The use of a bacterin vaccine in broiler breeders for the control of *Ornithobacterium rhinotracheale* in commercial broilers

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ABSTRACT

Ornithobacterium rhinotracheale (ORT) is a recently identified bacterial pathogen of poultry, linked to the respiratory disease complex of broilers and the economic losses associated with that disease complex. Present control measures applied for the disease include the continuous use of in-feed antibiotics. A recently developed bacterin vaccine that is applied to broiler-breeder hens to pass on protective immunity to their broiler progeny was tested under large-scale commercial conditions in South Africa. An indirect ELISA test for antibodies to ORT, optimised for use in South Africa, was used to determine antibody levels in breeders and broilers. ELISA test results showed that the vaccine stimulated the development of high antibody titre levels in broiler breeders. The efficacy of the vaccine in protecting the progeny of these birds from ORT challenge could not be determined during the trial, although the progeny of vaccinated hens appeared to perform slightly better under commercial conditions than the progeny of unvaccinated hens.

Key words: bacterin vaccine, broiler-breeders, indirect ELISA test, *Ornithobacterium rhinotracheale.*

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INTRODUCTION

The respiratory disease complex is associated with significant morbidity and mortality in commercial poultry worldwide and is a major cause of economic losses. The aetiology of respiratory disease is complex and frequently multifactorial, involving infectious agents as well as management and environmental factors.[12]

In 1991 a previously unidentified bacterium associated with signs of respiratory disease was isolated from the airsacs of broilers in South Africa.[8] The organism was named *Ornithobacterium rhinotracheale* (ORT) in 1994.[9] Since then the organism has been successfully isolated from chickens and turkeys worldwide and a few isolates have also been obtained from wild birds in Europe.[1,4–6] The control of ORT by means of prophylactic antibiotic treatment, often by means of in-feed tetracyclines, has become widespread in South Africa. In view of growing consumer concern about antibiotic residues in food products as well as evidence that ORT rapidly acquires resistance to antibiotics, this approach is clearly not sustainable.[3]

An ORT bacterin vaccine has been developed in the Netherlands. As injection of the vaccine into broilers is impractical on a large scale, the vaccine was applied to broiler breeder pullets during rearing with the aim of stimulating a strong immune response in the hens that could be transferred to their progeny transovarially. Previous work done under controlled conditions in the Netherlands indicated that breeder hens were able to transfer a significant and protective immunity to their broiler progeny after vaccination with the bacterin.[11]

The objective of this study was to confirm the safety (through clinical monitoring of vaccinated breeders) and efficacy (serologically and by evaluating the effect on broiler production) of the ORT bacterin under large-scale commercial conditions in South Africa.

MATERIALS AND METHODS

Vaccination of broiler breeders against Ornithobacterium rhinotracheale

Seven Ross broiler breeder flocks belonging to a large broiler integrator based on the Highveld region of South Africa were monitored serologically from 9 weeks of age to depopulation (Fig. 1). Each breeder flock comprised approximately 43 000 birds raised at a single site in climate-controlled buildings. At 19 weeks of age the flocks were transferred from the rearing farms to open-sided laying facilities. Flocks were placed at the rearing farms at approximately monthly intervals, between July and December 1998. Flocks 257, 259 and 261, placed on farm A, were vaccinated against ORT, as described below. Flocks 256, 258, 260 and 262, placed on farm B, which lies adjacent to farm A, were not vaccinated against ORT.

The inactivated bacterin vaccine (Nobilis® ORT Inac) was provided by Intervet South Africa. The vaccine contained ORT strain B3263/91, a serotype A strain[10] originally isolated from a broiler in South Africa, in a mineral oil adjuvant containing approximately 1×10^9 cells per dose. At between 8 and 10 weeks of age and again at about 18 weeks, breeder flocks were vaccinated intra-muscularly in the breast with 0.25 mℓ of vaccine. After vaccination, all flocks were monitored daily for clinical signs. In flocks vaccinated with the bacterin, 15 birds were palpated at 7 and 14 days post-vaccination for lesions in the breast muscles.

