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Virulence of South African isolates of *Haemophilus paragallinarum*. Part 2: Naturally occurring NAD-independent field isolates

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ABSTRACT

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Naturally occurring NAD-independent variants of *Haemophilus paragallinarum*, which have been isolates from poultry showing clinical signs of infectious coryza, were used to determine their virulence using a newly developed challenge model for infectious coryza.

It was established that the NAD-independent isolates belonging to a particular serogroup, were less virulent when compared to the virulence of the NAD-dependent isolates from the same serogroup. It was shown that the virulence of the NAD-independent isolates belonging to serogroup C and serogroup A were very similar to each other. This differs to the results obtained with NAD-dependent isolates reported on previously, in which the serogroup C isolates were found to be more virulent then the serogroup A isolates.

Keywords: *Haemophilus paragallinarum*, NAD-independence, virulence

INTRODUCTION

Infectious coryza, caused by *Haemophilus paragallinarum*, remains a serious problem in the layer industry in many parts of the world, despite the wide spread use of vaccines. Normally, *H. paragallinarum* requires NAD for growth and the typical bacterium is regarded as being NAD-dependent (Blackall & Reid 1982). Since 1990, NAD-independent variants of *H. paragallinarum* have been isolates in South Africa. Horner, Bishop & Haw (1992) reported on a bacterium which appeared to be *H. paragallinarum*, but did not require NAD for growth, They suggested that these isolates could not be *H. paragallinarum* because of their NAD-independence. Mouahid, Bisgaard, Morley, Mutters & Mannheim (1992) and Bragg, Coetzee & Verschoor (1993) suggested that these isolates were NAD-independent variants of *H. paragallinarum* based on DNA/ DNA hybridization (Mohaid *et al.* 1992) and reactions with monoclonal antibodies specific for *H. paragallinarum* (Bragg *et al.* 1993). Horner, Bishop, Jarvis & Coetzee (1995) later conceded that their isolates were NAD-independent *H. paragallinarum*.

Bragg *et al.* (1993) demonstrated that the NAD-independence of these isolates was encoded for on transferable genetic material by performing transformation experiments with crude DNA extracts made from NAD-independent isolates and used to convert NAD-dependent reference strains into NAD-independent strains. It was further demonstrated by Bragg, Purdan, Coetzee & Verschoor (1995) that

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the haemagglutinin of the transformed strains was not affected by the transformation process.

Gromkova & Koornhof (1990) and Windsor, Gromkova & Koornhof (1991) demonstrated plasmid mediated NAD-independence in *H. parainfluenza* isolates from human patients in South Africa. It would be most interesting to investigate the similarity of the plasmids isolated from *H. parainfluenza* and *H. paragallinarum* and attempt to identify a common source of these plasmids.

As a result of the expanding infectious coryza problem in South Africa, research on the virulence of the different serovars of NAD-dependent *H. paragallinarum* in South Africa was undertaken. It has been established that there are four serovars of *H. paragallinarum* (serovars A-1, B-1, C-2 and C-3) which occur in this country (Kume, Sawata, Nakase & Matsumoto 1983; Blackall, Eaves & Rogers 1990; Bragg 1995: Bragg, Coetzee & Verschoor 1996). Bragg (2002) established that the virulence of the South African serovar C-3 isolate was high. In this study, the challenge model developed by Bragg (2002) was used to investigate the virulence of the naturally occurring NAD-independent isolates.

MATERIALS AND METHODS

Source of chickens

A total of 30 unvaccinated Lohmann layer chickens, aged 11 weeks, were obtained from the same supplier of point-of-lay chickens as used for the study of the virulence of the NAD-dependent isolates (Bragg 2002). These chickens were kept in the same facilities as used previously (Bragg 2002) and were monitored throughout the rearing period. No clinical signs of infectious coryza were seen in any of the birds during the full period that they were in the layer cages.

When the chickens were 25 weeks of age, they were moved to a separate layer facility, where they were challenged with naturally occurring field isolates of NAD-independent *H. paragallinarum*.

Bacterial isolates used for challenge

The bacterial isolates used in this experiment consisted of two South African field isolates of NAD-independent *H. paragallinarum* that were isolated and serotyped by the author (Bragg *et al.* 1996). The isolates were referred to as 1750 (A-1) and 1343 (C-3) (the number referring to the isolate number and the serovar is given in the brackets). All isolates were grown in TMB broth medium described by Reid & Blackall (1984) without the addition of NAD or sterile chicken serum.

