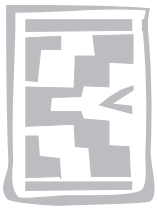

Evidence of possible evasion of protective immunity by NAD-independent isolates of *Haemophilus paragallinarum* in poultry

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ABSTRACT

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An indication of the ability of NAD-independent variants of *Haemophilus paragallinarum* to evade the immune system has been obtained from data obtained from several experiments.

Firstly, it was noted that there was a difference in the serovar distribution between the NAD-dependent isolates in the 1990s and the NAD-independent isolates, as there was a significant decrease in the incidence of serogroup A NAD-dependent isolates. This can possibly be attributed to the extensive use of vaccines. On the other hand, most of the earlier NAD-independent isolates were serovar A. This is a possible indication of evasion of the protective immunity by the NAD-independent isolates.

Further evidence of possible evasion of the protective immunity was obtained from results obtained when different isolates, both NAD dependent and NAD independent, were tested with a panel of monoclonal antibodies (Mabs). The V1 Mab reaction pattern was only seen in the reference strain 0083 among all of the NAD-dependent isolates tested in South Africa. This Mab was, however, found to react with some of the NAD-independent isolates. Furthermore, the isolation of NAD-dependent isolates in Australia which react with the V1 Mab also suggest possible evasion of the protective immunity by the NAD-independent isolates as no vaccines containing strain 0083 are used in Australia.

In order to investigate the hypothesis of immune-evasion by NAD-independent *H. paragallinarum*, vaccinated and unvaccinated chickens were challenged with a NAD-independent serogroup C isolate. As a control, chickens were also challenged with NAD-dependent *H. paragallinarum* of the same serogroup. The results obtained indicate that there is no significant difference in the disease profiles obtained in vaccinated and unvaccinated chickens challenged with the NAD-independent isolate, thus providing further evidence of evasion of the productivity immunity by the NAD-independent isolates.

The ability of the NAD-independent isolates to evade the immune system suggests that a different vaccination strategy, or alternative control methods may be needed for the control of IC caused by these isolates.

Keywords: *Haemophilus paragallinarum*, immunity, infectious coryza, NAD independent

INTRODUCTION

NAD-independent variants of *Haemophilus paragallinarum* have been isolated from chickens suffering from infectious coryza (IC) in South Africa since 1989 (Mouahid, Bisgaard, Morley, Mutters & Mannheim 1992; Horner, Bishop & Haw 1992; Bragg, Coetzee & Verschoor 1993a).

Bragg *et al*. (1993a) established that the genes encoding for NAD independence appear to be car-

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ried on a transferable plasmid and they succeeded in transforming reference strains of NAD-dependent *H. paragallinarum* into NAD-independent isolates. Bragg (2002b) demonstrated that the NAD-independent isolates appear to be less virulent that the NAD-dependent isolates of the same serovar. Taole, Albertyn, Van Heerden & Bragg (2002) demonstrated that experimentally produced NAD-independent isolates made from virulent serovar C-3 isolates were less virulent that the wild type NAD-dependent isolates.

Bragg, Coetzee & Verschoor (1996) established that there has been a significant change in the serovar distribution of NAD-dependent *H. paragallinarum* isolates since the 1970s. They established that in the 1990s, the incidence of serovar A-1 NAD-dependent *H. paragallinarum* had decreases from 34 % in the 1970s to 5 % in the 1990s and suggested that this decrease was a result of the intensive use of vaccines, all of which contain serogroup A isolates. On the other hand, Miflin, Horner, Blackall, Chen, Bishop, Morrow, Yamaguchi & Iritani (1994) reported that all of the NAD-independent South African isolates which they had received were serogroup A isolates. These isolates were, however, collected from a localized area of South Africa. Bragg, Greyling & Verschoor (1997) reported on a much wider collection of NAD-independent isolates and they also isolated serogroup C NAD-independent isolates for the first time, and they indicated that the incidence of serogroup A NAD-independent isolates was 58 %.

