Onderstepoort Journal of Veterinary Research, 63:197–207 (1996)

Pathogenicity differences between South African isolates of *Ornithobacterium rhinotracheale*

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ABSTRACT

TRAVERS, A.F., COETZEE, L. & GUMMOW, B. 1996. Pathogenicity differences between South African isolates of *Ornithobacterium rhinotracheale*. *Onderstepoort Journal of Veterinary Research*, 63:197–207

Three selected South African *Ornithobacterium rhinotracheale* field isolates were identified and inoculated via the caudal abdominal airsac into 28-day-old broilers, which were monitored under controlled laboratory conditions. It was concluded from data that the *O. rhinotracheale* isolates were capable of causing primary disease, with statistically significant resultant mass loss. Respiratory and arthritis symptoms were reproduced. However, no sinusitis was observed.

Airsacculitis- and arthritis-lesion scoring techniques showed significant pathogenicity differences between isolates. Furthermore, differences in symptomatology were also seen between isolates. A highly significant reisolation of *O. rhinotracheale* was made from the brains of broilers challenged with isolate 2.

In conclusion, the economic importance and financial loss due to *Ornithobacterium rhinotracheale* infections in poultry were highlighted.

Keywords: Isolates, *Ornithobacterium rhinotracheale*, pathogenicity differences

INTRODUCTION

The clinical importance of *Ornithobacterium rhinotracheale* has been established in South Africa, Germany, the Netherlands and the United States of America (Charlton, Channing-Santiago, Bickford, Cardona, Chin, Cooper, Droual, Jeffrey, Meteyer, Shivaprasad & Walker 1993; Hafez, Kruse, Emele, & Sting 1993; Van Beek, Van Empel, Van den Bosch, Storm, Bongers & Du Preez 1994; Van Damme, Segers, Vancanneyt, Van Hove, Mutters, Hommez, Dewhirst, Paster, Kersters, Falsen, Devriese, Bisgaard, Hinz & Mannheim 1994). In South Africa, *Ornithobacterium rhinotracheale* was initially isolated by J.H. du Preez (Van Beek *et al.* 1994) in 1991, from broilers with mild respiratory-tract infection and growth suppression. In North America (1991), isolates from broilers with respiratory problems were biochemically similar but serologically different from those in the Netherlands (Charlton *et al.* 1993; Van Beek *et al.* 1994).

O. rhinotracheale was isolated in Germany in 1992, from 23-week-old meat turkeys with respiratory problems, pneumonia and mortalities (Hinz, Blome & Ryll 1994). Hafez, Kruse, Emele & Sting (1993) reported similar observations. Since 1993, the syndrome has also been described in the Netherlands in turkeys and broilers (Van Beek *et al.* 1994).

In 1994, a new taxon, *Ornithobacterium rhinotracheale*, was proposed by Van Damme *et al.* (1994) for

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Accepted for publication 8 May 1996—Editor

bacteria first isolated from broilers in South Africa (1991). This new taxon was comprehensively described by the use of a wide range of taxonomic parameters, including genotypic, chemotaxonomic and classical phenotypic parameters, in order to establish the phylogenetic affiliation. Special emphasis was placed on the differentiation between *O. rhinotracheale, Riemerella anatipestifer* and *Capnocytophaga* species, as these taxa share a number of phenotypic characteristics (Van Damme *et al.* 1994).

In experimental challenge trials in the Netherlands, *O. rhinotracheale* led to statistically significant growth suppression in turkeys and broilers within 14 d (Van Beek *et al.* 1994). It was further shown that when an experimental bacterin was used to inoculate broilers, there was significant protection, preventing growth suppression in experimentally challenged birds (Van Beek *et al.* 1994). However, on post-inoculation with 10^8 live bacteria directly into the airsacs, *O. rhinotracheale* could not be re-isolated and caused no respiratory symptoms (Van Beek *et al.* 1994). Arthritis and growth suppression were, however, recorded (Van Beek *et al.* 1994). This is in contrast to experience with South African isolates, where respiratory symptoms could be reproduced by experimental inoculation (S.B. Buys, personal communication 1994).

In South Africa, observations were made in broilers at about 3 weeks of age, of a transient upper-respiratory syndrome. Several attempts at identifying the aetological agent were unsuccessful (J.H. du Preez, personal communication 1991). Special emphasis was subsequently placed on correct bacterial-sampling methods from live ill birds, with the use of adapted culturing techniques. The presence of *Haemophilus paragallinarum* in South Africa, necessitated bacteriology menus in diagnostic laboratories which included a micro-aerobic atmosphere and extended incubation times. By the application of these methods, slow-growing bacteria were eventually isolated from broilers with the described respiratory syndrome. These bacteria were subsequently identified as *Ornithobacterium rhinotracheale* (Van Beek *et al.* 1994; Van Damme *et al.* 1994).

