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Changes in the incidences of the different serovars of *Haemophilus paragallinarum* in South Africa: a possible explanation for vaccination failures

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ABSTRACT

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Infectious coryza remains an important disease in the poultry industry despite the long-term and widespread use of vaccines against its causative agent, *Haemophilus paragallinarum*, in South Africa.

In order to detect antigenic changes between populations of *H. paragallinarum* isolated before the use of vaccines against infectious coryza in this country, and field isolates obtained after the introduction of infectious coryza vaccines, 106 different NAD-dependent isolates (of which 93 were identified as *H. paragallinarum*) from 63 different farms, and dating from 1972 to March 1995, were identified by means of rabbit antisera against serogroups A, B and C. Serogroup C isolates show weaker cross-protection, requiring the further subdivision of this serogroup into its four different serovars.

The percentages of the different serovars obtained in the 1970s, confirmed previously published data on South African isolates. A tendency towards a decrease in the number of serogroup A and serovar C-2 isolates, and an increase in the percentage of serovar C-3 isolates, was noted among isolates of the 1980s. These changes were markedly enhanced in the isolates obtained from 1990 to March 1995. The percentage of serogroup A isolates decreased significantly from 34% in the 1970s to only 5% in the 1990s, and that of serovar C-2 isolates, from 31–18%, while the abundance of serovar C-3 isolates increased significantly from 31% in the 1970s to 73% in the 1990s. Serogroup B remained more or less constant and never reached more than 10 % of the population.

These results indicate the need for the incorporation of serovar C-3 in a vaccine for use in South Africa, particularly in those areas of the country from which isolates were collected during this study.

Some of the NAD-dependent isolates obtained from poultry in South Africa between 1970 and 1995, were biochemically identified as *Pasteurella avium* and *P. volantium*. As *H. avium* has been subdivided and reclassified into the genus *Pasteurella*, this represents the first report of the identification of *P. avium* and *P. volantium* in South Africa.

Keywords: *Haemophilus paragallinarum*, incidences, infectious coryza, serovars, vaccination failures, *Pasteurella avium, Pasteurella volantium*

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INTRODUCTION

Infectious coryza, caused by the bacterium *Haemophilus paragallinarum*, is a disease which causes upper respiratory tract infections in poultry. The disease usually shows only mild clinical symptoms, but causes losses of 10–40% in egg production in many

countries (Yamamoto 1991) and is therefore of significant economic importance to the poultry farmer.

The first serious outbreak of infectious coryza reported in South Africa, was in 1968 on a multi-age farm of approximately 100 000 layers. Soon after that, the disease spread to most large production sites and established itself as the most common bacterial infection in layers. Although vaccination has limited production losses since 1975, infectious coryza remains a serious disease among layers in South Africa, particularly in the winter months (Buys 1982).

There appears to be a correlation between the Page typing scheme and type-specific immunity (Rimler, Davis & Page 1977). Vaccines based on Page serotype A organisms protect against Page serotype A challenge, but not against serotype C challenge. This was confirmed by Kume, Sawata & Nakase (1980), who found that their serotype I cross-reacted with Page serotype A, and their serotype II cross-reacted with Page serotype C. The correlation between serotype and protective immunity was also established in Australia, where *H. paragallinarum* of serogroups A and C abound (Thornton & Blackall 1984), and where inactivated vaccines containing these two serotypes and aluminium hydroxide as an adjuvant, have provided ample protection (Blackall 1986; Reid & Blackall 1986) in a serotype-specific manner (Blackall & Reid 1987).

Although the plate-agglutination method of Page (1962) for the serotyping of isolates has proved most useful, a considerable number of isolates were found untypable by this method (Thornton & Blackall 1984; Blackall & Eaves 1988), including isolates collected in South Africa in the 1970s (Buys, personal communication 1994).

Kume, Sawata, Nakase & Matsumoto (1983) established a different scheme for the serotyping of *H. paragallinarum*, based on haemagglutination antigens which were obtained by sonication and potassium-thiocyanate extraction. A haemagglutination (HA) test was performed with the use of glutaraldehyde-fixed chicken erythrocytes together with these hemagglutinins. The different isolates were characterized according to their haemagglutination-inhibition (HI) reaction with rabbit-raised antisera against the different isolates. This scheme recognized three different serogroups and seven different serovars. Kume *et al.* (1983) placed two "untypable" South African isolates into their serovar HA-6. Eaves, Rogers & Blackall (1989) and Blackall, Eaves & Rogers (1990a) isolated two new serovars in Australia, thus creating the awareness that new serovars could continuously emerge. This prompted Blackall *et al.* (1990a) to propose the alteration of the nomenclature of the Kume scheme. It was suggested (Blackall *et al.* 1990a) that Kume's groups I, II and III, be changed to A, C and B, respectively, to reflect the relatedness of these groups to the original groups detected by Page (1962). Serovars within each group could be numbered A-1 to A-4, B-1, and C-1 to C-4, with the two South African isolates being renamed C-3. This is an open-ended system and any new serovars detected at a later stage, would simply be assigned the next number in the serogroup to which they belong. The nomenclature of Blackall *et al.* (1990a), of serogroups A, B and C, consisting of serovars A-1 to A-4, B-1, and C-1 to C-4, has been used throughout this text in the discussion of isolates classified according to the haemagglutination-inhibition method first described by Kume *et al.* (1983). The data for serotypes A, B and C, were obtained by the Page plate-agglutination method.

