Efficacy of a genotype 2 Newcastle disease vaccine (Avinew®) against challenge with highly virulent genotypes 5d and 3d

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ABSTRACT

Since 2002, following its introduction, the lineage 5d Newcastle disease virus (so-called Goose paramyxovirus – GPMV) strain has caused numerous disease outbreaks among commercial and backyard poultry in South Africa, raising questions about the ability of commercially available Newcastle disease vaccines to fully protect poultry against the strain. This study aimed to determine whether there are differences in the level of protection offered by Avinew® Newcastle disease vaccine against GPMV virus as compared with a 3d Newcastle disease virus isolated in South Africa in 1993 (Rainbow challenge virus – RCV) strain. Six groups of 10-day-old, specific pathogen-free chickens were vaccinated with doses of $10^{3.0}$, $10^{4.5}$ and $10^{6.0}$ EID_{50} of Avinew® vaccine and challenged at 4 weeks of age intramuscularly at a dose of $10^{5.3}EID_{50}$/ 0.2 mℓ/bird of GPMV and RCV. No statistically significant difference could be found in the protection offered by Avinew® vaccine against GPMV as compared to RCV challenge. The protection offered against the ND challenge was found to be dose dependent. At the recommended field dose of $10^{6.0}$ EID_{50} the vaccine gave 100 % protection from mortality against both the challenge viruses, but not against infection and replication of the viruses, as gross lesions were evident even in apparently healthy birds that survived the challenge. The protective dose (PD_{90}) of the Avinew® vaccine against GPMV challenge was calculated at $10^{4.38}$ and against that of RCV at $10^{4.43}$.

Keywords: lineage 3d, lineage 5d (GPMV), Newcastle disease, vaccine.

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INTRODUCTION

Newcastle disease (ND) is a fatal, highly contagious viral disease of domestic and wild avian species. Its global impact is enormous and unsurpassed by any other poultry disease[5], although the current epizootic of H5N1 avian influenza in some parts of the world seems to be challenging this status[28]. ND is caused by Newcastle disease virus (NDV) which is classified as an *Avulavirus* in the family *Paramyxoviridae*[14,16,17].

Newcastle disease was officially recorded to have entered South Africa through the port of Durban during 1944[13]. Since then, ND outbreaks in poultry have occurred sporadically[1,2]. Around 1999 a velogenic viscerotropic NDV belonging to lineage 5d (GPMV) was introduced into South Africa from the Far East and was responsible for the 1999/2000 outbreak in KwaZulu-Natal (KZN) province[2]. In 2003, lineage 5d re-emerged in a single outbreak and swept through the country infecting chickens, peacocks, Hadeda ibis (*Bostrychia hagedash*) chicks, geese, ostriches, pheasants and doves[1].

Most countries where poultry is raised commercially and where the disease is endemic rely on vaccination to keep the disease under control[4,5,6]. However, there are several reports indicating that commercially available ND vaccines are not performing optimally against virulent NDV[9,12,19,27]. The newly emerging virulent NDV strains (lineage 5d) are of great concern and have been suggested to have the ability to overcome vaccination barriers[21]. In South Africa, protection offered by available commercial vaccines against lineage 5d strains has been suboptimal, as ND infection and disease are characterized by mortalities in broiler flocks and declines in egg production even in fully-vaccinated pullets (Bisschop, unpubl. data). While the causes of the apparent vaccine failures in the field are not clear, the efficacy of commercially available vaccines is being questioned, and the findings of previous studies suggesting that currently available vaccines induced better protection against viruses that were isolated in past epizootics than against viruses that are currently circulating[10,12] necessitated this study. The study was aimed at determining whether the perceived field vaccine failure can be confirmed under laboratory conditions as well as determining if any difference can be detected in the level of protection achieved by the use of Avinew® ND vaccine against lineage 5d virus versus that achieved against the lineage 3d Rainbow virus isolated in 1993 from South African broilers.

