through a conservation program established in South Africa by the Mabula Ground Hornbill Project, wild populations are being re-established by the reintroduction of captive-reared birds. The SGH is susceptible to infection with avian avulavirus 1, which causes Newcastle disease (ND). Four different vaccines to protect against ND were administered through various vaccination schedules and evaluated by serologic monitoring to assess the efficiency and safety of various combinations of vaccines (live versus inactivated/killed), vaccine strains (Ulster strain, live; Avivac Cellimune, live; VG/GA strain, live; and Avivac Struvac, killed), and administration routes (intraocular versus subcutaneous versus intramuscular injection versus oral). We vaccinated 75 individuals and evaluated antibody titers in 53 individuals (24 juveniles, 13 subadults, and 16 adult SGH; 26 males and 27 females) over a period of 9 years. Antibody titers to avian avulavirus 1 in sera were monitored by a hemagglutination inhibition test. Protective titers were generated with 3/6 vaccine regimes tested in the SGH. The highest vaccine titers were established in birds vaccinated with the Ulster strain in the conjunctiva and followed with an intramuscular Struvac injection (mean $\log_2$ titer 8.6 ± 2.6) booster. Our aim was 1) to assess whether optimal vaccination protocols could be developed and 2) to then be able, by oral administration, to remove the need to recapture free-roaming, reintroduced birds to administer the initial vaccine or booster, thus remove the threat or mortality associated with ND to this endangered avian species in both captive birds and birds released back into the wild.

Key words: avian avulavirus 1, Newcastle disease, vaccine, conservation, endangered, reintroduction, avian, southern ground-hornbill, *Bucorvus leadbeate*ri

INTRODUCTION

The southern ground-hornbill (*Bucorvus leadbeateri*; hereafter SGH) is endangered in South Africa, Swaziland, and Namibia.1 In South Africa, SGH numbers are still declining outside of protected areas.[1] The SGH is classified as Vulnerable in the remainder of its subequatorial African range, likely because of data deficiency rather than a true reflection of population status.[2] A long-term field study started in 1973 in the Kruger National Park, South Africa, developed into a national SGH conservation restoration program. The Mabula Ground Hornbill Project and the national Action Group continue this work, with the aim to slow, and then reverse, the population decline. The primary conservation tool used is reintroduction, using harvested second-hatched chicks. Because SGH exhibits obligate brood reduction, second-hatched chicks die in the nest of parental neglect.[3] These chicks are harvested within 2 days of hatching and are reared in captivity.[3,4] Suitable birds are then reintroduced into the wild, as helpers, into existing ''bush-schools,'' which are intensively monitored family groups where naïve juveniles obtain wild experience from experienced conspecific mentors. Once skilled, these captive-reared SGHs are then used to lead entirely new groups, as mentors themselves.[5]

In 2006, 2 SGH (1 juvenile male at 8 months and 1 subadult female at 2.5 years) were reintroduced to a reserve in the North West Province, South Africa (Fig 1).[4] The female died 47 days postrelease and the male at 62 days postrelease. The release site was adjacent to a district with known Newcastle disease (ND) in poultry. Postmortem examinations of both released birds, including polymerase chain reaction diagnostic testing for both birds, confirmed death from virulent ND; only 1 isolate was able to be sequenced, which revealed a virulent strain of pigeon paramyxovirus (PPMV, an antigenic variant of Newcastle disease virus (genotype VI; lineage 4bi).[6,7] These SGHs were reported to have scavenged on dead doves around a waterhole, which was considered the likely source of infection considering a closely related PPMV-1 strain was also isolated from doves collected nearby. The ground-hornbill isolate had intracerebral pathogenicity and mean death time values characteristic of PPMV-1 strains. No other mortalities in wild SGHs have been attributed to ND to date, but this disease in birds is reported for the entire range of this avian species, from the extreme south of the sub-Saharan range in South Africa[8] to the northern extremes of the range in Kenya.[9] Figure 1 shows the extent of reported ND in poultry from 2006 to 2018 mapped against the current distribution range of the species, with data collated from atlases, incidental records, and information solicited through citizen science. Additionally, the sites where SGH reintroductions have occurred are also shown.