One possible explanation for the difference in the incidence of the serogroup A isolates among the NAD-dependent and NAD-independent isolates could possibly be the evasion of protective immunity by the NAD-independent isolates. Bragg *et al.* (1996) suggested that the decrease in the incidence of serogroup A NAD-dependent isolates could be as a result of intensive use of vaccines, all of which contain a serogroup A isolate. The fact that a large number of NAD-independent isolates were found to be serogroup A at a time when the incidence of serogroup A isolates amongst the NAD-dependent strains was decreasing, suggests that the NAD-independent isolates might be able to evade the protective immunity in the birds.

Further indications of the possible evasion of the protective immunity by NAD-independent isolates of *H. paragallinarum* was obtained from monoclonal antibody studies performed by Bragg, Coetzee & Verschoor (1993b) and Bragg, Gunter, Coetzee & Verschoor 1997b) who investigated the possibilities of using a panel of Mabs established by Verschoor, Coetzee & Visser (1989) for the serotyping of *H. paragallinarum*.

Although these Mabs detected certain antigens in *H. paragallinarum* (either protein or lipopolysaccharide in nature) (Bragg *et al.* 1997b), they could not be used for any meaningful serological classification of *H. paragallinarum*. However, during this work, large numbers of isolates of *H. paragallinarum*, both NAD dependent and NAD independent, were examined with this panel of Mabs (Bragg *et al*. 1993b, 1997b). One Mab was of particular interest. This was the Mab termed V1, which detected a lipopolysaccharide of between 13.8–14 kDa (Bragg *et al*. 1997b). This Mab was found to react only with the reference strain 0083. None of the South African NAD-dependent field isolates of *H. paragallinarum* reacted with this Mab. A small sample of NAD-dependent field isolates of *H. paragallinarum* (kindly supplied by Dr P. J. Blackall, Department of Primary Industries, Brisbane, Australia) were tested with these Mabs. One of the wild type Australian field isolates (as well as the mutant derived from it) reacted with the V1 Mab (Bragg *et al*. 1997b).

When examining the NAD-independent isolates, 12.5 % of the isolates were found to react with the V1 Mab (Bragg *et al.* 1997b). This was in sharp contrast to the situation in the NAD-dependent isolates. In South Africa, many of the registered IC vaccines contain isolate 0083. In Australia, no imported IC vaccines have been used and all of the IC vaccines used contain Australian isolates (Blackall 1991; Blackall & Reid 1987; Blackall, Eaves, Rogers & Firth 1992). Thus, strain 0083 has not been used in vaccines in Australia (Blackall, personal communication 1997). The isolation of NAD-dependent field isolates in Australia which express the antigen detected by the V1 Mab, and the high incidence of NAD-independent field isolates reacting with this Mab, suggested a possible immune-evasion by the NAD-independent isolates.

Until recently, one of the problems faced when attempting to verify the hypothesis of immune-evasion by the NAD-independent isolates was the existence of a suitable challenge model where the protective effects of a vaccine could be accurately measured. Bragg (2002a) established a new challenge model for IC that facilitates the comparison of the virulence of different isolates and also allows for the accurate comparison of the efficacy of different vaccines.

This challenge model is based largely on the various challenge models used by various authors (Bragg 2002a), but in this model a numerical score is given to the clinical signs. The numerical scores can be used to construct a graphic representation of the disease profile over a 20-day period. By superimposing the disease profiles of two different isolates, or from two different vaccination programmes, comparisons can be established. This data also lends itself to statistical analysis. This challenge model is used to confirm the hypothesis of immune-evasion by NAD-independent isolates of *H. paragallinarum*.

MATERIALS AND METHODS

Twenty unvaccinated commercial layer chickens were obtained from a supplier of point-of-lay chickens. These chickens were obtained at 11 weeks of age before they were vaccinated against IC. These chickens were collected from a site with no previous history of IC and were housed in disinfected layer facilities until they were used in the experiments.

Another 20 unvaccinated commercial layer chickens were obtained from the same flock and were vaccinated with an experimental vaccine containing South African isolates of serovars A-1, B-1, C-2 and C-3 *H. paragallinarum* when at 12 weeks of age. They were re-vaccinated at 16 weeks of age. Both vaccinated and unvaccinated chickens remained in the facilities until they were 25 weeks of age and were producing eggs. The protective capability of this vaccine was previously verified and will be reported on separately. All four different serovars of *H. paragallinarum* have been included in the experimental vaccine as it has been well established that there is limited cross protection between the different serogroups (Rimler, Davis & Page 1977; Kume, Sawata & Nakase 1980a, b).