MATERIALS AND METHODS

Facilities

This experiment was performed in containment isolation units at the facility of the Poultry Reference Centre of the Faculty of Veterinary Science, University of Pretoria. The isolators are airtight and equipped with positive pressure. They are of the biosafety level 2+ (BSL 2+). Eight isolator units were used for the study, ensuring that each trial group was completely isolated from the other. Approval for this research was obtained from the resident Animal Use and Care Committee (AUCC).

Vaccine

Avinew® Newcastle disease vaccine used in this study is a freeze-dried live vaccine against Newcastle disease produced by Merial Animal Health Limited (Lyon, France). The vaccine contained ND viruses of the VG/GA strain (lineage II virus), a lentogenic and naturally occurring strain that is apathogenic for chickens. Each dose (1 mℓ) was determined *via* viral titration to contain $10^{6.5}$ EID_{50}.

Challenge viruses

The first challenge virus used in this study was a velogenic NDV that was

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isolated from chicken tracheas, identified by the number APMV-1/chicken/South Africa/171/06 with a mean death time (MDT) of 48 hours and intracerebral pathogenicity index (ICPI) of 1.85. It was identified by PCR and molecular sequencing as belonging to lineage 5d. The second challenge virus was an NDV strain termed 'Rainbow challenge virus' (PPMV-1/chicken/South Africa/RCV/93) that was isolated in 1993 by Rainbow Farms laboratory at Hammarsdale and identified by molecular sequencing as a lineage 3d strain. It has an ICPI of 2.0 and MDT of 48 hours making it comparable to the Hertz strain (the standard challenge strain used in the UK) in terms of these pathogenicity parameters. Partial *F* gene sequences for the lineage 5d virus and the RCV were submitted to Genbank under the accession numbers FJ985978 and FJ985977. Previous trial work, accepted for the registration purposes of the Avinew® vaccine, indicated the protective dose (PD_{90}) against Hertz was about $10^{4.0}$ EID_{50} (J Vanmarcke, Merial Animal Health Limited, pers. comm., 2008).

Challenge dose

Virus challenge dose was $10^{5.3}$ EID_{50}/0.2 mℓ/bird, for both the challenge strains used. The route of challenge was by intramuscular injection.

Experimental model, design and procedure

Specific pathogen-free (SPF) White leghorn chickens (n = 126) were hatched and raised in the isolation unit to 9 days of age, when they were identified with numbered wing tags after being randomly assigned into 6 treatment groups of 18 birds each and 2 control groups of 9 birds each. At 10 days of age, birds in the 6 treatment groups were vaccinated with Avinew® vaccine using 3 different doses viz: $10^{3.0}$ EID_{50} (groups 4 & 8), $10^{4.5}$ EID_{50} (groups 3 & 7) and $10^{6.0}$ EID_{50} (groups 2 & 6). Two groups (1 & 5) served as unvaccinated controls. At 27-days of age (17 days post-vaccination), all the chickens were challenged *via* the intramuscular route with 1 of the 2 different NDV challenge strains at a dose of $10^{5.3}$ EID_{50}/0.2 mℓ/bird. GPMV was used to challenge groups 1, 2, 3 and 4 while groups 5, 6, 7 and 8 were challenged with RCV. Birds were provided with water and fed *ad libitum* and observed for 10 days post-challenge (pc). Chicks were scored at the 2 daily observations as either 0 = normal, 1 = sick or 2 = dead. All dead birds were necropsied and organs examined for the presence of gross lesions.

This challenge model was based on a combination of the OIE methods for the testing of ND virus vaccines for potency in both live and inactivated vaccines[20]. This particular challenge model was chosen to maximise the ability of the challenge trial to detect subtle differences in the efficacy of Avinew® vaccine against the different strains of the ND challenge viruses. The effects of the challenge on the different groups were assessed by evaluating clinical signs, mortality rates, gross pathology in organs and clinical and mortality scores.