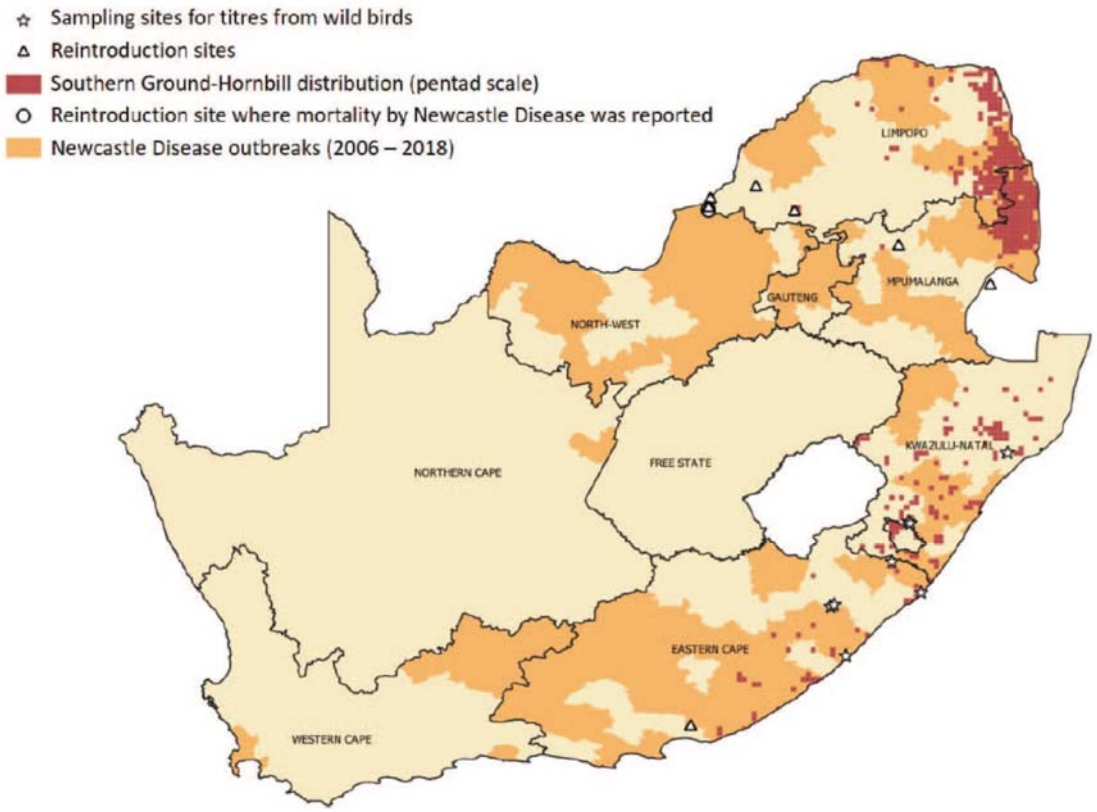

Figure 1. A map showing municipalities (in orange) in South Africa (as a proxy for state veterinary districts) where Newcastle disease (ND) outbreaks had been reported (from January 2006 until March 2018). Southern ground-hornbill (SGH) occurrence data (red boxes) is noted for the same period. Current and past SGH reintroduction sites are noted (triangle), as well as the site of the reintroduction and mortalities from ND of the SGHs released in 2006 (circle). Sample sites for avian avulavirus 1 antibody titers from wild SGH are indicted by stars.

Harvest, rearing, and reintroduction of SGHs is resource intensive; therefore, to prevent further mortalities, an ND vaccination program was implemented in 2008. Because reintroduction candidates need to remain as wild as possible, suitable ND vaccination protocols that minimized human contact required investigation and development. These birds moved freely post-reintroduction, so it was imperative that the ND vaccine program posed no threat to the SGH but also had no unintended negative consequences for other avian species. Additionally, monitoring of released individuals is not always possible; therefore, long-term and immediate efficacy of the vaccine was vital to the success of the project.