When they were 25 weeks of age, the vaccinated and unvaccinated chickens were subdivided into two groups of ten vaccinated and two groups of ten unvaccinated chickens. They were placed in cages of a battery in such a way such that ten chickens were placed into the top five cages (two chickens per cage) and ten chickens in the bottom row of cages. The vaccinated chickens were placed in the cages on one side of the battery, while the unvaccinated chickens were placed in those on the other side.

One group of the vaccinated and one group of the unvaccinated birds was challenged with strain 1343 [a South African field isolate of NAD-independent *H. paragallinarum (*Bragg 2002b)] while the remaining two groups were challenged with strain 46 C-3 [an NAD-dependent field isolate of *H. paragallinarum* (Bragg 2002a)] according to the methods used by Bragg (2002a). The clinical signs in the birds were recorded and scored for a 20-day period. The severity of the clinical signs was given a numerical value according to the methods described by Bragg (2002a) and disease profiles were plotted.

RESULTS

The results of the scoring of the clinical signs in the groups that were challenged with NAD-dependent serovar C-3 were used to produce a graphic representation of the course of the disease which is given in Fig. 1. Similarly the disease profiles obtained in the chickens challenged with the NAD-independent serovar C-3 are given in Fig. 2. Mean disease scores for each of the different experimental groups, as calculated from the disease profiles, are reflected in Table 1.

A statistical analysis of the disease profiles obtained was performed using a t test and the results obtained are shown in Table 1.

TABLE 1 Summary of the disease scores and profiles obtained when vaccinated and unvaccinated chickens challenged with serovar C-3 isolates of NAD-dependent and NAD-independent isolates of *H. paragallinarum*

Isolate	Vaccinated	Mean disease score	Significance	Highest score	Duration (days)#
C-3 (Dep*)	Yes	0.45	0.000358	1.6	12
C-3 (Dep)	No	1.85		4.2	15
C-3 (Indep**)	Yes	0.375	0.389	1.2	15
C-3 (Indep)	No	0.415		1.3	19

Calculated as the last day on which clinical signs were seen in the group of chickens

* NAD dependent

** NAD independent

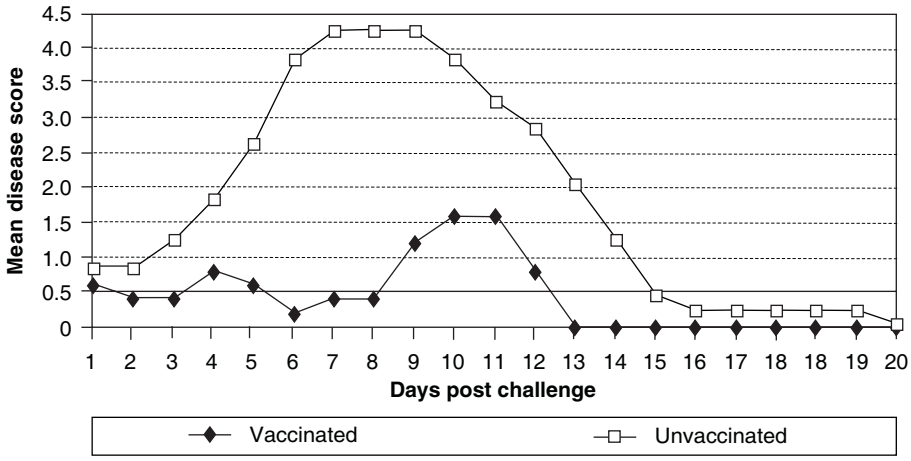

FIG. 1 Disease profiles of vaccinated and unvaccinated chickens challenged with an NAD-dependent serovar C-3 isolate of *H. paragallinarum*