Furthermore, a large, integrated broiler producer expressed concern over the role that *O. rhinotracheale* played in their farming enterprise. A serious sequel in the affected flocks, was condemnations at processing, due to chronic airsacculitis and peritonitis, with a reported incidence of between 30 and 35% (S.B. Buys, personal communication 1994). Similar observations were made by the author (A.F. Travers) in *O. rhinotracheale*-positive flocks. *O. rhinotracheale* therefore appears to have the potential to cause serious economic losses within the broiler industry. Interesting observations were made from field outbreaks in South Africa, which seemed to indicate that different syndromes occurred in *O. rhinotracheale*-positive flocks. Three broad categories of symptoms were identified, the first being a primarily upper-respiratory syndrome. The second outbreak occurred without clear sinusitis and nasal discharge in affected birds, but severe peritonitis was seen on necropsy. The last category was a respiratory syndrome, with arthritis and lameness observed in clinically ill birds. In all groups there was a poor average-daily-gain and feed-conversion rate.

This inevitably poses the question: Are pathogenicity differences responsible for these observations and, if so, do immunogenic differences exist? This could explain the North American isolates being biochemically similar, but serologically different (Charlton *et al.* 1993).

The possible role of predisposing factors, including management and environmental stressors and, more specifically, the possible role of primary respiratory and arthritic pathogens, for example Newcastle disease virus, turkey rhinotracheitis (*Pneumovirus*), infectious bronchitis virus and Reovirus, to name a few, need to be considered. Cook, Ellis & Huggins (1991) & Charles, Nagaraja, Halvorsen & Barnes (1993), indicated that the severity of bacterial respiratory infections, in combination with viral respiratory infections, invariably led to a more severe respiratory syndrome.

Little has been published regarding *O. rhinotracheale*. Only one pilot trial, in which growth suppression and arthritis were confirmed, appears to have been conducted (Van Beek *et al.* 1994). However, the absence of respiratory symptoms and sinusitis in these studies could not be explained (Van Beek *et al.* 1994). Confirmation of the symptoms caused by *O. rhinotracheale* as described, as well as symptoms that were absent in cases, was therefore necessary.

The main objectives of this work were to establish whether pathogenicity differences occur between South African isolates of *Ornithobacterium rhinotracheale,* to confirm the clinical findings of others and, by means of phenotypic tests, to confirm that the South African isolates were phylogenetically affiliated.

The research hypothesis (Ho): "No pathogenicity differences exist between South African isolates of *Ornithobacterium rhinotracheale*", was postulated for the purpose of this trial.

MATERIALS AND METHODS

Isolation and identification of three selected South African *O. rhinotracheale* isolates

Origin and selection

The three isolates used in the challenge trials were selected from field outbreaks. The specific field

outbreaks were selected on the grounds of different clinical signs observed in the affected flocks. The possibility that pathogenicity differences were responsible for the observed disease patterns, was therefore considered.

Isolate 1 (491/93) was obtained from infra-orbital sinus samples of clinically ill 28-d-old broilers. Symptoms noted in the affected flock were primarily upper respiratory in nature. Sinusitis, nasal discharge and slight dyspnoea were the main symptoms that occurred (S.B. Buys, personal communication 1994).

Isolate 2 (C1108/94) was obtained from the liver and abdominal airsacs of broilers (25 d old). This was the first isolation of *O. rhinotracheale* to be made directly from the liver. The isolate was selected because of the severity of the outbreak in the absence of clear sinusitis and nasal discharge in the affected birds.

Isolate 3 (C1194/94) was selected for the following reasons: The isolation was made from abdominal airsacs of 8-week-old Amber-link commercial layer pullets. This was the first isolation of *Ornithobacterium rhinotracheale* to be made from layer-type chickens in South Africa. An increase in mortalities of birds with respiratory symptoms was observed.

Isolation

Samples were obtained from clinically ill, live birds, in the early stages of disease.

Specimens for bacteriological examination were collected with sterile cotton swabs. The samples were inoculated onto blood agar (Catalog No. CM331; Oxoid) supplemented with 5 % defibrinated sheep blood, and incubated for 48 h at 37°C in a microaerobic atmosphere (candle jars) containing approximately 10% CO_2 (Van Damme *et al.* 1994). Colonies were then selected which were small, circular (± 0,1–0,2 mm in diameter), opaque to greyish, and butyrous. Characteristic homologous *O. rhinotracheale* colonies were then identified biochemically, to confirm the main phenotypic characteristics. Bacteriological purity was checked by plating and microscopic examination of the smears stained by Gram's method. These seed cultures were then replicated and freeze-dried for future reference and use.

Identification of isolates

Biochemical identification of the three *O. rhinotracheale* selected isolates was performed. Classical phenotypic tests were used as described (Hinz *et al.* 1994; Van Damme *et al.* 1994).