Newcastle disease is caused by multiple virulent strains of avian avulavirus 1 (AAvV1) antibody titers, formally known as avian paramyxovirus type 1.[10,11] Avian avulavirus 1 causes an acute, highly contagious, pneumoencephalitis in both domestic and wild avian species, with considerable economic and conservation implications.[12,13] Newcastles Disease is widely distributed geographically and is endemic in most southern African countries.[1,14] This viral disease has been associated with mass mortalities in birds, with annual losses as high as 80%reported for backyard poultry in Africa.[15] Avian avulavirus 1 antibody titer prevalence in wild bird populations was investigated (n-9000) in Madagascar, Zimbabwe, Mauritania, and

Mali, with up to 10% testing positive but averaging 3% depending on season, site, and species involved.[15] This result suggests that ND is endemic in southern African wild bird populations. Newcastle disease has also been diagnosed in a variety of scavenging raptors and is associated with feeding on infected carcasses.[16,17]

Virulence of the AAvV1 strain is determined by a cerebral pathogenicity index ≥0.7.[18] Strains of AAvV1 have been classified into 2 major divisions represented by classes labeled I and II, with class I consisting of 1 genotype and class II of 15 genotypes (I–XV).[19,20] Pigeon paramyxovirus was divided into 2 distinct lineages: 4bi (the ground-hornbill isolate) and 4bii.[6] The AAvV1 class II, III, IV, and XI genotypes are closely related to PPMV on phylogenic analysis.[20] The VIId lineage of AAvV1, which circulated in poultry in 2006 has recently (2015) been replaced by the VIIh lineage in South Africa.[7,14] Low virulent forms of AAvV1 have been detected in wild birds and are often subclinical infections.[21]

Newcastle disease vaccines are available in live, inactive, or attenuated commercial products.[11] The ideal vaccine should increase the birds' resistance to natural virus challenge without adverse reaction to the injected preparation.[11] In poultry, live vaccine is usually administered first to increase mucosal immunity, followed by an inactivated vaccine to increase the concentration of circulating antibodies. Live vaccine can result in side effects, whereas inactivated vaccines do not cause any known side effects other than local tissue reaction. Any ND vaccine should protect against any virulent ND, including PPMV, but vaccine efficacy varies depending on antigenic differences between strains.[11] Newcastle disease vaccine has been investigated previously in an endangered avian species, the houbara bustard (*Chlamydotis undulata undulata*).[23] Three ND vaccines (2 live, 1 killed) were tested in houbara bustards that resulted in immunity for >9 months, with the inactive vaccine in adjuvant consistently resulting in the highest titers after 2 subcutaneous vaccinations 4 weeks apart.[23] The main objective of this study was to develop a species-specific ND vaccination protocol that could be used for SGHs in the captive breeding program and, more importantly, for the reintroduction program,[22] where the vaccine, boosters, or both had to be administered without the need to recapture the now free-roaming birds.

MATERIALS AND METHODS

Seventy-five second-hatched SGH chicks were collected from wild nests and raised in captivity at various zoological facilities in South Africa as part of a conservation initiative. For more details about the reintroduction protocols, please see Kemp et al (2020)[22]. Antibody titers were measured in 53 captive birds (males =26, females =27). The ages were as follows: 24 juveniles up to 1 year old, 13 subadults between 1 and 3 years old, and 16 adults >3 years old. Eleven wild birds also were tested for AAvV1 antibody titers from the Eastern Cape, KwaZulu-Natal, and Limpopo Provinces during a project primarily aimed at DNA collection.[5]

Vaccines

To select the best ND vaccine or vaccine combination for SGHs, 4 strains or vaccines of

locally available commercial products were used. A live Ulster strain (Deltamune Isolation Bank, Pretoria, Gauteng, South Africa) was administered either as an eye drop or orally in 1-day-old SGH chicks. At least 10^7 50%embryo infectious dose was administered per bird. The inactivated Avivac ND Struvac Plus (Deltamune, Pretoria, Gauteng, South Africa) was administered either subcutaneously or intramuscularly. Most inactivated ND vaccines for chickens usually contain 10^9 50% embryo infectious dose/mL, which results in poor seroconversion in ostriches (*Struthio camelus*). Avivac ND Struvac Plus contains approximately double the amount of antigen, and the adjuvant causes very little tissue reaction in ostriches, which are very sensitive to scar formation. This strain was therefore selected for use in the SGHs to avoid any scarring that could result in reduced flying ability. A live Cellimune strain (Avivac Cellimune ND, Deltamune) was administered intramuscularly, and a fourth live VG/GA strain (Avinew, Merial Animal Health Limited, Lyon, France) was given orally to the birds. Vaccine protocols for the SGHs were based on vaccination protocols used for ostriches.

Vaccine schedules

The initial vaccine and booster protocols for testing immunogenicity in the SGHs are described in Table 1. Six different initial vaccine trials and 3 different booster vaccine routes or types were compared.