Statistical analysis

The clinical and mortality scores were analysed statistically to check for the level of significance of the protection achieved by each dose. Statistical analysis of the data was carried out with multiple linear regression using Stat 10.0 (StataCorp, College Station, TX). The protective dose (PD_{50} and PD_{90}) were calculated using the method recommended by Reed & Muench[25] for calculating the 50 % end-point of virus titration.

RESULTS

Clinical signs and mortality

Birds appeared clinically normal for the first 48 hours post-challenge in all the treatment groups except the RCV-challenged control group (group 5). In this group, clinical signs of ruffled feathers and depression were observed 2 days pc. Most of the other groups started showing clinical signs 3 days pc. The time of onset of clinical signs for all the groups, the number of birds that developed clinical signs and the number of mortalities from challenge are presented in Table 1. Some of the sick birds progressed to complete depression, passage of greenish watery diarrhoea, sternal recumbency with drooling salivation, complete paralysis and then death. By the evening observation of day 3 pc, there were 3, 4 and 1 deaths in group 1 (GPMV-challenged control), group 4 (lowest vaccine dose treatment group challenged with GPMV) and group 8 (lowest vaccine dose treatment group challenged with RCV), respectively, while on day 4 pc, there were 6, 1, 8, 9 and 15 deaths in groups 1, 3, 4, 5 and 8, respectively (data not shown). Both control groups had 100 % (n = 18) mortality by 4 days pc. On day 5 pc, group 4 had 1 death while the remaining 2 chickens in group 8 died, resulting in 100 % (n = 18) mortality for group 8 as well. Group 7 ($10^{4.5}$ EID_{50} vaccine dose challenged with RCV) had its first and only death on day 7 pc, which was also the end of mortalities until day 10 pc, when all the surviving birds were humanely euthanased and the experiment terminated. The summary of mortalities of the test and the control birds is presented graphically (Fig. 1).

Valid results were obtained from 124 of the 126 chickens originally used for the trial. Only 2 deaths occurred due to causes not related to the trial and these were therefore excluded from the study. During the challenge trial, 51(41.13 %) chickens died while 73(58.87 %) chickens survived the challenge (i.e. they were still alive 10 days pc when the trial was terminated, but with some of the birds showing clinical signs). The $10^{3.0}$ EID_{50} treatment groups (groups 4 and 8) had 4 birds (11.11 %) surviving without any clinical signs while the $10^{4.5}$ EID_{50} (groups 3 and 7) and $10^{6.0}$ EID_{50} (groups 2 and 6) treatment groups had 32 birds (88.89 %) and 31 birds (91.12 %) surviving, respectively, without manifesting any clinical signs, while 32 (88.89 %), 4 (11.11 %) and 3 (8.82 %) chicks

Table 1: **Clinical disease and occurrence of mortality in SPF chickens vaccinated with varying doses of Avinew® vaccine and challenged intramuscularly with GPMV and RCV strains of Newcastle disease virus.**

Group	Vaccine dose	Challenge virus	Clin. signs (1st evident)	Number sick/total[A]	Number dead/total[A]
1	None	GPMV	3 dpc	9/9	9/9
2	$10^{6.0}$ EID_{50}	GPMV	3 dpc	1/17	0/17[B]
3	$10^{4.5}$ EID_{50}	GPMV	3 dpc	2/18	1/18
4	$10^{3.0}$ EID_{50}	GPMV	3 dpc	14/18	13/18
5	None	RCV	2 dpc	9/9	9/9
6	$10^{6.0}$ EID_{50}	RCV	7 dpc	2/17	0/17[B]
7	$10^{4.5}$ EID_{50}	RCV	7 dpc	2/18	1/18
8	$10^{3.0}$ EID_{50}	RCV	3 dpc	18/18	18/18

Total[A] = the number of 4-week-old chicks per group/isolator that were used for the trial. The figures under the number sick/total[A] and number dead/total[A] refer to the number of birds that became sick and died, respectively, during the course of the whole trial (i.e. up to 10 days post-challenge when the trial was terminated).

