

## Efficacy of a commercially available coryza vaccine against challenge with recent South African NAD-independent isolates of *Haemophilus paragallinarum* in chickens

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### ABSTRACT

In South Africa the incidence of NAD-independent *Haemophilus paragallinarum* isolation from clinical cases is increasing. This study was carried out to test whether a commercially available coryza vaccine (Nobilis<sup>®</sup> Coryza, Intervet International BV) could protect chickens against challenge with recent NAD-independent isolates. SPF chickens were vaccinated twice at 3 and 7 weeks of age and were challenged at 9 weeks of age with 5 different NAD-independent isolates of serotype A or C-3. The results after challenge show that the coryza vaccine induces good protection against challenge with the different South African NAD-independent isolates of *H. paragallinarum*, including serotype C-3.

**Key words:** coryza vaccines, *Haemophilus paragallinarum*, poultry, vaccine efficacy.

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### INTRODUCTION

In South Africa the incidence of NAD-independent *Haemophilus paragallinarum* isolation from clinical cases is increasing. Bragg *et al.*<sup>1,2</sup> found a correlation between the increasing incidence of NAD-independent strains and increasing vaccine failure. Furthermore, these authors suggest that independent strains are more capable of evading the immune response induced by vaccines. In particular the recent increased isolation of serotype C-3 was linked to vaccine failure. This study was carried out to test whether a commercially available coryza vaccine (Nobilis<sup>®</sup> Coryza, Intervet International BV) could protect chickens against challenge with different NAD-independent strains of *H. paragallinarum*.

### MATERIALS AND METHODS

#### Bacterial strains

The NAD-independent strains of *H. paragallinarum* were obtained from R Bragg, Faculty of Veterinary Science, University of Pretoria, South Africa. All these strains are South African field isolates from diseased chickens. The strains were typed by R Bragg as follows: strain 1932 (type A), 1085 (type A), 587 (type

C-3), 1245 (type C-3) and 541 Sin 3 (type C-3). At our laboratory the strains were confirmed to be NAD-independent *H. paragallinarum* strains (API 20NH, Biomérieux) that were able to cause typical signs of coryza in SPF chickens. The strains were stored in the freeze-dried state until use.

#### Chickens

One hundred 3-week-old SPF chickens (Intervet), identified by numbered wing tags, were used. Before challenge the chickens were housed under SPF conditions. On the day of challenge, groups of 10 chickens were housed in isolators.

#### Vaccine

A trivalent coryza vaccine (Nobilis<sup>®</sup> Coryza, batch 77328, Intervet International BV) containing immunogenic concentrations of inactivated cells of *Haemophilus paragallinarum* serotypes A, B and C in purified saponin adjuvant was used.

#### Vaccination

Half the chickens were vaccinated twice intramuscularly in the leg with 0.25 ml vaccine at 3 and 7 weeks of age. The other half acted as an unvaccinated control group.

#### Challenge

Chickens were challenged at 9 weeks of

age (2 weeks after booster vaccination) using egg yolk of infected embryonated eggs. Egg yolk of 7-day-old embryonated eggs was inoculated with *H. paragallinarum* and subsequently incubated at 37 °C. The egg yolk was harvested after the embryo had died (usually after 24 hours) and always contained 10<sup>7.7</sup>–10<sup>8.7</sup> CFU/ml. Chickens were challenged by injecting the infected egg yolk directly into the sinus infra-orbitalis (200 µl) and intranasally (200 µl). Each group, vaccinated or controls, consisted of 10 chickens.

#### Clinical observations

At days 1, 2 and 5 after challenge all chickens were examined for nasal discharge and scored positive or negative. The clinical score is presented as the sum of positive scores of the 3 observations (maximum = 30).

#### Bacterial re-isolation

At necropsy (5 days after challenge), sinus infra-orbitalis swabs were streaked on blood agar. After incubation for 24 hours at 37 °C under increased CO<sub>2</sub> (5 %) the plates were inspected for typical colonies of *H. paragallinarum*. For each challenge group, a few suspected colonies were further identified as NAD independent *Haemophilus* by using API 20NH (Biomérieux).

### RESULTS

After challenge, all control chickens showed clinical signs of coryza on 1 or more days, except for 3/10 that were challenged with strain 587 (Table 1). Likewise, re-isolation of *Haemophilus* from the sinus infra-orbitalis was positive in all control chickens except for 2/10 that were challenged with strain 587 (Table 1). The vaccinated chickens showed various degrees of protection against the respective challenges, *i.e.* against development of clinical signs as well as re-isolation.

### DISCUSSION

The results indicate that the coryza vaccine induced good protection against

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Table 1: Results after challenge with NAD-independent isolates from South Africa.

Challenge strain	Serotype	Treatment <sup>a</sup>	Total clinical score <sup>b</sup>	Number of chickens with clinical signs	Number of chickens with <i>Haemophilus</i> re-isolated <sup>c</sup>
1932	A	Vaccinated	0	0	1
		Control	26	10	10
1085	A	Vaccinated	2	2	4
		Control	24	10	10
587	C-3	Vaccinated	1	1	2
		Control	15	7	8
1247	C-3	Vaccinated	2	1	1
		Control	23	10	10
541 Sin 3	C-3	Vaccinated	8	3	3
		Control	22	10	10

<sup>a</sup>SPF chickens were vaccinated with a commercially available coryza vaccine (Nobilis® Coryza, Intervet International BV) at 3 weeks and 7 weeks of age; all chickens were challenged at 9 weeks of age; each group consisted of 10 chickens.

<sup>b</sup>Chickens were observed for nasal discharge on days 1, 2 and 5 after challenge; the total score is represented by the sum of all positive observations (maximum 30).

<sup>c</sup>Re-isolation from sinus infraorbitalis 5 days after challenge.

all NAD-independent isolates tested, including 3 recent isolates of serotype C-3.

In a previous study the coryza vaccine was found to induce good protection against 3 other South African isolates of *H. paragallinarum*, i.e. strains 24317 and 24268 (both serotype B and NAD-dependent) and strain 2049, a non-typable NAD-independent isolate (Jacobs, unpublished results).

The discrepancy between the results of Bragg *et al.*<sup>1,2</sup> and our results might be explained by the use of inappropriate

vaccines in South-Africa, i.e. non-protective vaccines or vaccines containing the wrong serotypes. Misdiagnosis of clinical signs in the field could also play a role, as similar clinical signs can be caused by other microorganisms, e.g. IB4/91, TRT, *E. coli*, *Mycoplasma*, *Pasteurella* and possibly *Ornithobacterium*.

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independent isolates of *H. paragallinarum*.

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