Preparation of challenge strains for treatment in experiment 1

The challenge material was prepared from freeze-dried seed cultures of the three isolates identified. Prior to preparation, the three isolates were biochemically tested to be *Ornithobacterium rhinotracheale*. The isolates were cultured aerobically for 24 h at 37 °C in 10 mℓ of proteose peptone no. 3 medium (Difco Laboratories, Detroit, USA), supplemented with 0,1% w/v glucose, 0,5% w/v filtered, sterilized yeast extract (Oxoid) and 5 µℓ of nicotine-adenine dinucleotide (NAD) (Sigma Chemical Company, St. Louis, USA) (Piechulla, Bisgaard, Gerlach & Mannheim 1984). About 0,1 mℓ of this culture was transferred onto blood-agar plates (Oxoid) supplemented with 5% defibrinated sheep blood. Twenty plates per isolate were prepared and incubated for a further 24 h at 37°C in a microaerobic atmosphere (candle jars). The colonies were then transferred by means of a sterile inoculation loop to 7 mℓ of phosphate-buffered saline (PBS) at 37°C. Two mℓ of this solution was used to determine the optical density (OD), measured with a spectrophotometer (Multiskan MCC/340 MK II, Flow Laboratories, Switzerland) at an absorbence (A) of 540 nm. The OD for the three challenge isolates are reported in Table 1.

A tenfold dilution series was then prepared with 9 mℓ of saline and 1 mℓ of the challenge strains in PBS. Three blood-agar plates per dilution per strain were inoculated and incubated for 24 h at 37°C in a microaerobic atmosphere to determine the colony-forming units (cfu) per isolate per 0,1 mℓ. The results are recorded in Table 2.

Forty (40) 0,1-mℓ aliquots per isolate containing approximately 10^8 cfu *O. rhinotracheale* were prepared from the remaining 5 mℓ of PBS bacterial solution. The procedure for preparing aliquots with approximately 10^8 cfu *O. rhinotracheale* was tested in a pilot trial. These 0,1-mℓ aliquots were inoculated directly into the caudal abdominal airsacs of 160 day-old Ross broiler chickens that had been selected by simple random sampling and had originated from a single broiler breeder flock, as discussed under experiment 1.

TABLE 1 Optical density (OD) of *Ornithobacterium rhinotracheale* challenge strains

O. rhinotracheale challenge strain	OD at A540 nm
491/93 (Treatment 1)	2,710
C1108/94 (Treatment 2)	2,690
C1194/94 (Treatment 3)	2,718

TABLE 2 Colony-forming units per isolate per 0,1 mℓ

O. rhinotracheale challenge strain	Colony-forming units (cfu) per 0,1 mℓ
491/93 (Treatment 1)	$1,9 \times 10^8$
C1108/94 (Treatment 2)	$1,8 \times 10^8$
C1194/94 (Treatment 3)	$1,9 \times 10^8$

Experiment 1

Experimental design

Day-old broilers were selected by simple random sampling from 5 000 eggs set and hatched from one specific broiler breeder flock. The selected broiler breeder flock was serologically tested, prior to the commencement of the trial, for *Mycoplasma gallisepticum, Mycoplasma synoviae* and *Ornithobacterium rhinotracheale* antibodies. The setter was selected in the hatchery by simple random sampling, and a setter/hatcher tray was similarly selected.

This stage of the experiment was a 4 x 2 block design. As described under experimental housing, eight isolators were available. The four treatments in the design included three isolates of *O. rhinotracheale* selected from South African isolates. The last treatment was a control. For each treatment, two blocks (replicates) or isolators were provided. Twenty broilers were selected by simple random sampling and placed per numbered isolator (1–8). The total number of chickens in the experiment was therefore 4 x 2 x 20 = 160 birds (40 birds per treatment). No statistical method for determining sample size was used in this experiment, owing to the space restriction of the isolators. A maximum of 20 broilers per isolator could be accommodated. However, it was accepted that 40 birds per treatment would be sufficient for statistical inferences. All the broilers were individually numbered by leg bands at day-old, and the mass of each was recorded by means of a calibrated laboratory scale, accurate to the third decimal point in g.

Any bird that succumbed during the first 48 h of the trial was replaced. No bird that died after that was replaced, but was recorded as a mortality.

Experimental procedures

Birds were challenged at 28 d of age (day 0 of the challenge study) with the three strains of *O. rhinotracheale* selected by criteria as discussed under origin and selection and preparation of challenge strains for treatment in experiment 1. Treatments of 0,1 mℓ per bird, containing approximately 10^8 cfu of *O. rhinotracheale*, were injected directly into the left caudal abdominal airsac. The controls were similarly injected with 0,1 mℓ of sterile, normal saline to simulate the same stressors as in positive treatment groups. The procedures were carried out by means of a double-blind method so as to eliminate hidden bias. The decision to use the caudal abdominal airsac as challenge route of choice, was determined by means of a pilot trial, and resulted in more severe and specific symptomatology. When the infra-orbital sinus was used as route of challenge, nasal discharge, sinusitis and dyspnoea, although present, were less severe. To measure pathogenicity differences between the three strains, it was decided to use the route of challenge resulting in more prominent pathology.