[B]These groups had 17 chickens each instead of the 18 chickens originally placed as a result of the death of 1 chicken from each group due to causes not related to the challenge.

dpc = days post-challenge; EID_{50} = embryo infective dose (50 %); GPMV = Goose paramyxovirus; RCV = Rainbow challenge virus.

in the $10^{3.0}$ EID_{50}, $10^{4.5}$ EID_{50} and $10^{6.0}$ EID_{50} groups developed clinical disease (Table 1).

Table 2 presents the computed average of the daily clinical and mortality scores (where 0 = normal, 1 = sick and 2 = dead) for all the birds. The control groups had an average score of above 1.5, while the treatment groups that received the highest dose of vaccine ($10^{6.0}$ EID_{50}) and challenged with GPMV and RCV had average scores of 0.047 and 0.011, respectively. The other treatment groups fell between the highest score of 1.517 and lowest score of 0.011. The average scores were plotted against the different vaccine doses into a line graph (Fig. 2). The higher average scores as shown in both Table 2 and Fig. 2 indicate little or no protection while lower scores indicate better protection and fewer clinical signs with low or no mortalities.

Table 2: **Treatment groups and their computed post-challenge average clinical scores**

Tmt (Vac.) Group	Groups–challenge	Virus (average scores)
Control	Group 1–GPMV (1.517)	Group 5-RCV(1.517)
$10^{3.0}$ EID_{50}	Group 4–GPMV (1.103)	Group 8-RCV(1.478)
$10^{4.5}$ EID_{50}	Group 3–GPMV (0.122)	Group 7-RCV (0.044)
$10^{6.0}$ EID_{50}	Group 2–GPMV (0.047)	Group 6-RCV (0.011)

Average scores were calculated from the twice daily scorings for 10 days post-challenge for individual birds in each group.

Tmt (Vac.) = treatment (vaccination); GPMV = Goose paramyxovirus; RCV = Rainbow challenge virus

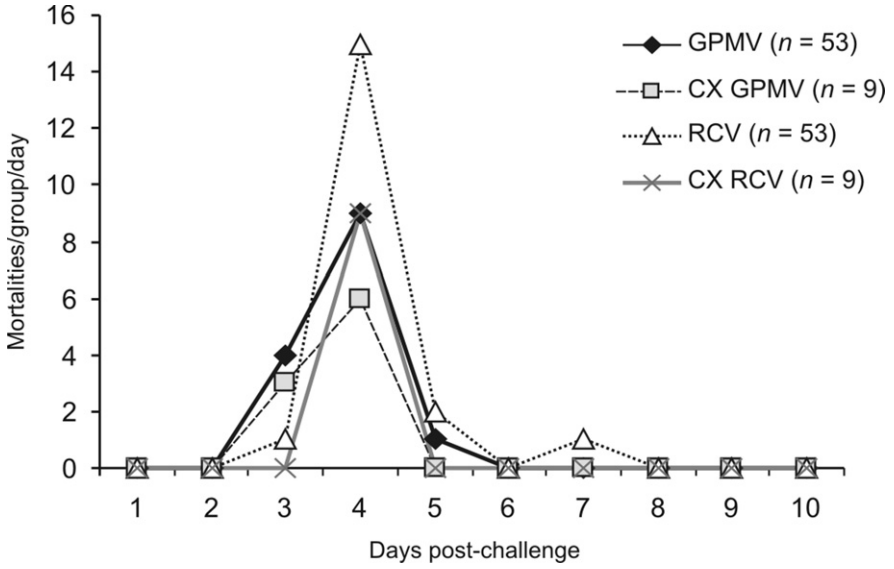

Fig. 1: **Daily mortality figures for all the treatment groups after challenge.** GPMV = Goose paramyxovirus (combination of groups 2, 3 & 4); CX GPMV = control group challenged with GPMV; RCV = Rainbow challenge virus (combination of groups 6, 7 & 8); CX RCV = control birds/group challenged with RCV.