Challenge methods

The same challenge model and experimental layer facilities as used by Bragg (2002) was used for this experiment. The 30 chickens were divided into three groups of 10 chickens each. Each group was placed into a row of five cages, such that two birds were placed into each cage. The groups were not connected to each other in any way. The cages

TABLE 1 Mean daily disease scores obtained when unvaccinated chickens (n = 10) were challenged with different serogroup (A and C) of NAD-independent of *H. paragallinarum*

Days post challenge	Serogroups used to challenge chickens		
	A	C	Control
1	0	0	0
2	0	0.1	0
3	0	0.7	0
4	0.2	0.8	0
5	0.2	1.2	0
6	0.4	1.3	0
7	0	1.2	0
8	1.6	1.2	0
9	1.6	0.2	0
10	1.0	0.1	0
11	1.2	0	0
12	0.6	0.1	0
13	0.6	0.1	0
14	0.6	0.1	0
15	0.2	0.4	0
16	0	0.2	0
17	0	0.2	0
18	0	0.2	0
19	0	0.2	0
20	0	0	0
Mean	0.49	0.42	0

TABLE 2 Total disease score, Mean disease score number and highest daily disease score obtained when unvaccinated chickens (n = 10) were challenged with different serogroups (A and C) of NAD-independent of *H. paragallinarum*

	Isolate	
	A-1	B-1
Total disease score	9.8	8.4
Mean disease score	0.49	0.42
Highest daily disease score	1.6	1.3

within each group had a communal water supply and a feed trough which passed in front of each cage in the row.

One chicken in the middle cage of each of the rows was marked and challenged by intrasinus injection of 0.1 mℓ of an 18-hour-old culture, grown in TMB (Reid & Blackall 1984) without addition of NAD or sterile chicken serum of one of the two different South African serovars of NAD-independent *H. paragallinarum*. Both serovars of NAD-independent isolates were used to inoculate different groups of birds. The remaining group of ten birds was left as an unchallenged control.

Scoring of clinical signs

The clinical signs of infectious coryza were rated and scored according to the methods described by Bragg (2002) without any modification. Clinical signs were evaluated and scored on a daily basis in the population of chickens. The total disease score for each day was calculated and the mean daily disease score was calculated for each day by dividing the daily disease score by the total number of chickens.

The mean daily disease score was plotted graphically to present the disease profile for the flock. The mean of the daily disease scores was also calculated, resulting in a numerical evaluation of the disease profile.

The disease profiles of the different isolates were plotted graphically for the purpose of comparison. The numerical disease profiles were compared with each other. The disease profiles obtained for the NAD-independent variants were compared to the disease profiles obtained for the NAD-dependent field isolates which have previously been reported by Bragg (2002).

RESULTS

When the chickens were challenged with a particular isolate of NAD-independent *H. paragallinarum* and their disease profiles calculated, some indication of the virulence of the isolates could be obtained. The mean daily disease scores obtained when unvaccinated chickens were challenged with two different serovars of NAD-independent *H. paragallinarum* are given in Table 1. A comparison of the disease profiles of the two different NAD-independent isolates is graphically illustrated in Fig. 1 and Fig. 2.

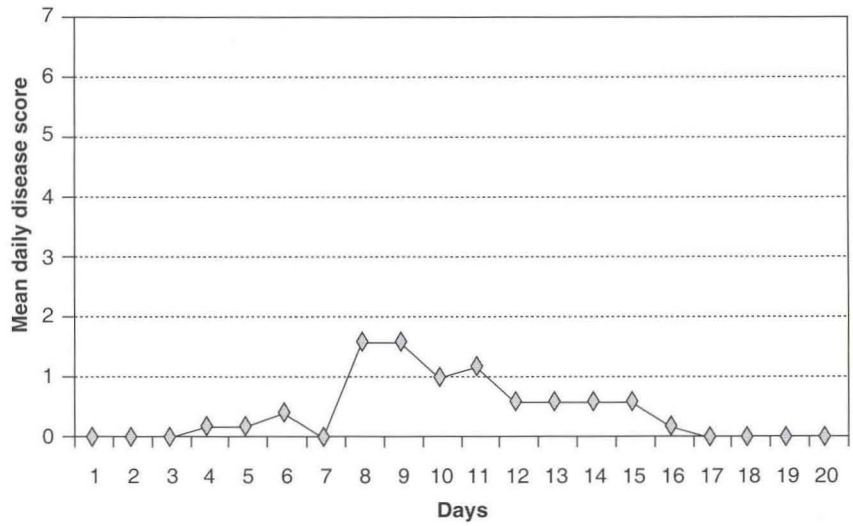

FIG. 1 Graphic representation of the disease profiles obtained when unvaccinated chickens were challenged with serogroup A of NAD-independent of *Haemophilus paragallinarum*