Serum was collected and identified from individually numbered birds at day 0 (28 d of age). The following serological tests were performed:

- ELISA tests

  O. rhinotracheale, Newcastle disease (NCD), infectious bronchitis (IB), infectious laryngotracheitis (ILT), turkey rhinotracheatis (TRT) and infectious bursal disease (IBD)

- Haemagglutination-inhibition (HI) test

  Influenza (2.31 antigen)

The serological screening was done to ensure that isolation procedures were effective, and that no extraneous factors played a role in the results observed.

Experimental groups were monitored daily for the following response variables, and recorded on data-capture forms:

- Nasal discharge: Any indication of serous discharge, unilateral or bilateral
- Sinusitis: Any indication of unilateral or bilateral swelling of infra-orbital sinuses
- Dyspnoea: Any indication of respiratory distress and gasping
- Lameness: Any indication of difficulty in moving, limping or hock swelling. Lameness due to tibial dyschondroplasia, slipped tendon or valgus/varus deformities, was not recorded
- Mortality: Any mortalities were recorded, removed and necropsied to ascertain the cause. Organs were collected for bacteriology, and for future histological examination.

On day +5 post inoculation (PI) (33 d of age), five birds per isolator were selected randomly. The birds were weighed individually and serum samples were collected for serological testing as at day 0 (28 d of age). The birds were sacrificed and samples for bacterial re-isolation were collected from the brain, sinus, airsacs, lungs, liver, spleen and hock joints. Airsacculitis lesions were scored according to Glisson, Cheng, Brown & Stewart (1989) (Table 3), and arthritis lesions were scored according to the procedure in Table 4.

Lastly, on necropsy, any macroscopic lesions other than airsacculitis and arthritis scores, were recorded. The bursa of Fabricius was collected and histologically analysed to rule out extraneous immunosuppressive aetiologies.

On day +14 (42 d of age) the birds in all treatment groups were processed as described on day +5, and data were recorded on capture forms. The duration of experiment 1 was 42 d.

TABLE 3 Airsacculitis-lesion scoring post inoculation with *Ornithobacterium rhinotracheale* (Glisson *et al.* 1989)

Score	Lesions
0	No lesions
1	Lesions in *one* abdominal airsac, *or* the thoracic airsac (Only one major airsac involved)
2	Lesions in *both* abdominal airsacs, *or* one abdominal airsac and the thoracic airsac (Two major airsacs involved)
3	Lesions in *both* abdominal airsacs, *and* the thoracic airsac
4	Extremely severe lesions in all airsacs

TABLE 4 Arthritis-lesion scoring post inoculation with *Ornithobacterium rhinotracheale*

Score	Lesions
0	No lesions
1	Lesions in either left *or* right hock joints
2	Lesions in both left *and* right hock joints
3 proxi-	Lesions in both left *and* right hock joints *and* mal femoral joints

Housing and management

The selected broilers were not vaccinated at day-old, nor during the trial period of 42 d. Twenty randomly selected broilers were placed per isolator.

ISOLATORS

The isolation unit was separated from the main departmental building, by approximately 500 m. The isolators were situated in a separate room with access control. Individual isolators were placed in two rows of four units. All isolators were filtered-air, positive-pressure units, 1 x 1,2 m x 1 m high. Observation of the birds was facilitated by viewing windows, and handling or feeding, by arm-length rubber gloves. Each isolator had one feeder and three nipple drinkers. (The height above floor level could be adjusted.)

ENVIRONMENTAL CONTROL

Positive pressure was maintained in the units, with incoming air filtered through hepa-filters. All filters were tested according to US Feed-standard-290E for class 100 filters, and were within the required maximum penetration allowance of 0,01%. Air flow was calibrated at 2 m/sec. Air quality was maintained at CO_2 levels of less than 0,2%, and NH_3 levels of less than 15 ppm.

Temperature was maintained at 30°C approximately 2°C for the first 3 d. After that, temperature was reduced gradually by 1°C every 3 d, to be maintained at 24°C approximately 2°C by 21 d of age. Temperature and relative humidity (RH) were recorded on a daily basis by means of an electronic temperature/RH meter.

BASAL RATION

Feed supplied to the broilers was representative of local formulations and was formulated by a large, reputable poultry-feed manufacturer. An anticoccidial drug and a growth promotant (zinc bacitracin) were included in the ration to mimic standard commercial practice.