Table 1. Description of Newcastle disease vaccine type, timing, and method of administration for 53 southern ground-hornbills (24 juveniles, 13 subadults, and 16 adults of 26 males and 27 females) receiving 4 different vaccines in 6 trials along with booster vaccinations over a period of 9 years.

Trial	Vaccine type	Route	Day	Method of administration
1	Ulster strain (live)	ED	0	Inoculation with 1 drop into each eye
	Avivac ND Struvac Plus (killed)	SC	21	SC injection (1 mL)
2	Ulster strain (live)	ED	0	Inoculation with 1 drop into each eye
	Avivac ND Struvac Plus (killed)	IM	21	IM injection (1 mL)
3	Ulster strain (live)	ED	0	Inoculation with 1 drop into each eye
	Avivac Cellimune ND (live)	IM	21	IM injection (0.5 mL)
4	Chicken embryos inoculated with VG/GA strain: Avinew (live)	PO	0	Oral: Chicken embryos fed to captive SGHs
5	Chicken embryos inoculated with Ulster strain (live)	PO	0	Oral: Chicken embryos fed to captive SGHs
6	Chicken embryos inoculated with Ulster strain (live)	PO	0	Oral: Chicken embryos fed to captive SGHs
	Avivac ND Struvac Plus (killed)	IM	21	IM injection (1 mL)
	Vaccine booster		Frequency	
1	Avivac ND Struvac Plus	SC	Annually	SC injection (1 mL)
2	Avivac ND Struvac Plus	IM	Annually	IM injection (1 mL)
3	Ulster strain (live)	PO	Annually	Vaccine produced from chicken embryos injected into day-old chickens and fed to captive SGHs (1 mL)

Abbreviations: ED indicates topical to the eye; SC, subcutaneous; IM, intramuscular; PO, oral; SGH, southern ground-hornbill.

Clinical response

A pilot vaccine study was conducted at the Johannesburg Zoo in 2008 on two 18-month-old hand-reared SGHs (1 male and 1 female). After being vaccinated with the live Ulster strain primer as eye drops, followed by an inactivated vaccine 4 weeks later, the birds were monitored over a period of 28 days for changes in behavior, food intake, and bodyweight. At

the end of the monitoring period, the 2 pilot study birds were subjected to a complete physical examination. All SGHs in the ND vaccine trial groups described in this study were monitored for body condition score, swelling of eyes, irritation at injection site, food intake, and behavior. Birds were not moved for vaccination trial. Chicks were moved for release postfledging.

Blood sampling and serology

For all trial groups, plasma samples were collected 28 days after vaccination by venepuncture from the medial metatarsal or brachial vein and stored at -20°C (-4°F) in heparin before analysis. Antibody titers to ND were determined by the hemagglutination inhibition (HI) test, which is conventionally used to detect and quantify ND antibodies.[24] Serology was performed by Deltamune Pty Ltd (Lyttelton, Gauteng, South Africa). Hemagglutination inhibition titers were regarded as being positive with inhibition at a serum dilution of 1/16 (2^4, or $\log_2$ 4 when expressed as the reciprocal), as recommended by the World Organization for Animal Health.[24]

Statistical analysis

Log-transformed titers were compared by Kruskal-Wallis equality of populations rank. Log-transformed titers of booster vaccines were compared over time by a linear mixed model with Bonferroni correction for multiple comparisons. Data were analyzed by Stata 14 (StataCorp, College Station, TX, USA), and statistical significance was assessed at P <.05.

RESULTS

The pilot group showed promising antibody titers after eye drops and booster vaccine 3 weeks later (HI $\log_2$ 6, n -2), and no adverse physical effects were observed. The number of birds recruited to each trial per year is shown in Table 2. Trial 1 yielded adequate titer values (mean ±SD; 7.2 ± 2.6, n = 21) for initial vaccine responses and for the booster inoculation. In trial 2, the booster was given intramuscularly instead of subcutaneously, yielding slightly higher titers (8.6 ± 2.6, n = 5). The administration of the Struvac booster was changed from subcutaneous (trial 1) to intramuscular (trial 2) because higher titers were achieved without any significant adverse physical reaction from the 2015 and 2016 annual vaccinations.