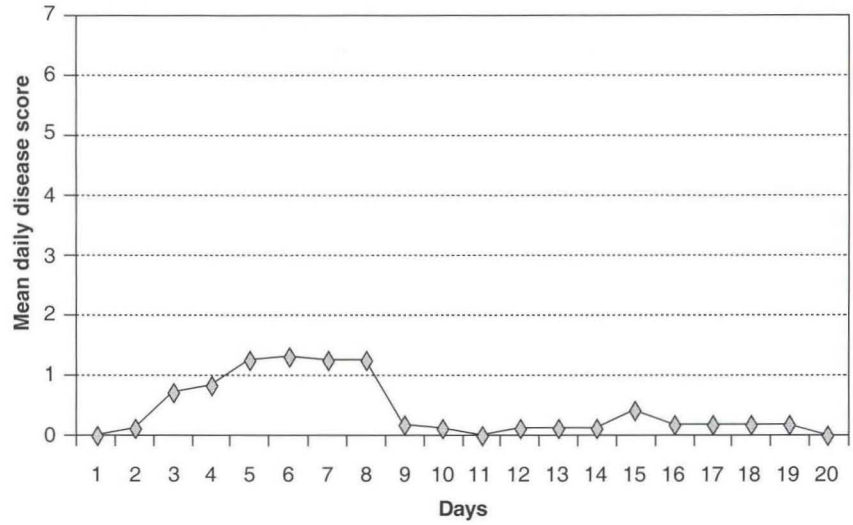

FIG. 2 Graphic representation of the disease profiles obtained when unvaccinated chickens were challenged with serogroup C of NAD-independent of *Haemophilus paragallinarum*

No clinical signs of infectious coryza were seen in any of the control birds in the third group that were not challenged directly with any isolate of *H. paragallinarum* (Table 1).

The mean disease scores for the different serovars of NAD-independent *H. paragallinarum* were calculated, thus presenting a numerical comparison of the disease profiles of the different isolates (Table 2). The highest daily disease score for the bird population appears in Table 2. The total disease score, which is the sum of all the daily disease scores presented in Table 1, is presented in Table 2.

DISCUSSION

The mean disease profile numbers for both the NAD-independent isolates tested were quite similar (0.49 for the serogroup A isolate and 0.42 for the serogroup C isolate (Table 2) in the chickens.

Interesting results were obtained when the disease profiles calculated for the naturally occurring NAD-independent isolates are compared to those obtained in the study by Bragg (2002) when unvaccinated chickens were challenged with the four different serovars of NAD-dependent field isolates.

In the later study, a mean disease profile number for the NAD-dependent serovar A-1 isolate was found to be 0.16, with the highest daily disease score of 1.2. If this is compared to the results obtained in the present study for the NAD-independent serogroup A isolate used, it can be seen that the NAD-independent serovar A-1 isolates appears to be more virulent than the wild-type serovar A-1 isolate used. In this study the NAD-independent serogroup A isolate resulted in a mean disease profile number of 0.49, with a maximum daily disease score of 1.3. These findings could explain why there was such a high incidence of NAD-independent serogroup A isolates reported in the 1990s (Horner *et al.* 1995; Bragg 1995: Bragg, Greyling & Verschoor 1997)

A different situation was observed when considering the virulence of the serogroup C isolates. Bragg (2002) demonstrated that the two serogroup C NAD-dependent isolates used in his study were highly virulent, with the serovar C-3 isolate being the more virulent. The mean disease profile number for these NAD-dependent isolates was 1.67 for serovar C-2 isolate and 2.25 for serovar C-3 isolate. The maximum daily disease score was found to be 4.8 for the serovar C-2 isolate and 6.2 for the serovar C-3 isolate (Bragg 2002). The results obtained for the naturally occurring NAD-independent serogroup C isolate tested in the present experiment are in sharp contrast to the results obtained for the NAD-dependent isolates. For the NAD-independent isolate, a mean disease profile number of only 0.42 was found with a maximum daily disease score of 1.3.

It would thus appear from these findings that the NAD-independent variants of serogroup C NAD-independent isolates are of a much lower virulence than the wild type NAD-dependent serogroup C isolates, which were found to be highly virulent. The perceived lower virulence seen in the naturally occurring NAD-independent serogroup C isolates could be a possible explanation for the lower incidence of NAD-independent serogroup C isolates found among the NAD-independent isolates made from chickens (Horner *et al.* 1995; Bragg 1995; Bragg *et al.* 1997). The prevalence of serogroup C isolates in the NAD-dependent isolates was almost 90% of the population isolated in the 1990s (Bragg *et al.* 1996). A possible reason for this high incidence could be related to the high virulence of the naturally occurring NAD-dependent serogroup C isolates.

It must be stressed that these experiments have been performed in unvaccinated chickens that have no protective antibodies against any of the serogroups of *H. paragallinarum*. It would be interesting to investigate the effects of a vaccination programme on the virulence of the different serovars of *H. paragallinarum*, using this challenge model.

It is unclear why there should be such a difference in the levels of virulence of the NAD-dependent serogroup C isolates and the NAD-independent isolate. Bragg *et al.* (1995) demonstrated the transferability of NAD-independence between naturally occurring NAD-independent isolates and naturally occurring NAD-independent isolates. It was also demonstrated that the haemagglutinin of the transformed isolates was not affected by the transformation process. An attempt to transform the highly virulent serovar C-3 strain used by Bragg (2002) into NAD-independence and investigate the effects of the transformation to NAD-independence have on the virulence of the isolate, will be reported on separately.

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