The rations were fed *ad libitum*; starter crumbs from day 0–21, grower pellets from day 22–35 and finisher pellets from day 36–42.

WATER QUALITY

The water supply was municipal chlorinated water, potable and fit for human and animal consumption.

DISEASE CONTROL

The birds were not immunized during the trial period. All isolators were cleaned and disinfected by means of a gluteraldehyde product before the chicks were placed in them. This specific product has been approved for use in poultry housing and, furthermore, is effective in inactivating Birna virus (infectious bursal disease virus). This specific virus is known to be highly resistant. The disinfection procedure was monitored by the collection of bacteriological samples from the isolators that were tested.

Additional control measures

To eliminate all possible extraneous factors that might influence the data, certain control measures were included prior to commencement of the trial.

The 36-week-old broiler breeder flock selected for the source of embryonated eggs was:

- bled prior to commencement of trial. Sixteen serum samples were tested for antibodies to *Mycoplasma gallisepticum, Mycoplasma synoviae* (on rapid plate agglutination) and *Ornithobacterium rhinotracheale* on ELISA test. This ELISA test was developed by methods described by Travers (1995), according to which antigen from the 3263/92 or LMG 12591 reference strain (Van Damme *et al.* 1994) of *Ornithobacterium rhinotracheale* was used (the first isolate obtained in a South African outbreak)

- fully immunized according to a prescribed vaccination schedule
- clinically normal, with normal production parameters

Data analysis

MASS GAIN

The difference in geometric mean mass within treatment groups between day +5 PI and day 0; and day +14 PI and day 0, was calculated. Geometric mean mass was used because of the relatively small sample size. To assess the statistical significance of the difference between the treatments' mass means, a paired *T*-test was used.

Average daily gain within treatment groups was not calculated, as the values graphically illustrated in Fig. 1, respresent the trends.

The mass differences within treatment groups at day +5 PI and day +14 PI were statistically evaluated against the control group (Table 5) by means of the paired *T*-test. Furthermore, the mass differences within treatment groups at day +5 PI and day +14 PI, were statistically evaluated against one another.

The calculated mean mass difference (growth rate) was used to evaluate and express the degree of pathogenicity of the *O. rhinotracheale* isolates. In the null hypothesis (Ho) it was assumed that no pathogenicity differences exist between isolates. The alternative hypothesis (Ha) was that pathogenicity differs. A significance level (α) of less or equal to 0,05 was chosen.

CLINICAL SYMPTOMS POST INFECTION

Response variables of nasal discharge, sinusitis, dyspnoea, lameness and mortality were recorded on a daily basis. The response variables were recorded to ascertain whether differences would exist between treatment groups on a nominal scale in this experimental study. The statistical test performed on the data was the chi-square test (χ^2), by means of a 2 x 2 table to check for statistically significant differences at the 95% confidence interval. To evaluate risk factors, the prevalence data recorded were analysed by means of odds ratios (OR).

RESULTS

Isolation and identification of three selected South African *O. rhinotracheale* isolates

The three *O. rhinotracheale* isolates were identified biochemically by means of phenotypic tests as described by Van Damme *et al.* (1994). The results are given in Table 6. The three strains are phenotypically identical to strains referred to by Van Damme *et al.* (1994) and Hinz *et al.* (1994). It is interesting, however, that isolate 1 (ref. no 491/93) is oxidase negative. This was the only biochemical difference observed between the three isolates.

Isolation of *O. rhinotracheale* from field samples is not always easy. Samples taken from sinuses and tracheas, when inoculated onto blood agar, are commonly overgrown by rapid-growing bacteria like *Escherichia coli*. These bacterial colonies mask the *O. rhinotracheale* colonies. Van Beek *et al.* (1994) discussed the probable reasons why *O. rhinotracheale* isolations were not previously made. These include the slow growth (24–48 h) and the need for a microaerobic atmosphere (5–10% CO_2).

The initial isolation of *O. rhinotracheale* in South Africa in 1991 (Van Beek *et al.* 1994) was achieved only when ill birds in the early stages of disease were

TABLE 5 Statistical analysis of results (mass) within treatment groups

Treatment	1				2				3			
	5 d PI	5 d control	14 d PI	14 d control	5 d PI	5 d control	14 d PI	14 d control	5 d PI	5 d control	14 d PI	14 d control
Sample size	10	10	30	30	10	10	30	30	10	10	30	30
Average mass	262,1	490,8	967,6	1021,9	347,5	490,8	914,3	1021,9	348,0	490,8	922,0	1021,9
SD	219,7	92,7	157,7	148,4	187,1	92,7	202,2	148,4	144,6	92,7	131,5	148,4
Computed *T*	–2,360		–1,370		–2,170		–2,350		–2,630		–2,760	
P value	0,029[a]		0,170		0,044[a]		0,022[a]		0,017[a]		0,007[a]	

[a] Statistically significant at the 95 % confidence interval

bacteriologically tested. Furthermore, a continuous monitoring of suspect farms led to an increase in the number of *O. rhinotracheale* isolations made. Diagnostic laboratories in South Africa include microaerobic culturing procedures in their bacteriology menus because of the common occurrence of *Haemophilus paragallinarum*.