Gross pathology

All the birds used in this trial were necropsied after death or after euthanasia, either during or at the termination of the trial. All the birds in the 2 control groups had variable macropathological lesions in the trachea, spleen, intestine, caecal tonsils, proventriculus and heart. Gross pathology included haemorrhage and congestion of the trachea and necrohaemorrhagic foci in the caecal tonsils and proventriculus. Some of the control birds had haemorrhagic enteritis and pin-point haemorrhages on the serosal surface of the pericardium. The most severe macroscopic lesions were observed in the caecal tonsils and proventriculus and these particular lesions were consistent in all the birds in both control groups. Chickens in groups 4 and 8, which received $10^{3.0}$ EID_{50} doses of vaccine, had gross lesions similar to those of the control birds. Most of these birds had obvious necrohaemorrhage foci in the caecal tonsils and proventriculus, with only a few birds having additional aforementioned lesions in the tracheas and intestines. Two birds among those euthanased at the termination of the study from group 4 had no macroscopically obvious lesions. Despite the fact that the birds in groups 2 (n = 16), 3 (n = 16), 4 (n = 4), 6 (n = 15), and 7 (n = 16) appeared 'healthy', 15 of these birds in group 3 and 8 in group 8 had necrohaemorrhagic lesions in the caecal tonsils. Only 3 birds in group 3, and 9 birds in group 7, had no visible lesions. Twelve birds from groups 2 and 6, respectively, had haemorrhages in the caecal tonsils, while 5 birds from groups 2, 3, 4, 6, 7, and 8 had no gross pathology.

The 50 % and 90 % protective doses (PD_{50} and PD_{90}) for Avinew® vaccine against challenge with GPMV and RCV were $10^{3.51}$ and $10^{4.38}$ for GPMV and $10^{3.79}$ and $10^{4.43}$ for RCV. The PD_{50} and PD_{90} are measurements of the dose of the test vaccine required to protect 50 % and 90 %, respectively of the test population from challenge with the respective viruses.

DISCUSSION

At the manufacturer's recommended dose of $10^{6.0}$ EID_{50}, Avinew® gave 100 % protection from mortality against challenge with both GPMV and RCV, while 94.44 % protection from mortality was achieved

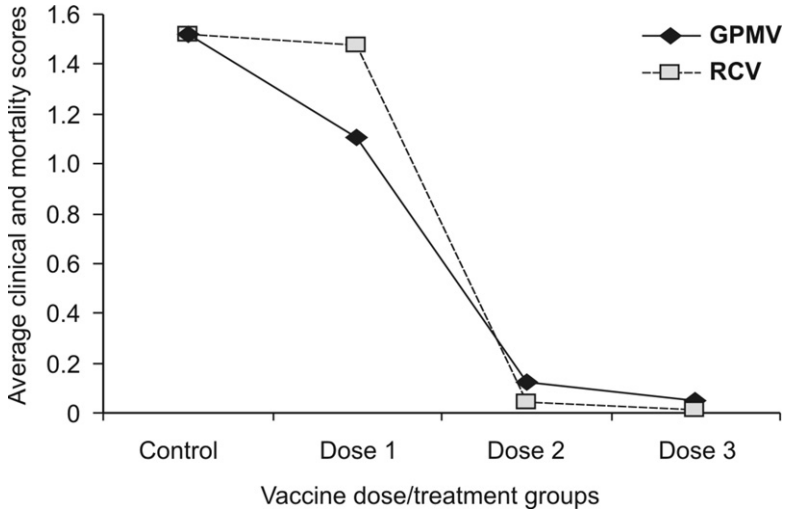

Fig. 2: **Averages of the clinical and mortality scores of 4-week-old chickens vaccinated and challenged with GPMV and RCV. The average scores are plotted against the control as well as the different doses of vaccines administered to the different treatment groups**. Dose 1 = $10^{3.0}$ EID_{50}; Dose 2 = $10^{4.5}$ EID_{50}; Dose 3 = $10^{6.0}$ EID_{50}

