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Toxigenic type D *Pasteurella multocida* in New South Wales pig herds — prevalence and factors associated with infection

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SUMMARY: Between March and July 1987, a study was undertaken to determine the prevalence of and factors associated with toxigenic type D *Pasteurella multocida* infection in New South Wales pig herds. Toxigenic type D *P. multocida* was isolated from the nasal cavities of pigs in one (2%) of 50 randomly selected herds. Toxigenic isolates were also recovered from 2 (8%) of a separate group of 25 herds that had purchased pigs from a known infected piggery in South Australia (herd SA). Snout abnormalities were present in 9.4%, 3.2% and 1.8% of grower pigs in the 3 affected herds. Isolation of toxigenic *P. multocida* was significantly associated ($p < 0.0001$) with the occurrence of clinically affected pigs in the herd. Purchase of at least 5 pigs from herd SA was associated with an elevated risk ($p < 0.05$) of isolation of toxigenic *P. multocida*.

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Introduction

Toxin-producing isolates of *Pasteurella multocida* play an essential role in the aetiology of enzootic or progressive atrophic rhinitis (Pedersen and Barfod 1981; Rutter and Rojas 1982; Rutter 1983; Rutter *et al* 1984). *P. multocida* colonises the nasal cavity after some pre-existing damage (Rutter and Rojas 1982; Rutter 1987), and produces toxins which cause turbinate atrophy. Isolation of toxigenic type D *P. multocida* from swine in 2 New South Wales pig herds was recently described (Eamens *et al* 1988). Infection was thought to have been introduced with pigs from a known infected South Australian piggery (herd SA) but there were no definitive data to support this hypothesis.

The present study was undertaken to determine the apparent prevalence of toxin-producing *P. multocida* in a random sample of New South Wales pig herds, to measure the association between recovery of toxigenic isolates and clinical disease, and to determine whether purchase of pigs from herd SA and other introduction practices were associated with an increased risk of isolation of *P. multocida*.

Materials and Methods

Selection of Herds

The Swine Brand Register, a list maintained by the Department of Agriculture and Fisheries of all pig producers in New South Wales, was used for selection of the sample. The Register identified herds by geographical area (Pastures Protection Board) and included data, that was up to 2 years old, on the number of sows in each herd. From the Register, all herds with 20 or more sows (n = 1227) were selected as the reference population and numbered consecutively. The number of herds to be sampled to estimate prevalence within fixed limits with 95% confidence was determined by the formula (DiGiacomo and Koepsell 1986):

$$n = 3.84\,\hat{P}(1-\hat{P})/E^2$$

where $\hat{P}$ = prevalence, estimated to be between 0-5%
E = maximum tolerable error (5%)

On the basis of these calculations and consideration of available resources, 50 herds were drawn from the reference population using computer-generated random numbers. Owners of selected herds were advised by mail of the reasons for the study, and the requirement for nasal swabbing and clinical evaluation of pigs in their herd. Ten replacement herds were required because 8 owners no longer had pigs, one owner did not have the minimum number of sows, and one owner refused to participate. Replacement herds were randomly selected from the Register from the same Pastures Protection Board as the non-participating herd.

All 25 owners who purchased pigs from herd SA during 1985 or 1986 (PW Brownrigg, personal communication 1987) were also surveyed. None of the 25 herds (referred to in the balance of the report as high risk herds) has been previously selected in the random sample. The 2 study groups were therefore mutually exclusive.

Farm Visits

The 75 herds (50 random, 25 high risk) were visited by a Pastures Protection Board or Department of Agriculture and Fisheries veterinarian between March and July 1987. The farm visit consisted of 3 parts. Sucker, weaner, grower and finisher pigs were clinically evaluated for signs of enzootic atrophic rhinitis (Schoss 1983). Second, nasal swabbing, as detailed below, was carried out to determine whether a herd was infected with toxigenic *P. multocida*. Third, the owner or manager completed a questionnaire which characterised herd size, production systems, housing characteristics, number and sources of introduced pigs in the 2 years prior to the survey, and quarantine and antibiotic treatment practices for introduced pigs. When clinical signs of atrophic rhinitis were observed, owners indicated when they first detected disease.

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Nasal Swabbing and Culturing

The number of pigs to be swabbed was calculated using the formula (DiGiacomo and Koepsell 1986):

$$n = \log (1-C)/\log (1-P)$$

where C = level of confidence
and P = probability that a sampled pig was infected

At a prevalence of 20% and 90% confidence level, 10 randomly selected pigs were needed in each herd to detect at least one infected pig. However, to increase the likelihood of recovery of toxigenic *P. multocida*, priority was given to sampling pigs with upper respiratory tract disease or snout deformities. When the veterinarian considered all pigs in a herd were clincially normal, pigs weaned in the previous 2 weeks were swabbed. The nasal swabbing procedure was carried out as follows: external nares were wiped with 70% alcohol to remove gross contamination. Mini-tipped aluminium shafted swabs* were inserted to the caudal end of the nasal cavity, placed in Amies charcoal transport medium and despatched at ambient temperature to Regional Veterinary Laboratories. Swabs were cultured onto Pedersen's NB medium (Eamens *et al* 1988) and isolates confirmed as *P. multocida* by standard procedures (Cowan 1974). Capsular type and toxigenity of *P. multocida* isolates to bovine turbinate cells were determined (Eamens *et al* 1988).