Experiment 1

Housing and management

BASAL RATION

The rations were sampled and cultured bacteriologically. All rations tested bacteriologically negative for *O. rhinotracheale*. Owing to the possibility of immunosuppression as a result of aflatoxin B_1, all rations were sampled and tests showed them to be below 5 ppb aflatoxin B_1 (Idexx rapid diagnostic kits, Maine USA).

WATER QUALITY

Bacteriological tests of water samples showed them to be negative for *O. rhinotracheale*.

TABLE 6 Phenotypic characteristics of *Ornithobacterium rhinotracheale* strains

Tests	Isolate 1 Ref. no. 491/93	Isolate 2 Ref. no. 1108/94	Isolate 3 Ref. no. 1194/94
Oxidase	–	+	+
Catalase	–	–	–
Arginine dehydrolase	+	+	+
Lysine decarboxylase	–	–	–
Ornithine decarboxylase	–	–	–
ONPG (ß-galactosidase)	+	+	+
Urease	+	+	+
Indole	–	–	–
Nitrate reduction	–	–	–
Methyl red	–	–	–
Voges Proskauer	+	+	+
Aesculin hydrolysis	–	–	–
Porphyrin test	+	+	+
Growth on McConkey	–	–	–
D(+)-glucose	+	+	+
D(+)-mannose	+	+	+
Lactose	+	+	+
D(+)-galactose	+	+	+
Maltose	+	+	+
Rhamnose	–	–	–
Sucrose	–	–	–
Xylose	–	–	–
Trehalose	–	–	–
Dulcitol	–	–	–
D(–)-sorbitol	–	–	–
D(+)-mannitol	–	–	–
M-inositol	–	–	–
Salicin	+	+	+
Haemolysis	–	–	–

+ Positive reaction, – Negative reaction, ONPG O-nitrophenyl-ß-D-galactopyronoside

DISEASE CONTROL

All samples collected post disinfection from the isolators were bacteriologically (including *O. rhinotracheale*) and fungally negative.

- Additional control measures

M. gallisepticum and *M. synoviae* rapid plate agglutination and *O. rhinotracheale* ELISA (Travers 1995) tests were done on the samples. The results were negative.

Maternal antibodies to *O. rhinotracheale* therefore played no role in the broilers selected for the trial (Table 7). *M. gallisepticum* and *M. synoviae* which could be transmitted trans-ovarially and cause respiratory disease in the broilers, were confirmed negative on serology in breeders.

Response variables

Mass gain

The results of individual birds' mass over time are graphically illustrated in Fig. 1.

TABLE 7 *Ornithobacterium rhinotracheale* ELISA results: Broiler breeder flock (Travers 1995)

Sample (n=16)	Optical density		Standard deviation	% coefficient of variation
	0,19		0,050	13,30
Positive controls (n = 6)	High	1,552	0,130	6,32
	Medium	1,030	0,148	12,70
	Low	0,773	0,130	14,90
Negative controls (n = 2)	0,20		0,044	22,37

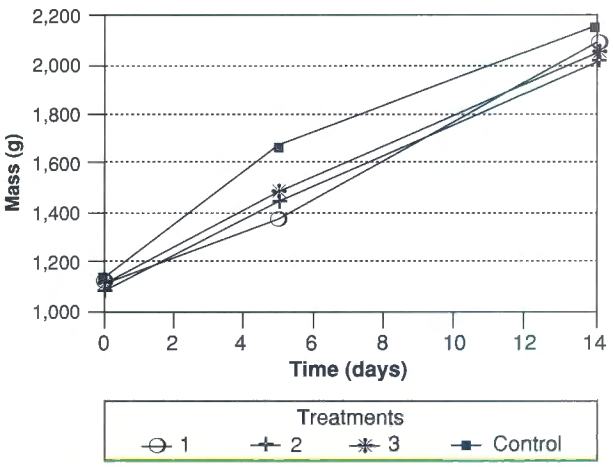

FIG. 1 Average mass per treatment group post challenge

The rate of growth of all treatment groups to 28 d of age (day 0), had a similar trend. At 5 d PI and 14 d PI, the average mass of the treatment groups was significantly lower than that of the control group, with the exception of treatment 1 at 14 d PI. The growth-rate disturbance occurred within the first 5 d post infection, after which growth rates were normal (Fig. 1). However, there were no significant differences between strains ($P < 0,05$).

It was interesting to note that strain 2 had the most severe effect on growth over 14 d. This strain was isolated from specimens taken from a field outbreak with more pronounced symptoms than the other field strains.