Table 2. Newcastle disease vaccine study in the southern ground-hornbills described in Table 1 showing trial type and booster vaccination per year and number of birds vaccinated.

	Year									
	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Trial no. (sample size)	1 (4)	0	0	0	1 (10) 3 (1)	1 (5) 3 (2)	1 (7) 4 (2)	1 (2) 2 (6) 5 (3)	1 (3) 5 (5) 6 (5)	5 (20)

Trials 3, 4, and 5 were discontinued. Trial 3 had lower titers (7.3 ± 2.1, n = 4) compared with trial 2 (8.6 ± 2.6, n = 5) but higher than trial 1 (7.2 ± 2.6, n = 21). Trials 4 and

5 did not show an adequate antibody response (trial 4 mean titers 2.5 ± 2.12, n = 2; trial 5 mean titers 2.8 ± 2.59, n = 5). Titers <4 HI log_2 were regarded as not protective.[24] Trial 6 showed excellent antibody responses with high mean values (8.0 ± 0, n = 3) when compared with all previous trials, with the exception of trial 2 (Fig 2). Titers between trials 1 and 5 were significantly different (*P* = .018), but not in titers for trials 1–3 (*P* >.05). The HI (log_2) of the initial trials—trial 1 Ulster strain by eye drops followed by Struvac subcutaneously; trial 2, Ulster by eye drops followed by Struvac intramuscularly; and trial 6, Ulster orally followed by Struvac intramuscularly—were compared with various boosters: Struvac subcutaneously (booster 1), Struvac intramuscularly (booster 2), or Ulster orally (booster 3).

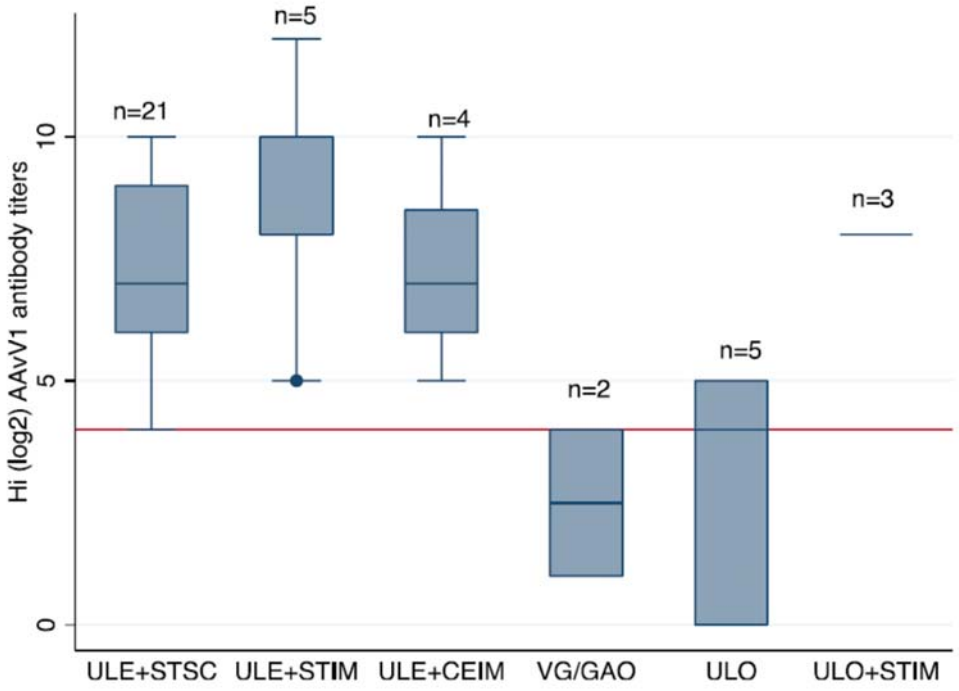

Figure 2. Hemagglutination (HI) avian avulavirus 1 antibody titers log_2 (±SD) between different vaccine trials 4 weeks postvaccination in the southern ground-hornbill. Ulster by eye drop and Struvac subcutaneous (ULE+STSC), Ulster by eye drop and Struvac intramuscular (ULE+STIM), Ulster by eye drop and Cellimune intramuscular (ULE+CEIM), Avinew VG/GA oral (VG/GAO), Ulster oral (ULO), and Ulster oral and Struvac intramuscular (ULO+STIM). Error bars indicate standard deviation. HI titers were regarded as protective with inhibition at a serum dilution of 1/16 (log_2 4) (red horizontal line)