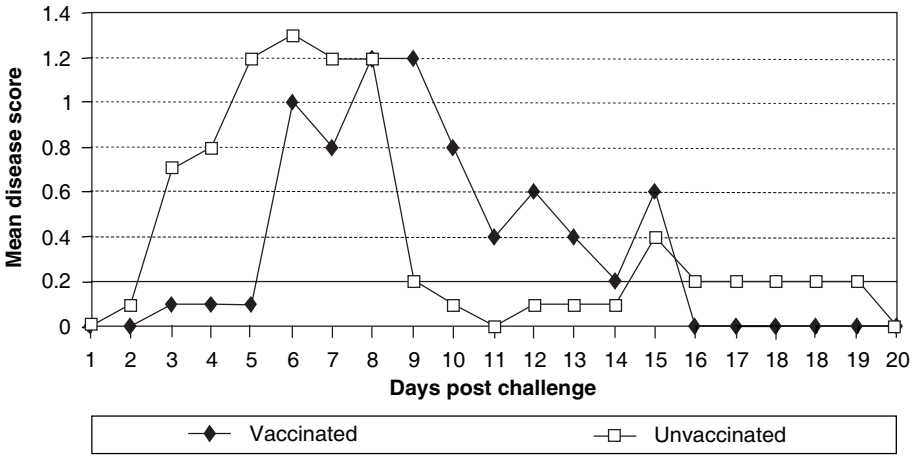

FIG. 2 Disease profiles of vaccinated and unvaccinated chickens challenged with an NAD-independent serovar C-3 isolate of *H. paragallinarum*

DISCUSSION

It can be seen from this experiment that chickens which were vaccinated with an experimental vaccine were not protected against challenge with an NAD-independent isolate of *H. paragallinarum.* It was also demonstrated in this experiment that the experimental vaccine used in this experiment provided protection to the NAD-dependent isolate of the same serovar. It is well known that protection afforded by a *H. paragallinarum* vaccine is dependent of the serovar used to make the vaccine (Rimler *et al*. 1977; Kume *et al*. 1980a, b).

The use of the experimental challenge model proposed by Bragg (2002a) has shown that the NAD-independent isolates have the ability to evade the immune system. It can be seen from Fig. 2 that the overall diseases profiles in the vaccinated and unvaccinated chickens are similar. It was found that there is not a significant difference between the two profiles (P = 0.389). It is evident from the graph in Fig. 2 that the development of clinical signs in the vaccinated birds was delayed when compared to that of unvaccinated birds and that the duration of clinical signs was shorter in the vaccinated birds. However, the highest daily disease scores of the two groups of birds were found to be very similar (Table 1). This is in contrast to the results obtained when the birds were challenged with the NAD-dependent isolates. The data obtained revealed that there is a significant difference (P = 0.000358) between the disease profiles obtained for the vaccinated and unvaccinated birds. These results indicate that the vaccine used in this experiment provided protection when the birds were challenged with the NAD-dependent serovar C-3 isolate.

These findings strongly suggest that the NAD-independent isolate was capable of evading the protective immunity induced in the vaccinated birds and support the hypothesis of immune-evasion by NAD-independent strains which was postulated from results obtained from previously described work.

One possible explanation for the ability of the NAD-independent isolates to evade the immune system could be that the acquisition of the genes for NAD independence, which appear to be plasmid mediated and allow *H. paragallinarum* to use other niches in the sinuses of the chicken. It is possible that the NAD-dependent isolates need to colonize areas in the sinus with a rich blood supply, thus allowing for the drawing of the needed NAD from the circulating blood stream. However, if the birds had been vaccinated with inactivated bacterins against *H. paragallinarum*, the circulating blood would also have contained specific antibodies against the different serovars of *H. paragallinarum* (depending on the composition of the vaccine). If the acquisition of NAD independence is freeing the bacteria from their dependence on drawing NAD for the blood supply, this might allow them to grow in other areas of the sinuses. It would be interesting to investigate the locations of NAD-dependent and NAD-independent strains of *H. paragallinarum* in the sinuses of experimentally infected chickens.

CONCLUSIONS

It would appear, from the results obtained in this experiment that the NAD-independent isolates appeared to have been able to evade the immune system in the vaccinated chickens. If this hypothesis is correct, there is a need for an alternative vaccination strategy, or some other disease control strategy, for the control of IC caused by the NAD-independent variants, such as the stimulation of local immunity in the upper respiratory tract of the chickens. Other disease control methods could include improved biosecurity through the use of non-toxic disinfectants as described recently by Bragg (2004). Fortunately, it would appear that the NAD-independent isolates of serogroup C *H. paragallinarum* are less virulent that the NAD-dependent strains (Bragg 2002b; Taole *et al.* 2002).

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