The Australian isolates used for vaccine production have been re-examined by the Kume scheme and were found to consist of serovars C-2, C-4 and A-4 (Eaves *et al.* 1989; Blackall *et al.* 1990a). It was found (Blackall 1991) that there is cross-protection between serovars C-2 and C-4 (between 50 and 100%), but little cross-protection was detected when chickens were vaccinated with serovar A-4 and challenged with either serovars C-2 or C-4. It has also been found that Kume serovars C-1 and C-2 are cross-protective, but Kume A-1 does not cross-protect with C-1 or C-2 (Kume *et al.* 1980).

Kume *et al.* (1980) observed that serotype C strains appear to be inherently less immunogenic than those of serotype A. Although cross-protection between serovar C strains H-18 and Modesto has been demonstrated (Kume *et al.* 1980), this cross-protection was not complete. The SA-3 strain has been used in cross-protection studies (Kume 1985 as cited by Matsumoto 1988). In this study, the cross-protective abilities of strains 221, 2403, E-3C (serogroup A), H-18, Modesto, SA-3 (serogroup C) and 2671 (serogroup B) were examined. The results were presented on a scale of 0–4, with 0 representing no protection after two vaccinations and 4 representing no clinical signs and no persistent organisms in 70% of the chickens after only one vaccination.

In this study, it was found that chickens vaccinated with SA-3 and subsequently challenged with SA-3, yielded a score of 4. Chickens vaccinated with SA-3 and challenged with either H-18 or Modesto, yielded a score of only 2. Similar results were obtained when SA-3 was used as the challenge strain for chickens vaccinated with H-18 or Modesto. These results demonstrate that only partial cross-protection is obtained between SA-3 and the other serogroup C strains.

The first vaccine produced in South Africa in 1975 by Buys, consisted of strain 0083 (serovar A-1) only (Buys, personal communication 1994). Buys (1982) later established that serogroup B organisms were also present in this country, and the vaccine was adjusted to contain both strains 0083 (serovar A-1) and

0222 (serovar B-1) (Buys, personal communication 1994). This vaccine was modified in 1993, by the removal of strain 0222, and its replacement with a South African field isolate (information on the serotype of this isolate is not available). This vaccine was again modified in 1995 to include a South African serovar C-2 field isolate (Jäger, personal communication 1996).

Under the supervision of one of the authors (L.C.), a larger layer operation in South Africa produced two in-house vaccines containing two isolates, i.e. SB86 from a site in the Tala Valley in KwaZulu-Natal and M85 from a site close to Pretoria. One vaccine, incorporating isolate M85, was used for 5 years (beginning in 1985) and the other, incorporating isolate SB86 and giving ample protection, was used for 10 years (beginning in 1986) by clients of this layer operation.

Imported, commercially available vaccines containing serogroup A and serogroup C strains, are also used in South Africa, as well as two trivalent vaccines consisting of serogroups A, B and C. All of the commercially available bacterins against infectious coryza, consist of inactivated broth cultures. Although some vaccines contain only one strain, most commercial vaccines consist of a combination of two or three different serotypes. In most cases, the vaccines contain more than 10^8 CFU/mℓ, inactivated either by formalin or merthiolate, and they usually include adjuvants, stabilizers and saline diluents (Yamamoto 1991).

Although vaccines against infectious coryza have been used in this country since 1975 (Buys, personal communication 1994), it became apparent in the 1980s that the vaccines were becoming less effective in controlling the disease. This could have been due to the emergence of a previously unknown serovar, or even serogroup, of *H. paragallinarum* in South Africa, as two newly emerged serovars in Australia were described by Eaves *et al.* (1989) and Blackall *et al.* (1990a). Another explanation is the possibility of changes in the population dynamics, resulting in a change in the relative abundance of non-cross-protective serovars.

Although a sample of South African isolates, characterized in Australia, revealed the presence of serovars A-1, C-2, C-3 and B-1 (Eaves *et al.* 1989), they could not be regarded as representative of the total *H. paragallinarum* population in South Africa. This study was introduced on a national level. It was aimed at evaluating a more respresentative population of South African isolates so as to obtain information on the distribution of the different serovars in this country, as well as to determine any shift in the population dynamics of serovars over the past three decades, covering the period before and after vaccination against infectious coryza.