Field placements of broilers hatched from vaccinated breeders

Broiler chicks hatched from breeder flocks in the trial were delivered at day old to identical commercial broiler rearing sites designated BG1 and BG2. Approximately 53 500 birds were placed per cycle on each site at a stocking density of 20 birds per m^2. Management procedures on both sites were the same. In each broiler cycle, as far as possible, progeny from vaccinated parent flocks were placed simultaneously with progeny from unvaccinated parent flocks of a similar age.

In order to evaluate the effect of breeder vaccination on the control of ORT in the broilers, oxytetracycline was withdrawn from the feed of 3 cycles of broilers on both sites. A further 2 broiler cycles where

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oxytetracycline remained in the feed were also compared. At the time of the trials oxytetracycline was usually added to broiler feed at 300 g/tonne from day-old until 5 days before slaughter with the primary aim of controlling ORT challenge in the flocks.

Final production results for each broiler cycle were recorded. These included total live weight, average live weight and age at slaughter, mortality rate, feed consumption and feed conversion ratio. No further data such as individual bird weights or the range of bird weights was made available.

Serological testing

Blood samples were collected from the brachial vein and allowed to clot at room temperature. Serum was collected and frozen at –20 °C until tested. An indirect ELISA test, developed by Intervet in the Netherlands, and validated for use in South Africa during the trial, was used to detect ORT antibodies in serum. Chemical reagents were obtained from Merck chemicals (Fedsure Park, Midrand, Gauteng, South Africa). Boiled extract antigen and positive reference serum of ORT serotype A were obtained from Intervet, and stored at –70 °C until use. Shortly before testing, sera were thawed and diluted $1:2^6$ (1:64). Test sera were then diluted in serial 2-fold dilutions down the length of the micro-titre plate, from an initial dilution of $1:2^7$ to a final dilution of $1:2^{17}$.

Serum samples were collected from broiler breeders at approximately 8-week intervals between 9 and 60 weeks of age. The results of samples taken within 3 weeks of each other, were pooled for comparisons among flocks. ORT serological monitoring of broilers was carried out at slaughter. Values greater than $1:2^8$ were considered positive in broilers and values greater than $1:2^{12}$ positive in broiler breeders. ELISA results were expressed as $\log_2$ values.

Statistical analysis

Repeated measure analysis of variance was performed on breeder serological data to detect interactions between the age of the breeders at successive sampling times, vaccination and the mean antibody titres to ORT. In broilers, the proportion of positive reactors in different flocks was compared using the chi-square test.[7] Broiler flock data was recorded as mean flock data and the significance of observed differences could not be evaluated statistically. A chi-square test was used to compare mortality rates. A significance level of $\alpha = 0.05$ was used unless otherwise indicated. Analyses were carried out using the NCSS 2000 (Kaysville, Utah, USA) statistical package and Microsoft Excel spreadsheets (Microsoft Systems, Santa Rosa, California, USA).