Titers were maintained at a significantly higher level at 13 months when the Struvac vaccine was administered intramuscularly (mean HI = 9.4, n = 7) compared with subcutaneously (mean HI = 4.4, n = 10; *P* <.001). Oral vaccine titers were significantly lower (mean HI = 4.4, n = 10) at 37 months compared with injectable vaccine titers (mean = 9.43, n = 7; *P* =.003). Even with a missed booster vaccine at 13 months, titers at 37 weeks were not significantly different between the injectable Struvac vaccines. Serological response declined once annual subcutaneous boosters with the Struvac vaccine were discontinued (Fig 3).

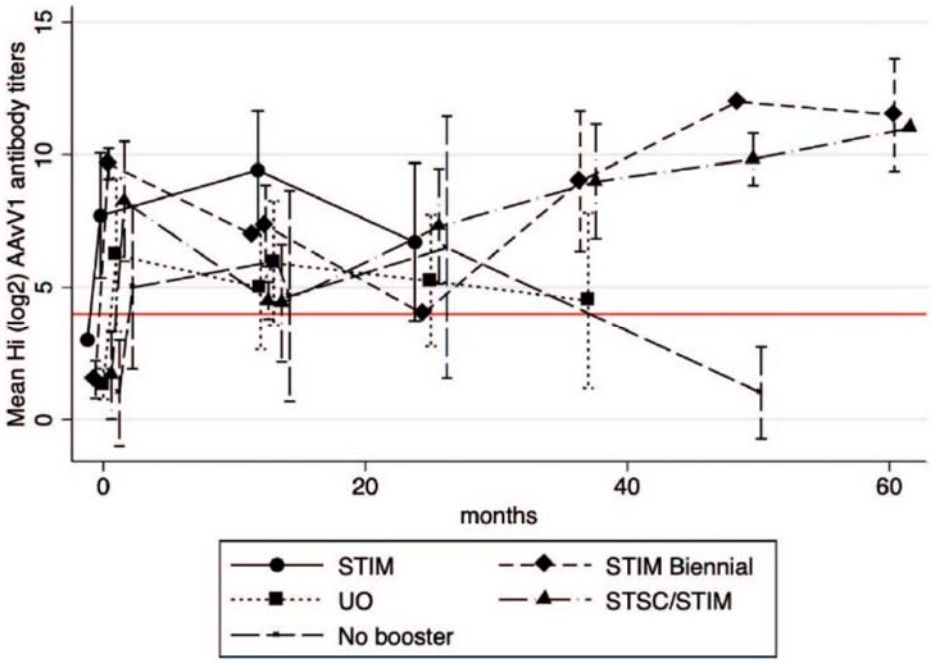

Figure 3. Mean log_2 hemagglutination (HI) avian avulavirus 1 antibody titers between different booster vaccine administrations over time in the southern ground-hornbills. Booster vaccine Struvac intramuscular (STIM), Ulster oral boosters (UO), no booster after initial vaccine (No booster), Struvac intramuscular booster biennial (STIM Biennial), and a combination of Struvac subcutaneous (13 and 25 months) and intramuscular boosters (37, 49, and 61 months) (STSC/STIM). Error bars indicate standard deviation. HI titers were regarded as protective with inhibition at a serum dilution of 1/16 (log_2 4) (red horizontal line)

Eleven wild birds, 10 adults and 1 subadult of 7 males and 4 females, were sampled for ND antibodies. The birds were captured in the Eastern Cape (n = 4), KwaZulu-Natal (n = 6), and Limpopo (n = 1) provinces (Fig 1). Newcastle disease antibody titers for all birds were negative except for 1 bird from Limpopo, which had a HI titer (log_2) of 4.

DISCUSSION

This study evaluated the administration of ND vaccine and antibody response to the vaccine in SGH, an endangered African avian species. Because of the scarcity of available SGH study subjects, data were accumulated over an extended time period. Retrospective data was included in the analysis, and it was not possible to standardize the techniques and sampling intervals fully. Nevertheless, valuable data were collected, and useful findings were obtained. Vaccination of SGHs with live Ulster strain in eye drops followed by subcutaneous administration of Struvac vaccine provided antibody levels considered sufficient to protect the species against natural infection from AAvV1. This protocol was shown to be effective, but the birds required annual vaccination boosters to prevent titers from dropping below HI 5, the protective levels for other avian species against AAvV1 field challenge.[24] Antibody titers of ≤HI 5 were believed to be effective in protecting endangered houbara bustards against AAvV1; however, no clinical challenges were performed in that study.[23] Intramuscular boosters were superior to subcutaneously administered boosters for SGH.