MATERIALS AND METHODS

Bacterial isolates

Reference isolates

Reference isolates, representing the different serovars described by Kume *et al.* (1983) and renamed by Blackall *et al.* (1990a), were obtained from Dr Blackall, Animal Research Institute, Yeerongopilly, Australia. They consisted of strains 221, HP-90, E-3C and HP-14, representing serovars A-1, A-2, A-3 and A-4, respectively; strains H-18, Modesto, SA-3 and HP-60, representing serovars C-1, C-2, C-3 and C-4, respectively; and strain 0222, representing serovar B-1. Samples of strains 221 (serovar A-1), Spross (serovar B-1) and H-18 (serovar C-1), were kindly supplied by Dr A.A.C. Jacobs of Intervet, Boxmeer, The Netherlands. Strains 0083 (serovar A-1) and 0222 (serovar B-1) were obtained from three different sources: Dr Blackall, Avimune (formerly Golden Lay Laboratories, P.O. Box 14167, Centurion, 0140 South Africa) and our own stored samples.

All of these isolates were supplied in a freeze-dried state and were reconstituted in this laboratory in modified Casman's medium which had been supplemented with 10% sterile chicken serum (Coetzee, Rogers & Velthuysen 1983).

Field isolates

A total of 48 NAD-dependent isolates, from 21 different farms, were obtained from diseased chickens between 1990 and March 1995, and were stored either in the Microbank system (supplied by Davies Diagnostic, P.O. Box 3222, Randburg, 2125 South Africa) or freeze-dried. These isolates were all reconstituted in modified Casman's medium supplemented with 10% sterile chicken serum and plated onto Blood-tryptose-agar (BTA) plates (obtained from Onderstepoort Biological Products, Private Bag X5, Onderstepoort, 0110 South Africa), then inoculated with a feeder culture of *Staphylococcus aureus* and incubated at 37 °C for 18 h in a candle jar. As far as could be ascertained, all of these isolates were obtained from chickens vaccinated with registered infectious coryza vaccines.

The registered vaccines available in South Africa during the 1990s are listed in Table 7.

A total of 12 isolates from ten different farms, and obtained in the 1980s by Dr Fritz Huchzermeyer of the Onderstepoort Veterinary Institute from cases of infectious coryza, were also examined. These isolates were stored in culture fluid that had been frozen in liquid nitrogen, and were reconstituted by thawing and inoculating 100 µℓ into 10 mℓ of modified Casman's medium supplemented with 10% sterile chicken serum. The medium was incubated at

37°C for 18 h. Two other isolates obtained in the 1980s, M85 and SB86, were kindly supplied by Avimune. All isolates were obtained from chickens vaccinated with the only registered infectious coryza vaccine available during the 1980s (see Table 7).

A total of 44 isolates from 31 different sites, and obtained in the 1970s by Dr Buys and Mr Van der Walt (then of the Onderstepoort Veterinary Institute) were also examined. These isolates were all freeze-dried and reconstituted as described above. At the time these isolates were obtained, no infectious coryza vaccines were available in South Africa, therefore these isolates were all obtained from non-vaccinated chickens.

Details of the geographical areas from where all of the isolates biochemically identified as *H. paragallinarum* were obtained, can be seen in Tables 3–5. Data relating to isolates not identified as *H. paragallinarum,* are not shown in these tables.

Biochemical characterization

Carbohydrate fermentation tests were carried out in tubes containing phenol red broth (BDH), 0,5% (w/v) NaCl, 1% (v/v) sterile chicken serum and 0,0025% (w/v) NAD, supplemented with 1% (w/v) of either galactose, glucose, lactose, mannitol, mannose, sorbitol, sucrose or xylose (Blackall & Reid 1982).

Polyclonal antibodies

Polyclonal antibodies were obtained from two different sources. Samples of rabbit-raised antibodies against serogroups A, B and C were obtained from Dr P.J. Blackall. These antiserum samples were diluted 1:100 before use.

Rabbit-raised antibodies against *H. paragallinarum*, strain SA-3, were produced by the South African Institute of Medical Research (SAIMR) according to the methods described by Bragg, Purdan, Coetzee & Verschoor (1995), without any modifications.

Haemagglutination (HA) and haemagglutination-inhibition (HI) test

All of the reference isolates, as well as all of the field isolates obtained either in the 1970s, 1980s or 1990s, were inoculated into 10 mℓ Casman's medium which had been supplemented with 10% sterile chicken serum and incubated at 37°C for 18 h. The purity of the cultures was ascertained by the inoculation of samples onto BTA plates which were inoculated with a feeder culture of *S. aureus* and incubated at 37°C for 18 h in a candle jar. Samples of fresh 18-h-old cultures of all the organisms were subsequently tested in the HA test.