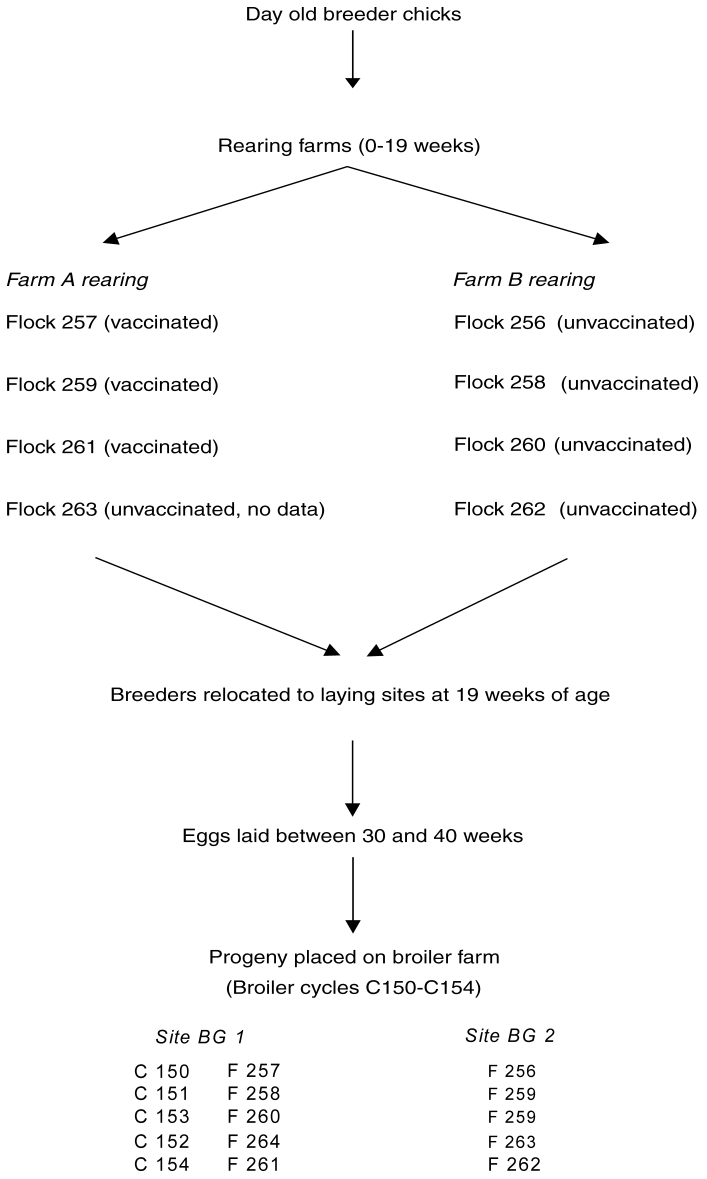


Fig. 1: **Schematic representation of trial procedures.**

RESULTS

Vaccine safety

No adverse clinical signs were observed in any of the breeder flocks subsequent to vaccination; however, a proportion of broiler breeders showed adverse reactions on palpation as indicated in Table 1.

Serological response to vaccination

The results of the grouped data are shown in Figs 2 and 3.

There was a significant difference in titres between vaccinated and unvaccinated groups at each bleed time interval between 14 and 60 weeks ($P < 0.001$) (Fig. 2). In the vaccinated group there was a significant increase in titres between 9 and 14 weeks ($P < 0.001$) in response to the 1st vaccination, and between 18 and 24 weeks ($P < 0.02$) in response to the 2nd vaccination. The subsequent decline in titres was significant only between 24 and 42 weeks ($P < 0.01$). In the unvaccinated group there were smaller, but also significant increases in antibody titres between 9 and 14 weeks ($P < 0.001$) and between 14 and 18 weeks.

The proportion of seropositive birds in the vaccinated group was significantly higher at all bleed time intervals between 14 and 60 weeks ($P < 0.001$) (Fig. 3).

Broiler production

Table 2 compares production results of different treatment groups. The unvaccinated group that received no oxytetracycline (negative control group) achieved the poorest results in terms of all production parameters. The progeny of vaccinated breeders that received no oxytetracycline in-feed had 2.55 % lower mortality rates, 6 g higher average live weight at

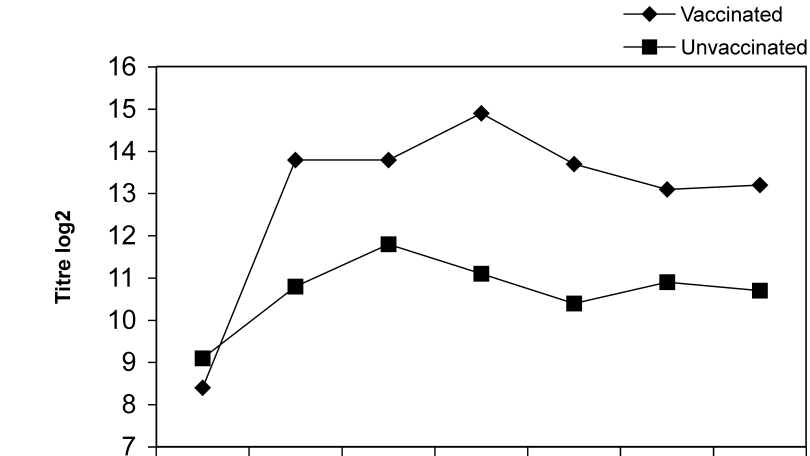
Table 1: **Assessment of local reactions in broiler breeders after vaccination.**