Statistical Analysis

Apparent herd prevalence was calculated as the proportion of randomly selected herds with one or more isolates of toxigenic *P. multocida* during the survey period. A 95% confidence interval was estimated for the herd prevalence in New South Wales (Fleiss 1981). For clinically affected herds, rates of snout abnormalities were calculated using as denominators the total number of weaner, grower and finisher pigs in the herd.

Data from the 2 groups of herds were combined for further analysis. The association between the presence of clinical disease in a herd and isolation of toxigenic *P. multocida* was tested for significance by Fisher's exact test (Fleiss 1981). Factors associated with isolation of *P. multocida* were tested at the 5% level of significance by Fisher's exact or chi-square tests (Fleiss 1981). Fisher's exact test was used for 2 x 2 tables and chi-square tests for tables with 3 or more levels of a factor.

Results

Descriptive variables for the 75 selected herds are presented in Table 1 and the geographical distribution of these herds is shown in Figure 1.

Isolates of Pasteurella multocida

P. multocida was isolated from 98 (13.3%) of 737 nasal swabs collected during the survey. One or more isolates were obtained from 31 (41.3%) of 75 herds (Table 2). Five isolates from 3 herds (1 random, 2 high risk) were classed as toxigenic type D. Toxigenic type A *P. multocida* was not detected. All positive herds were located in central-western New South Wales (Figure 1). Based on the 50 randomly selected herds, the apparent prevalence of toxigenic *P. multocida* in New South Wales herds ith more than 20 sows was 2% (95% confidence interval = 0.1 — 12.0%).

Clinical Signs

Isolation of toxigenic *P. multocida* was significantly associated ($p < 0.0001$) with the occurrence of clinically affected pigs in the herd. Toxigenic *P. multocida* was recovered from 3 of 4 clinically affected herds that had snout deformities in 9.4%, 3.2% and 1.8% of weaner, grower and finisher pigs. Owners of affected herds first detected pigs with snout abnormalities in July 1986, January 1987, and April 1987, respectively. In the 3 affected herds, toxigenic *P. multocida* was isolated from 5 (22.7%) of 22 pigs with snout abnormalities or upper respiratory tract disease. Toxigenic isolates were not recovered from a fourth herd that had a single finisher pig (0.9% of pigs at risk) with a twisted snout, or from 71 herds that were free of snout deformities. The owner of the fourth herd had not noticed the pig with the twisted snout prior to the farm visit.

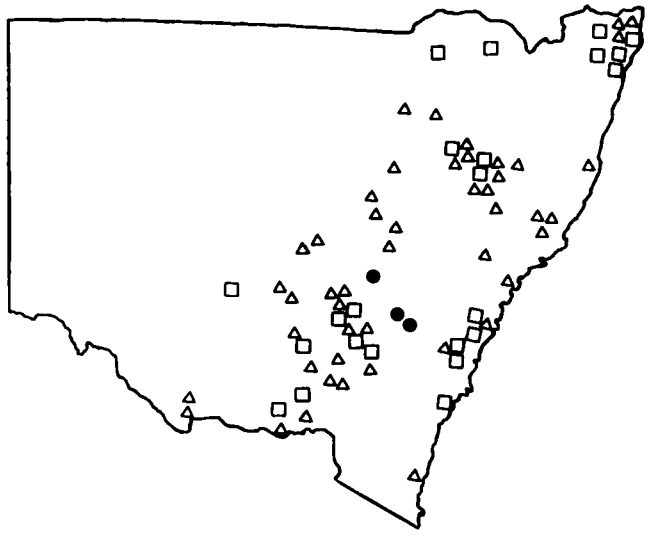

Figure 1. Approximate location of New South Wales piggeries surveyed for toxigenic *Pasteurella multocida*, March-July 1987

- ● Positive herds
- □ Negative herds — high risk group
- △ Negative herds — random group

Factors Associated with Isolation of Toxigenic P. multocida

The crude association between isolation of toxigenic *P. multocida* and introduction of pigs from herd SA was not significant (Table 3). However, owners who purchased more than 5 pigs from herd SA were more likely to have toxigenic *P. multocida* isolated from pigs in their herds than owners who purchased less than 5 pigs from that source. Introduction of more than 50 pigs from other sources in the same period was also associated with an increased risk ($p < 0.001$) but the number of sources from which these pigs were derived was not significant ($p = 0.06$). Lack of quarantine and antibiotic treatment of introduced pigs was not associated ($p = 0.87$) with an increased risk of recovery of toxigenic isolates (Table

TABLE 1
Characteristics of herds surveyed for toxigenic *Pasteurella multocida* in New South Wales, March — July 1987