It was therefore experimentally confirmed that *O. rhinotracheale* strains can cause severe growth retardation. This finding was also shown by Van Beek *et al.* (1994). The financial implications of sacrificing a minimum of 100 g per broiler over 14 d are hereby highlighted.

Clinical symptoms post infection

No statistical differences between the isolates were confirmed. However, strong evidence of differences were observable in the response variables (Tables 8 and 9).

TABLE 8 Response variables in treatment groups on 5 d post inoculation

Response variable	Treatment			
	1	2	3	Control
	(n = 40)			
Nasal discharge	6	4	4	0
Sinusitus	0	0	0	0
Dyspnoea	3	1	1	0
Lameness	4	5	4	0
Mortality	0	0	0	0

TABLE 9 Response variables in treatment groups on 14 d post inoculation

Response variable	Treatment			
	1	2	3	Control
	(n = 30)			
Nasal discharge	3	5	6	0
Sinusitus	0	0	0	0
Dyspnoea	3	1	1	0
Lameness	3	2	5	0
Mortality	0	0	0	0

TABLE 10 Airsacculitis-lesion scores for three isolates of *Ornithobacterium rhinotracheale* inoculated via the caudal abdominal airsac route: 5 and 14 d post inoculation

Treatment	1		2		3	
	5 d PI	14 d PI	5 d PI	14 d PI	5 d PI	14 d PI
No. of observations	10	30	10	30	10	30
Average	2,90	1,90	2,40[a]	1,60	3,20[a]	1,10
Variance	0,54	0,60	0,27	0,67	0,40	0,93
Standard deviation	0,74	0,78	0,52	0,82	0,63	0,96

[a] Significant difference ($\alpha \leq 0,05$) between treatment 2 and treatment 3 at 5 d PI

TABLE 11 Arthritis-lesion scores for three isolates of *Ornithobacterium rhinotracheale* inoculated via the caudal airsac route: 5 d and 14 d post inoculation

Treatment	1		2		3	
	5 d PI	14 d PI	5 d PI	14 d PI	5 d PI	14 d PI
No. of observations	10	30	10	30	10	30
Average	0,40[a, b]	0,57	1,40[a]	0,97	1,20[b]	0,93
Variance	0,27	0,74	1,82	0,99	1,96	1,02
Standard deviation	0,52	0,86	1,35	0,99	1,39	1,01

[a] Significant difference ($\alpha \leq 0,05$) between treatment 1 and treatment 2 at 5 d PI
[b] Significant difference ($\alpha \leq 0,10$) between treatment 1 and treatment 3 (confidence interval of 90%)

TABLE 12 Reisolation of *Ornithobacterium rhinotraheale* strains at 5 d and 14 d post challenge

Treatment	1		2		3	
	5 d PI (n = 10)	14 d PI (n = 30)	5 d PI (n = 10)	14 d PI (n = 30)	5 d PI (n = 10)	14 d PI (n = 30)
Brain	–	–	1	–	–	–
Sinus	–	–	2	–	–	–
Lung	1	–	1	–	–	–
Airsac	–	–	1	1	–	1
Hock joint	1	1	–	2	1	2

Treatment 1 shows the highest number of upper respiratory symptoms over the observation period of 14 d. This isolate was selected from an observed field outbreak with a high incidence of upper respiratory signs. *O. rhinotracheale* isolate 491/93 was used by S.B. Buys (personal communication 1994) to reproduce sinusitis symptoms when injected by the infra-orbital sinus route. With other isolates used in his trial, no sinusitis could be reproduced. An interesting observation was that treatment 1 led to the most severe mass loss 5 d post inoculation, with good recovery over the last 9 d of the trial (Fig. 1).

Airsacculitis- and arthritis-lesion scores

The results for airsacculitis scores are reported in Table 10 and arthritis scores are reported in Table 11.

AIRSACCULITIS

No significant differences ($P \leq 0,05$) occurred between the isolates at day +5 or day +14 PI. However, at a confidence interval of 90%, there is a significant difference between isolates 1 and 2.

ARTHRITIS

At 5 d PI, treatments 2 and 3 resulted in significantly higher arthritis scores over *O. rhinotracheale* isolate 1. At a confidence interval of 90%, isolates 2 and 3 obtained significantly higher arthritis scores over isolate 1. This observation suggests that pathogenicity differences exist when arthiritis lesions are scored. Isolate 1 caused more severe upper respiratory symptoms than isolates 2 and 3. Observations made in the field outbreak confirm this hypothesis.

It would therefore seem that *O. rhinotracheale* isolate 1, when judged purely on arthritis and airsacculitis lesions, differs in pathogenicity from isolates 2 and 3.

Reisolation of *O. rhinotracheale* post challenge

Bacterial samples were collected from the brain, sinus, lung, airsac, liver, spleen and hock joints, 5 d PI and 14 d PI (Table 12).