The HA test was performed according to a modification of the method of Eaves *et al.* (1989) as described by Bragg *et al.* (1995). In the wells of V-bottomed, 96-well plates, 50 µℓ of twofold serial dilutions of the different bacterial cultures were added to 50-µℓ volumes of Veronal buffer (VB). Each well subsequently received 50 µℓ of glutaraldehyde-fixed red-blood cells (RBC). The plates were incubated at room temperature for 1 h. The HA titre was read as the highest dilution of the bacterial suspension causing haemagglutination of the glutaraldehyde-fixed RBC.

All fresh cultures, not showing HA activity, were treated according to the methods of Blackall, Eaves & Aus (1990b). These cultures were washed by centrifugation, reconstituted in phosphate-buffered saline (PBS) containing 0,01% (w/v) thiomersal, and stored at 4 °C for 3 d, after which the HA test was repeated.

The HI test was also performed according to the method of Eaves *et al.* (1989), with some modifications, as described by Bragg *et al.* (1995). Volumes (50 µℓ) of twofold serial dilutions of the polyclonal antiserum in diluted VB were made in V-bottomed, 96-well plates. Each well subsequently received 50 µℓ of a dilution of four HA units of the different bacterial suspensions and was incubated for 15 min, prior to the addition of 50 µℓ of a 1% solution of glutaraldehyde-fixed RBC. After incubation for 1 h, the HI titre was read as the highest dilution of antiserum inhibiting haemagglutination of four HA units of the different bacteria.

RESULTS

Bacterial isolates and carbohydrate fermentation tests

A correlation between the serotypes of *H. paragallinarum* and protective immunity has already been demonstrated (Rimler *et al.* 1977; Kume *et al.* 1980; Blackall 1991), necessitating the serotyping of isolates from diseased chickens to predict the efficacy of a particular vaccine composition. Before any attempt was made to serotype the isolates of NAD-dependent organisms obtained in the 1970s, 1980s and 1990s by the HA and HI tests, the carbohydrate-fermentation patterns of the isolates were determined and compared with those determined and published for *H. paragallinarum* (Reid & Blackall 1982), *Pasteurella avium, P. volantium* and *Pasteurella* type A (Mutters, Piechulla, Hinz & Mannheim 1985).

From1990 to March 1995, a total of 48 NAD-dependent isolates were obtained from chickens with signs of infectious coryza. From the carbohydrate-fermentation patterns, it was established that 44 of these isolates were *H. paragallinarum*, while the remaining four isolates were identified as *Pasteurella avium* (Table 1). Only 14 of the isolates obtained in the 1980s could be successfully reconstituted, and these

were all found to be NAD-dependent *H. paragallinarum*, by carbohydrate-fermentation tests. From a total of 44 reconstituted freeze-dried samples of NAD-dependent isolates obtained in the 1970s, 35 were identified as *H. paragallinarum* and nine were found to be *Pasteurella* spp. (Table 1).

TABLE 1 Number 0f isolates identified as *Haemophilus paragallinarum*, *Pasteurella avium*, *P. volantium* and *Pasteurella* type isolates obtained in the 1970s, 1980s and 1990s in South Africa

Decade	Total	*H. paragallinarum*	*P. avium*	*P. volantium*	*Pasteurella* type A
1970s	44	35	5	4	0
1980s	14	14	0	0	0
1990s	48	44	4	0	0

Antiserum specificity

The HA test revealed that fresh cultures of the serogroup A strains and Strain 0222 of serogroup B, haemagglutinated glutaraldehyde-fixed RBC without any further treatment. No fresh cultures of the Spross strain (serogroup B) and none of the serogroup C cultures agglutinated glutaraldehyde-fixed RBC unless these cultures were first washed and stored at 4°C for 3 d, as suggested by Blackall *et al.* (1990b). This treatment potentiated all of these strains to haemagglutinate GA-fixed RBC.

The specificity of the freshly prepared rabbit antiserum against strain SA-3 was tested against strains 0083, 221, Spross, Modesto, H-18, SA-3, HP-60 and SB86 by HI. No HI activity was detected against Strains 0083, 221, Spross, H-18 or Modesto, when undiluted and unabsorbed serum was used. Some HI activity with HP-60 (C-4) and a strong reaction against SA-3 were found (Table 2). The reaction against SA-3 was equivalent to the reaction obtained against SB86, indicating that isolate SB86, like SA-3, is a serovar C-3 isolate (Blackall *et al.* 1990a).