In practice, the oral vaccine provided in a food item is superior to injectable vaccine, in that it reduces handling stress by vaccination of released birds without recapture. However, the oral vaccine, by itself, did not provide adequate antibody titers and required a follow-up intramuscular injection. The intramuscular injections are acceptable with fledging ground-hornbill chicks that are less stressed when handled but, unfortunately, not with adult birds. SGH chicks should not be vaccinated by injection until they are 3 weeks old to prevent interference of maternally derived antibodies with the vaccine response.[11]

In the trial vaccine, titers >4 IU were protective against ND according to Office International des Epizooties guidelines,[24] but no challenge studies were done with SGHs vaccinated in this study because of the endangered status of the birds. Two birds below the benchmark titer (HI =4) were released back to the site where the outbreak occurred and subsequently survived for 6 years before being relocated. No further SGH deaths, following the birds described earlier that led to this study, from ND have been reported at any of the release sites postvaccination (Fig 1).

Hemagglutination inhibition is used to evaluate the serological response to ND vaccines. The HI serological response in chickens is directly correlated with the quality and quantity of antigen present in different vaccines.[25] Vaccination provides early protection against ND, despite low or absent detectable antibodies in the blood. This reaction is thought to be due to local immunity in the respiratory tract.[26] Cell-mediated immunity to AAvV1 has occurred in chickens from 3 days postvaccination, much earlier than in HI titers that were only detected from 7 days postvaccination.[26]SGH may also develop local and cell-mediated immunity to AAvV1.

In chickens, higher AAvV1 titers have been reported in chicks vaccinated at 7 versus 12 weeks of age.26 Vaccination should be performed as early as possible in SGHs, ideally between 3 and 4 months of age when fledging, but at least before they are 1 year old. Revaccination (eg, administration of the booster dose) could perhaps be timed to coincide with the period preceding the next breeding season. The highest peaks of ND in wild avian species have been reported for November and February in Zimbabwe, which coincides with the SGH breeding season, which lasts from October through March.15 Vaccination of SGHs for ND should ideally occur before the breeding season to ensure the highest immunity at the time of highest risk.

The only SGHs known to be affected by ND to date were reintroduced birds that were captive-raised from 2 days of age. Thus far, only 1 positive titer has been detected in wild SGHs (n = 25). The positive bird originated from a wild population on a private game reserve adjacent to the Kruger National Park. The SGH population in South Africa is still declining and should be considered at risk of ND. A full postmortem examination, including testing for AAvV1, on carcasses of SGHs is recommended to determine the cause of death. At this time, all reintroduced SGHs associated with the Mabula Ground Hornbill Project are vaccinated for ND.

The use of injectable and oral vaccine protocols, commonly used in ostrich and poultry, proved safe in SGHs and yielded positive ND titers. SGHs immunized with the Ulster

strain responded better to subsequent vaccinations with other products than birds that received no initial vaccine. None of the vaccine protocols showed any adverse side effects. Moreover, because the SGHs have been vaccinated against ND, no mortalities associated with this disease have been reported in captive or reintroduced birds. The use of oral vaccines allows for ongoing reintroduction management without the need to capture, recapture, and handle the birds, eliminating risks associated with injury, stress, and imprinting on humans.

Acknowledgments

This work was conducted with ethical approval from the Johannesburg Zoo (from 2008) and the National Zoological Gardens of South Africa (from 2016) (Ethics Committee Project Reference NZG/RES/P16/02). We thank the keepers from Mpumalanga Tourism and Parks Agency, Johannesburg Zoo, National Zoological Gardens of South Africa, Boscia Birds, and Montecasino Bird Gardens for their dedication to this species and excellent avian care; Patience Shito for research support; Dr Michelle Barrows for the pilot project; the University of Pretoria for a travel grant; and the financial partners of the Mabula Ground Hornbill Project, including major US partners Virginia Zoo, Disney Conservation Grant, SeaWorld and Busch Gardens Conservation Fund, and San Diego Zoo Global.

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