Reisolation of *O. rhinotracheale* was difficult after challenge. Similar observations were made by Van Beek *et al.* (1994). Pure cultures were made from certain hock joints with lesions. An isolation of *O. rhinotracheale* isolate 2 from the brain of one bird, is highly significant. The most likely source of *O. rhinotracheale* was the meninges, as a lymphocytic leptomeningitis was confirmed on histopathology. This is a strong indication that pathogenicity differences play a role. Field observations in an outbreak where strain 2 was isolated, seemed to indicate more severe symptoms than did those in flocks affected by isolates 1 and 3. Reisolation, mainly from the respiratory system and joints, confirms that these sites are usually affected in *O. rhinotracheale*-positive birds.

NECROPSY FINDINGS

At necropsy at 14 d post challenge, the most outstanding macroscopic lesions were fibrino-purulent in nature, which agreed with the findings of others (Hinz *et al.* 1994; Van Beek *et al.* 1994). Lesions were limited to the airsacs, lungs, trachea, sinuses and hock joints. However, in contrast to previous observations (Van Damme *et al.* 1994), no pericarditis was noted. Fibrino-purulent peritonitis was noted in all cases with a severe airsacculitis. In the early stages (5 d PI), a frothy airsac and peritoneal material were noted. The airsac membranes were cloudy in appearance. At 14 d PI the macroscopic appearance of the airsac and peritoneal material can be described as yellow to yellow-white rice-grain-sized, caseous granules. Similar observations were regularly made in field outbreaks, with an incidence of 30–35% at processing. These broilers are then condemned at slaughter, with severe economic loss resulting. Femoral-head necrosis was observed in cases with moderate to severe airsacculitis.

Histopathological changes were not evaluated in this study, although samples were collected for future analyses.

DISCUSSION

Ornithobacterium rhinotracheale, as a distinct genus and species, leads to an acute respiratory disease in broilers and turkeys (Hinz *et al.* 1994; Van Beek *et*

al. 1994; Van Damme *et al.* 1994). Moreover, *O. rhinotracheale* causes arthritis of particularly the hock joints, and leads to growth suppression (Van Damme *et al.* 1994). It can be concluded from this study that respiratory and joint lesions are a common sequel to *O. rhinotracheale* infection. However, sinusitis was not found to be a major symptom. Growth suppression, chronic airsacculitis and peritonitis lesions (leading to carcase condemnation at processing) were confirmed to be of major importance, especially as all this results in severe economic losses. Primary, uncomplicated *O. rhinotracheale* challenge did not lead to mortality in the broilers used in this experiment.

Interesting observations were made from all the response variables measured in this trial. When compared with the controls, the challenged birds were morbid post challenge, and nasal discharge with signs of dyspnoea due to deeper respiratory pathology, and arthritis were seen. However, no significant differences in pathogenicity could be established when the disease symptoms were evaluated. Since the isolates were selected from field strains with differing clinical pictures, one may argue that concomitant stressors or infections could exacerbate *O. rhinotracheale* symptoms.

Reisolation of *O. rhinotracheale* isolates post challenge was difficult, which confirms the experience of Van Beek *et al.* (1994). Of particular significance, was a reisolation made from brain tissue of one broiler in the challenged group, for which isolate 2 was used, as this is the first reported isolation made from the brain. Reisolation from the infraorbital sinus, lung, airsac and hock joints from treatment-2 birds, is a strong indication of increased invasiveness and pathogenicity. *O. rhinotracheale* isolate 2 also had the most severe effect on growth rate measured over 14 d.

Significant differences between isolates could be presented in the evaluation of airsacculitis and arthritis lesions. Isolate 1 caused significantly fewer arthritis lesions. Consequently, the field observation that this *O. rhinotracheale* isolate caused more respiratory lesions, with little or no arthritis, was confirmed. This isolate was negative in the oxidase test (most strains are positive, although not all) (Van Damme *et al.* 1994) when tested phenotypically; and was shown not to be *Capnocytophaga* species. There were no other differences between selected strains.

CONCLUSION

Data gleaned from this trial, show that there is strong evidence of pathogenicity differences. The research hypothesis (Ho) can therefore be rejected and rephrased as: Pathogenicity differences occur between South African isolates of *Ornithobacterium rhinotracheale*.

Further study is needed regarding the epidemiology and pathogenesis of *O. rhinotracheale* infections. There is strong evidence of primary disease due to *O. rhinotracheale* (Hinz *et al.* 1994; Van Beek *et al.* 1994), but the pathogenesis, together with other bacterial or viral infections, needs to be investigated.

ACKNOWLEDGEMENT

The authors express their appreciation to the Poultry Reference Laboratory, Department of Poultry Diseases, University of Pretoria, for providing the staff, facilities and financial support for this study.

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