TABLE 2 Serotyping of *Haemophilus paragallinarum*, strain SA-3, by determining the specificity of its antiserum against reference strains in haemagglutination inhibition

Isolate	Serovar	HI titre (reciprocal of highest dilution showing HI activity ($n = 4$)
H-18	C-1	0
Modesto	C-2	0
SA-3	C-3	38
HP-60	C-4	8
Spross	B-1	0
0083	A-1	0
221	A-1	0
SB86	?	36

Serogrouping of field isolates by HI

Serological classification of the 35 *H. paragallinarum* isolates obtained in the 1970s, was done with the use of HA and HI tests. From Table 3, it can be seen that 12 of these isolates were serologically identified as serogroup A, one as serogroup B and 11 as serogroup C. The remaining isolates were found not to show HI activity with any of the polyclonal antibodies supplied by Dr Blackall. When these isolates were tested with the freshly prepared antiserum against SA-3, all reacted with either undiluted antiserum or antiserum diluted 1:10 or 1:100, thus exhibiting serovar C-3 characteristics.

The 14 isolates obtained during the 1980s, were similarly classified serologically. Four were identified as serogroup A, one as serogroup B, three as serogroup C, and six did not react with any of the antisera obtained from Dr Blackall (Table 4). These latter isolates were all found to react with the 1:100 dilution of the antiserum prepared against SA-3, this suggesting that these isolates are of serovar C-3, similar to the isolates collected during the 1970s.

Of the 44 *H. paragallinarum* isolates obtained from 1990 to March 1995, only two isolates were serologically identified as serogroup A, two as serogroup B and eight as serogroup C. The vast majority of the

TABLE 3 Geographical distribution of isolates and serovar of *Haemophilus paragallinarum* identified during the 1970s

Case no.	Farm	Geographical area	Size	Serovar isolated
1	1	KwaZulu-Natal	M [b]	C-3
4	2	KwaZulu-Natal	M	C-2
7	3	North West	M	C-2
8	4	Gauteng	S [a]	A-1
10	5	Gauteng	S	C-2
11	1	KwaZulu-Natal	M	C-2
18	6	KwaZulu-Natal	S	C-2
24	7	Cape Province	M	A-1
26	8	Mpumalanga	M	C-3
32	9	Free State	M	A-1
37	10	Mpumalanga	L [c]	C-2
39	11	Gauteng	S	A-1
41	12	Mpumalanga	M	A-1
42	13	Free State	M	C-3
43	14	Gauteng	S	C-2
44	15	North West	M	A-1
45	16	Gauteng	S	A-1
46	1	KwaZulu-Natal	M	C-3
47	1	KwaZulu-Natal	M	C-2
48	1	KwaZulu-Natal	M	C-3
49	15	North West	M	A-1
53	17	North Cape	M	C-3
54	18	Gauteng	S	C-3
55	19	Mpumalanga	S	C-2
56	21	Gauteng	L	A-1
58	21	Gauteng	L	B-1
59	46	North West	M	C-3
60	21	Gauteng	L	A-1
63	10	Gauteng	L	C-2
73	22	Mpumalanga	L	C-3
97	22	Mpumalanga	L	C-3
100	23	Mpumalanga	L	A-1
101	24	North West	M	A-1
106	48	Gauteng	M	C-2
108	47	KwaZulu-Natal	L	C-3

[a] Small farms with fewer than 3 000 layers
[b] Medium-sized farms with 3 000–10 000 layers
[c] Large farms with more than 10 000 layers

TABLE 4 Geographical distribution of isolates and serovars of *Haemophilus paragallinarum* identified during the 1980s

Case no.	Farm	Geographical area	Size	Serovar isolated
216	20	Cape Province	S [a]	A-1
308	26	Gauteng	L [c]	C-3
386	27	KwaZulu-Natal	M [b]	A-1
387	28	KwaZulu-Natal	M	A-1
418	25	Northern Province	M	C-2
428	26	Gauteng	L	B-1
430	29	Gauteng	L	C-3
454	49	Free State	M	C-3
487	50	Mpumalaga	M	C-2
505	30	Gauteng	S	C-2
530	31	Gauteng	L	A-1
564	32	KwaZulu-Natal	M	C-3
M85	51A	Gauteng	L	C-3
SB86	51B	KwaZulu-Natal	L	C-3

[a] Small farms with fewer than 3 000 layers
[b] Medium-sized farms with 3 000–10 000 layers
[c] Large farms with more than 10 000 layers

TABLE 5 Geographical distribution of isolates and serovars of *Haemophilus paragallinarum* identified during the 1990s