	Date	Birds with reactions	Comment
Flock 257			
9 weeks	**22.ix.98–30.ix.98**		
7 days post-vaccination	02.x.98	3/15	2 birds showed mild diffuse swelling of breast muscle. 1 bird showed moderate focal swelling of breast.
14 days post-vaccination	09.x.98	0/15	
18 weeks	**26.xi.98–04.xii.98**		**Vaccination**
7 days post-vaccination	11.xii.98	1/15	1 bird showed mild diffuse swelling of breast muscle.
14 days post-vaccination	18.xii.98	0/15	
Flock 259			
9 weeks	**17.xi.98–25.xi.98**		**Vaccination**
7 days post-vaccination	26.xi.98	3/15	2 birds showed mild diffuse swelling of the breast muscles. 1 bird had a defined lump near caudal part of keel bone.
14 days post-vaccination			Not done.
18 Weeks	**21.i.99–29.i.99**		**Vaccination**
7 days post-vaccination	02.ii.99	2/15	1 bird with diffuse but pronounced swelling of breast muscle. 1 bird with discrete abscess on breast.
14 days post-vaccination	08.ii.99	9/15	Birds showed circumscribed swellings, often in anterior region of breast. 3 showed tubular elongated swellings in subcutis. Breast muscles were normal size. Able to open 3 dead birds on site and could visualise vaccine oil in subcutis of all 3.
Flock 261			
9 weeks	**12.i.99–20.i.99**		**Vaccination**
7 days post-vaccination	21.i.99	3/15	1 bird showed a moderate swelling of the breast muscle, while the other 2 showed very mild swelling.
14 days post-vaccination	02.02.99	2/15	Both affected birds showed moderate swelling of the breast muscles.
18 weeks	**17.iii.99–24.iii.99**		**Vaccination**
6 days post-vaccination*	23.iii.99	7/15	In all recorded cases organised circumscribed lumps were palpated. 3 of the affected birds also showed mild diffuse swelling of the breast muscle, this may have been due to ORT vaccination.
14 days post-vaccination	09.iv.99	6/15	Nodular lumps.
Cockerels prior to vaccination*	23.iii.99	12/15	Palpation of cockerels prior to vaccination revealed that most had circumscribed lumps in the breast at the position of the ORT vaccination site.

*Each flock was housed in 8 houses during rearing – 7 houses containing hens and 1 house of cockerels. The vaccination team vaccinated 1 house per day, thus the 8th house had not yet been vaccinated a week after the 1st house where the initial 7-day post-vaccination palpations were done.

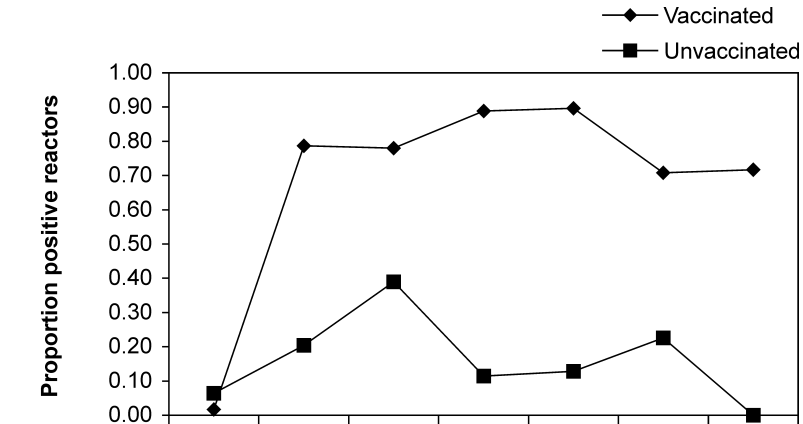
slaughter and 0.054 better FCR than the negative control group. The unvaccinated group that received in-feed oxytetracycline achieved 3.01 % lower mortality rates, 70 g higher average live weight at slaughter and 0.104 better FCR than the negative control group. The average slaughter age of each of these 3 groups was within 0.5 days of 40 days of age. A single placement of birds received both vaccine and oxytetracycline and had 6.76 % lower mortality rates, 52 g higher average live weight at slaughter and 0.273 better FCR than the negative control group, despite being slaughtered at 37 days.