in the groups that received a vaccine dose of $10^{4.5}$ EID_{50}. Birds that were vaccinated at a dose of $10^{3.0}$ EID_{50} had 13.89 % protection against challenge with both GPMV and RCV. The protection in birds challenged with GPMV at the lowest vaccine dose of $10^{3.0}$ EID_{50} was poor but statistically significant ($P < 0.05$) when compared with the control groups, while protection of RCV-challenged groups at $10^{3.0}$ EID_{50} was not statistically significant ($P > 0.05$). At both higher doses there was good protection, which was statistically significant ($P < 0.01$) when compared with the unvaccinated control birds and the lower dose of $10^{3.0}$ EID_{50}. The difference in protection between the 2 higher doses of $10^{4.5}$ EID_{50} and $10^{6.0}$ EID_{50} was not statistically significant and both doses offered good protection from clinical disease and mortality. Kapczynski and King[12] demonstrated that a positive correlation exists between a higher dose of live vaccine and the presence of antibody titres and the subsequent protection offered post-challenge, which agrees with the findings in this study.

The level of protection achieved in this study demonstrates the efficacy of the test vaccine and its ability to protect against the clinical consequence of ND, which includes clinical signs and death. This is in agreement with recent works on the efficacy of VG/GA vaccines (Avinew®)[8,24–26], all of which reported full protection against lethal NDV challenge at the recommended vaccination dose. The results of this study therefore contrast with the concerns in the field and published reports[15,28] that ND vaccines may not produce adequate protection against velogenic challenge. Previous studies using VG/GA vaccine[8,24–26] and others[12,15,19] against various NDV isolates, all reported effective protection against challenge.

Despite the antigenic and genetic diversity that exists within the APMV-1 serotype[3], and the reports that homologous vaccines perform better than heterologous vaccines[12,19], no statistically significant difference was detected in the level of protection achieved by the Avinew® vaccine against challenge with the 2 viruses. Indeed, contrary to the belief prior to the commencement of this trial that the vaccine may protect less effectively against GPMV than against the classic strain of the disease, it emerged that protection against the older virus was, if anything, slightly poorer. The difference was most probably linked to the slightly higher pathogenicity of the RCV strain (1.85 for GPMV versus 2.00 for the RCV strain). This was shown by the higher clinical scores and mortality rates in the groups challenged with RCV, most clearly shown at a virus dose of $10^{3.0}$ EID_{50}. The shedding of viruses post-challenge was not assayed in this study to assess the level of protection as done by Kapczynski and King[12] and Miller *et al.*[19].

The protection achieved in this study did not, however, inhibit the challenge viruses from infecting and replicating in the host tissues and organs, as varying degrees of gross pathology were encountered even in the apparently healthy challenged birds that were euthanased at the termination of the trial. This is in agreement with reports that vaccination of poultry against ND can only protect birds from the more serious consequence of virulent NDV infection (clinical signs and mortality) but not infection and replication of the virulent strains of the virus[4,7,12,19,22].

The unvaccinated control birds were not protected, as all died within 6 days of challenge, which met the OIE requirements for the acceptance of such challenge trials. The control birds had clinical signs, mortalities and lesions that are consistent with that of velogenic NDV infection in non-immunized birds as reported in earlier studies[11,18,22].

In conclusion, the poultry industry relies heavily on vaccines to control infectious disease including ND. The inability of vaccines to protect against viral replication and shedding, especially in natural infections in field situations, presents an even bigger problem, as it may mask the possible introduction and spread of virulent virus, which become endemic but only become apparent when immunity level is down. The need therefore for the development of improved vaccines against ND, especially using recent isolates, which can protect against infection, replication and reduce or prevent the shedding of virus during infection, cannot be overemphasized. This issue represents one of the biggest challenges in our efforts to control ND and its devastating impact on the poultry industry.

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