Case no.	Farm	Geographical area	Size	Serovar isolated
564/92	38	Mpumalanga	L [c]	C-3
606/91	33	Mpumalanga	M [b]	C-2
637/91	34	Gauteng	S [a]	C-3
639/91 A	35	KwaZulu-Natal	L	C-2
639/91 B	35	KwaZulu-Natal	L	C-2
644/91	42	Mpumalanga	M	C-2
704/92	37	Gauteng	M	C-3
709/92	19	Mpumalanga	L	C-3
732/93 A	39	Gauteng	L	C-3
732/93 B	39	Gauteng	L	C-3
739/91	36	Free State	L	C-3
844/90 A	35	KwaZulu-Natal	L	A-1
844/90 B	35	KwaZulu-Natal	L	C-3
864/90 A	35	KwaZulu-Natal	L	C-3
864/90 B	35	KwaZulu-Natal	L	C-3
910/90	35	KwaZulu-Natal	L	C-3
910/91	43	KwaZulu-Natal	L	B-1
965/91 A	38	KwaZulu-Natal	L	B-1
965/91 B	38	Gauteng	L	C-3
1053/92 A	39	Gauteng	M	C-3
1053/92 B	39	North West	M	C-3
1112/93	29	Gauteng	L	C-3
1130/93	38	Gauteng	L	C-2
1382/92 A	29	Gauteng	L	A-1
1382/92 B	29	Gauteng	L	C-3
1382/92 C	29	Gauteng	L	C-2
1438/92	5	Gauteng	L	C-2
1439/92	40	Mpumalanga	L	C-3
1640/92 A	41	Gauteng	L	C-2
1640/92 B	41	Gauteng	L	C-3
1649/94	5	Gauteng	L	C-3
1665/92 A	38	Northern Province	L	C-3
1665/92 B	44	Northern Province	L	C-3
1670/93 A	38	KwaZulu-Natal	L	C-3
1670/93 B	38	KwaZulu-Natal	L	C-3
1670/93 C	38	KwaZulu-Natal	L	C-3
1672/92	38	Gauteng	L	C-3
1672/93	45	Gauteng	M	C-3
1688/93 A	11	Gauteng	M	C-3
1688/93 B	11	Gauteng	M	C-3
1697/92 A	23	North West	M	C-3
1697/93	29	Gauteng	L	C-3
1721/94 A	29	Gauteng	L	C-3
1721/94 B	29	Gauteng	L	C-3

[a] Small farms with fewer than 3 000 layers
[b] Medium-sized farms with 3 000–10 000 layers
[c] Large farms with more than 10 000 layers

isolates (32) were found to be untypable when the antiserum supplied by Dr Blackall was used, but they did react with the 1:100 dilution of the antiserum prepared against SA-3. Therefore they belonged to serovar C-3 (Table 5).

Population dynamics and serotype incidence

The results in Table 6, graphically presented in Fig. 1, reveal that the percentage of serogroup B isolates in the population has remained more or less constant throughout the measured period. During the 1970s, only one isolate of serogroup B was obtained, and this represents 3% of the population. In the 1980s, one isolate of serogroup B was also found. Only 14 samples of isolates obtained in the 1980s were available, therefore the single isolation of a serogroup B isolate represents 7% of the population. Serogroup B isolates represent 5% of the total population isolated during the 1990s.

There appears to be a marginal decrease in the percentage of serogroup A isolates between the 1970s, and the 1980s, with this serogroup representing 34 % in the 1970s and 29% in the 1980s. However, there is a marked decrease in the numbers of serogroup A isolates obtained during the 1990s (Fig. 1), with only two of the isolates having been identified as belonging to serogroup A (Table 1). This represents only 5% of the population and is therefore a significant decrease from the 29% recorded in the 1980s.

Similar results were obtained with the serogroup C isolates which were characterized as serogroup C by virtue of their reaction with the serogroup C polyclonal antibodies obtained from Dr Blackall. There was a slight decrease in the percentage of these serogroup C isolates between the 1970s and 1980s, with the percentage decreasing from 31% in the 1970s to 21% in the 1980s. This decrease in the percentage of serogroup C isolates continued into the 1990s, when only 18% of the population were identified as serogroup C isolates.

The most interesting and significant results were obtained when the numbers of isolates which did not react with the antisera supplied by Dr Blackall, were studied. There was an increase in the percentage of these isolates between the 1970s and 1980s, with percentages increasing from 31% in the isolates obtained in the 1970s, to 43% in those obtained in the 1980s, continuing to a level of 73% in the 1990s.

TABLE 6 Serotyping of *Haemophilus paragallinarum* isolates obtained in the 1970s, 1980s and from 1990 to March 1995, by means of haemagglutination inhibition

Decade	Serotype	Total	Percentage
1970s	A	12	34
	B	1	3
	C	11	31
	C-3	11	31
1980s	A	4	29
	B	1	7
	C	3	29
	C-3	6	43
1990s	A	2	5
	B	2	5
	C	6	18
	C-3	32	73