Also shown in Table 2 are the results of the ORT ELISA test. There was a widely varied rate of seroconversion to ORT between broiler cycles, but that there was exposure to the disease in all groups. No



	9w	14w	18w	24w	42w	48w	60w
Vaccinated	8.4	13.8	13.8	14.9	13.7	13.1	13.2
Unvaccinated	9.1	10.8	11.8	11.1	10.4	10.9	10.7

Fig. 2: **Mean ORT ELISA titres in broiler breeders.**



	9w	14w	18w	24w	42w	48w	60w
Vaccinated	0.017	0.787	0.78	0.889	0.896	0.708	0.717
Unvaccinated	0.064	0.204	0.39	0.114	0.128	0.226	0

Fig. 3: **Proportion of broiler breeders with positive ORT ELISA titres.**

correlation could be found between serological evidence of exposure to ORT and the production results achieved in the broiler flocks.

DISCUSSION

Clinical evidence indicated that the birds experienced no adverse systemic reactions to the test vaccine or its application. It would be expected after application of inactivated vaccines such as the test product, that a proportion of birds would show local inflammatory responses to the adjuvant but that this reaction would be transient. In this trial, most reactions followed the expected pattern. Overall, the ORT test vaccine was applied a total of 6 times to 3 different broiler breeder flocks during this trial. On only 1 occasion were excessive vaccine reactions associated with the application of this vaccine. It is likely that the reactions observed on this single occasion were a result of poor vaccine application rather than a problem with the vaccine itself.

At 9 weeks of age all flocks tested were serologically negative mean titre below 12 for ORT. In response to the vaccinations given, the titres of the vaccinated flocks rose above the 9-week levels and remained significantly higher than the titres in unvaccinated breeder flocks for the duration of the production cycle. These results were similar to those obtained using the same vaccine in Ross broiler breeders in Belgium during 1999.[2]

No conclusions could be drawn as to whether the vaccination of broiler breeders resulted in sufficient immunity to be beneficial to their progeny. Compounding factors that made it difficult to draw conclusions were the poor quality of the data and the small number of broiler cycles that could be compared. Overall, the results suggest that breeder vaccination with the ORT test vaccine may be beneficial to their progeny, but is probably less effective than the application of oxytetracycline in the birds' feed. This difference may be due to the fact that oxytetracycline controls a wide range of pathogens besides ORT.

Table 2: **Comparison of production results of different broiler treatment groups.**

Oxytetracycline in feed: / **Vaccination:**	**Yes** / **Yes**	**Yes** / **No**	**No** / **Yes**	**No** / **No**
Number of placements	1	3	3	3
Average number placed	53 520	53 520	52 640	52 640
Average site area (m^2)	2676	2676	2676	2676
Average slaughter age (days)	37	39.6	39.7	39.6
Percentage mortality	5.27	9.02	9.48	12.03
Percentage survivors	94.79	90.62	89.79	89.15
Average live weight at slaughter (kg)	1.712	1.730	1.666	1.660
Average feed conversion ratio (FCR)	1.736	1.905	1.955	2.009
Kg/m^2	32.46	31.50	29.69	28.71
Total live weight per cycle (kg)	86 856	84 299	79 461	76 826
Total feed used per cycle (kg)	150 760	160 833	155 197	153 743
Positive ORT titres (%)	19	44	10	3

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