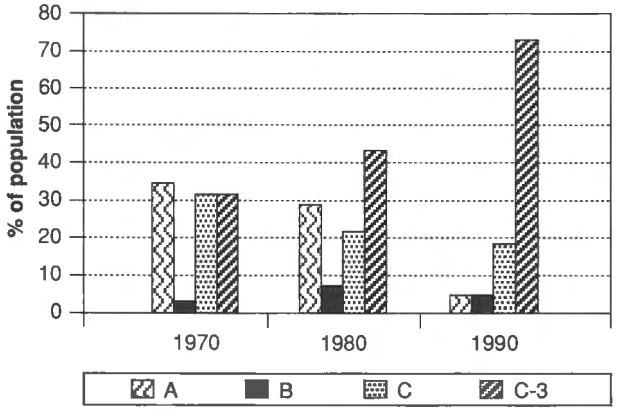

FIG. 1 Geographic representation of the distribution of the different serotypes of *Haemophilus paragallinarum*

From the results in Fig. 1, it is evident that these isolates have become the dominant serovar in the population of *H. paragallinarum* in South Africa. They were all found to react to the 1:100 dilution of freshly prepared rabbit antiserum against strain SA-3, and therefore identified as serovar C-3

DISCUSSION

During the 1980s, it became apparent that poultry flocks were no longer sufficiently protected by the available infectious coryza vaccines. This resulted in decreased egg production and significant financial losses to the poultry farmer. These findings of inadequate protection led Verschoor, Coetzee & Visser (1989) to produce a panel of Mabs in an attempt to detect antigenic differences between the strains of *H. paragallinarum* which were used in the vaccines, and the isolates obtained from vaccinated flocks. It was later established that these Mabs were not suitable for the monitoring of the population of *H. paragallinarum* in terms of their expressed antigens involved in protective immunity (unpublished data).

It has been established by a number of workers that the serotype-specific hemagglutinins play a role in protective immunity (Rimler *et al.* 1977; Kume *et al.* 1980). The establishing of the serovars of *H. paragallinarum* present in a country, and the rapid detection of any changes in the population dynamics of these organisms, is therefore of the utmost importance if the disease is to be effectively controlled by vaccination. Work carried out in Brazil, suggested that an active monitoring programme is needed to ensure that the correct serovars are used in vaccines for certain areas (Blackall, Silva, Yamaguchi & Iritani 1994). It has been well established that there is little or no cross-protection between organisms of different serogroups (Rimler *et al.* 1977; Kume *et al.* 1980). Furthermore, serogroup C strains appeared to be less immunogenic than the serogroup A strains (Kume *et al.* 1980) and provided incomplete cross-protection among different serovar C strains, with a range of 50–100% (Blackall 1991), depending on the serovars used for the production of the bacterin and the serovar of the challenge organisms.

In South Africa, the first vaccine to be produced and used, contained only strain 0083 (serovar A-1), as Buys (1982) found that 81% of his isolates were serologically identified as serotype A, according to the methods used by Page (1962). Later, this vaccine was adjusted to contain serotype B as well, when Buys (1982) found serotype B isolates in this country. Buys (1982) did not report on the isolation of serotype C isolates. Serogroup C isolates were, however, later reported from South African samples sent overseas (Kume, *et al.* 1983; Blackall & Eaves 1988; Eaves *et al.* 1989).

At present, there are eight registered infectious coryza vaccines, that are manufactured outside South Africa, and are registered for use in this country. Three of these vaccines are trivalent vaccines containing serovar A-1, B-1 and C-1 strains. The other vaccines are all bivalent vaccines containing serogroup A and either serovars C-1 or C-2. Strains W, 0083, 221 or 17756 are used for serogroup A; Spross or 0222, for serogroup B; and H-18 or Modesto, for serogroup C (see Table 7).

Buys (1982) established that 44% of the isolates which he had obtained were not the pathogenic *H. paragallinarum*, but the apathogenic "*H. avium*". Mutters *et al.* (1985) established by DNA/DNA hybridization, that the isolates identified as *H. avium* could be divided into three different species found to be more closely related to the genus *Pasteurella* than to the genus *Haemophilus*. It can be seen (Table 1) that 14% of all isolates obtained were not *H. paragallinarum*, but were identified as what was previously called *H. avium*, and are now biochemically

TABLE 7 Information on the strains of *Haemophilus paragallinarum* available in registered infectious coryza vaccines in South Africa

Vaccine no.	Date registered	Date withdrawn	Local or imported	Strains in the vaccine	Serogroup or serovar
1	Before 1980	1990	L	0083 0222	A-1 B-1
2	1990	?	L	0083 Tongaat	A ?
3	1991	Current[a]	I	W Modesto	A C-2
4	1992	Current	I	W Modesto	A C-2
5	1992	Current	I	W Modesto	A C-2
6	1993	Current	I	W ? Modesto	A B C-2
7	1993	Current	I	W ? Modesto	A B C-2
8	1993	Curtent	I	0083 Spross H-18	A-1 B-1 C-1
9	1993	Current	I	17756 0083 Modesto	A A-1 C-2

? = Information not available

[a] = Still available

characterized as *P. avium* (nine isolates) and *P. volantium* (four isolates).

Very interesting results were obtained when the field isolates of *H. paragallinarum* were serotyped by the HI method established by Kume *et al.* (1983) and modified by Blackall *et al.* (1990a; b).

The samples of freeze-dried isolates obtained in the 1970s were of particular interest, as these isolates were obtained from chickens in this country before the use of any vaccines against infectious coryza, thus representing the population incidence of serovars before any selective pressure, resulting from vaccination, could be exerted on the population.

The presence of serogroup A, B and C isolates in the South African population of *H. paragallinarum* has previously been reported in the literature (Kume *et al.* 1983; Blackall & Eaves 1988; Eaves *et al.* 1989). Blackall & Eaves (1988), using the plate agglutination method of Page (1962), found 31% of the South African samples they examined, to be serotype A, and 31% to be serotype C. They found 38% of these samples to be untypable according to the Page method. In the following year, Eaves *et al.* (1989) studied a number of isolates, including 12 South African isolates, according to the HI method established by Kume *et al.* (1983). In this study, 41% of the South African isolates were identified as HA-1 [later renamed serovar A-1 by Blackall *et al.* (1990a)], 17% as HA-7 (serovar B-1), 25% as HA-5 (serovar C-2) and 17% as HA-6 (serovar C-3). Although the percentages of the population differ from those obtained in Table 6, it can be seen that the identification of serogroups A, B, C, by means of the serum obtained from Dr Blackall, and the identification of isolates appearing to be C-3, by means of rabbit-raised antiserum against SA-3, appears to correspond favourably with the results obtained by Eaves *et al.* (1989). It is therefore most likely that the serovars of *H. paragallinarum*, identified as serogroups A, B and C, are serovars A-1, B-1 and C-2, respectively.

All four serovars of *H. paragallinarum* found among the 1970s isolates, were also found in the population isolated and stored from the 1980s until March 1995 (Table 6). These results argue against the emergence of a new *H. paragallinarum* serovar or serogroup as the cause of the reported vaccine failure in South Africa. A change in the population incidence of the four different serovars (as illustrated in Fig. 1), appears to provide a more likely explanation.

The tendencies for change in the population dynamics of serovars A-1, C-2 and C-3, as seen in the isolates obtained between the pre-vaccination era and the 1980s, are even more noticeable in the 1990s.

Serovar A-1 isolates have all but disappeared in the 1990s. The percentage of C-2 isolates has also, as is the case with the 1980s isolates, continued to decline into the 1990s. The most significant changes can be seen in the population dynamics for the C-3 isolates. The tendency, identified in the 1980s, for an increased percentage of C-3 isolates, continued in the 1990s. Unlike the situation in the 1980s, a much larger number of samples were obtained and examined in the 1990s. The population sizes of the pre-vaccination samples, and the isolates obtained in the 1990s, are comparable, thus facilitating comparisons of the population dynamics. Details of exactly which infectious coryza vaccines were used on the farms where the isolates were obtained, are not available from the case records. However, in most cases it could be ascertained that the chickens from which the isolates were obtained, had been vaccinated. It was also possible to ascertain when the different infectious coryza vaccines were registered. From this data, the registered vaccines available at the time of isolation of each of the isolates, is known. None of the registered vaccines (Table 7) contains the uniquely South African C-3 serovar, although M85 and SB86, later serotyped as C-3, were used as in-house vaccines in one large layer operation. In this country, the registered vaccines that were used, and that do contain serogroup C strains, are probably providing only partial protection against serovar C-3, resulting in the significant increase seen in the numbers of C-3 isolates in the population (Fig. 1). The decrease in serovar A-1 and C-2 populations is possibly due to the extensive use of vaccines against infectious coryza. The in-house use of vaccines containing M85 and SB86, later serotyped as C-3, does not influence the interpretation of these results, as no isolates from farms involved in this operation, were submitted to the laboratory for serotyping.

The population incidence of the serovar B-1 organisms, appears to be unchanged by vaccination. A possible explanation for this could be that the strain of serovar B-1 used in the vaccines, is not completely cross-protective against the South African B-1 organisms. Supporting evidence for this was obtained by Yamaguchi, Blackall, Takigami, Iritani & Hayashi (1990), who found low levels of cross-protection between Spross and two South African field isolates which had been identified as serogroup B. In contrast, high levels of cross-protection between the South African isolates were observed.

For the effective control of infectious coryza in this country, there appears to be a need for a registered vaccine containing the C-3 serovar, which should be available to all poultry producers in South Africa. Ideally, the vaccine should contain isolates of serogroup A and serovar C-2, as well as South African isolates of serogroup B and serovar C-3. Such a vaccine should provide enhanced levels of protection against infectious coryza—at least in this country.

The need for an ongoing system for the monitoring of the population dynamics of *H. paragallinarum*, in order to ensure sustained vaccine efficiency, is well illustrated in this study.

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