Observation	Herds	
	Random (n = 50)	High risk (n = 25)
Herd size*		
No. sows	57 (20-200)	158 (20-630)
No. weaner/grower/finisher pigs	342 (47-1900)	947 (109-3900)
Production		
Stud breeder	4 (8%)	7 (28%)
Breeding/finishing herd†	47 (94%)	25 (100%)
Introductions*‡		
Total no.	30 (0-833)	42 (1-600)
No. from herd S.A.	0	3 (1-13)
No. of source herd	2 (0-6)	3 (1-5)

* Results are means rounded to the nearest whole number. Numbers in parenthesis are lower and upper limits of the range.
† Three random sample herds sold all pigs at less than 50 kg liveweight
‡ Pigs entering herd in the 2 years prior to the study

* Disposable Products, Botany, New South Wales

TABLE 2

Capsular type and toxigenicity of *Pasteurella multocida* isolates recovered from pigs in New South Wales, March — July 1987

	No. Sampled		Type D		Type A			
	Herds	Pigs	Toxigenic	Non-toxigenic	Non-toxigenic	Other isolates	Non-viable	Total
Random sample	50	(498)†	1 (2)	20 (56)	0 (0)	1 (1)‡	3 (4)	21 (63)
High risk	25	(239)§	2 (3)	9 (30)	1 (1)	0 (0)	1 (1)	10 (35)
Total	75	(737)	3 (5)	29 (86)	1 (1)	1 (1)	4 (5)	31 (98)

* Results represent herd isolations. Figures in parenthesis are number of individual pig isolations. Some herds had isolates in more than one of the 5 categories.
† Eight instead of ten swabs were received from one sample herd
‡ Isolate was non-toxigenic; capsular type was neither D nor A
§ Two swabs (both positive) were collected from one herd, and, from another, only 7 were collected

3). Associations with herd size (categories 20-50, 51-100, > 100 sows) were also not significant ($p = 0.16$), and commercial herds were no more likely to be affected than stud herds ($p = 0.61$). Toxigenic *P. multocida* was more likely to be isolated ($p = 0.05$) from herds in which all age groups (dry sows, lactating sows, weaners, growers and finishers) were housed in the same shed than those in which there was either partial or total segregation of age groups into separate sheds.

A significant association existed between the number of pigs from herd SA and the number of introduced pigs from other sources ($p < 0.0001$). Owners of the 3 affected herds also had purchased more than 50 pigs from herds other than SA. Under the assumption that the number of pigs introduced from other sources was a potential confounding factor, a stratified analysis was conducted. Using this approach, purchase of more than 5 pigs from herd SA was not significantly associated ($p = 0.28$) with a higher isolation rate of toxigenic *P. multocida*.

TABLE 3

Pig introduction practices and isolation of toxigenic *Pasteurella multocida* from New South Wales pig herds, March — July 1987

Factor	Number of herds		p*
	Positive	Negative	
Introductions from herd SA			
Yes	2	23	0.26
No	1	49	
No. introduced from herd SA			
>5	2	1	<0.001
1-5	0	22	
0	1	49	
No. of introduced pigs†‡			
>50	3	5	<0.001
0-50	0	67	
No. of sources of introduced pigs‡			
5-6	0	7	0.06
3-4	3	23	
0-2	0	41	
Quarantine and antibiotic treatment of introductions			
No	3	66	0.87
Yes	0	3	

* Fisher's exact test used for 2 × 2 tables and chi-square tests for tables with 3 or more levels of the factor.
† Introduced pigs were those entering herd in the 2 years prior to sampling. Two owners did not introduce pigs during that period.
‡ Maximim number of sources was 6.

Discussion

The apparent herd prevalence of toxin-producing *P. multocida* of 2% indicated that toxigenic isolates were not widespread in breeding herds in New South Wales. All infected herds were directly or indirectly linked to introductions of pigs. The 2 herds in the high risk group had bought pigs from herd SA. The third herd, in the random sample group, had purchased 45 gilts between September and November 1986 from the owner who had first seen clinical signs of atrophic rhinitis in July 1986. Recently detected infection in 2 other herds in central-western NSW has also been associated with introductions from the latter herd (I A Gardner, unpublished).

Estimates of the prevalence of atrophic rhinitis in other Australian states have largely been based on notifications to veterinary authorities, abattoir monitoring and laboratory testing. Prevalence estimates based on such non-random sampling procedures may over or underestimate the true prevalence depending on the direction of the selection bias. The random sampling procedure in the present study allowed us to calculate an unbiased estimate of herd prevalence. Only herds with at least 20 sows were included in the sampling frame as these were herds which represented better the commercial pig industry.

The diagnostic test procedure consisted of 2 parts: isolation of *P. multocida* from nasal swabs followed by cell culture assay for toxigenicity. The specificity of both procedures is likely to approach 100%, but nasal swabbing is of unknown sensitivity. Although not specifying test sensitivity, Rutter (1987) indicated that if 30% of pigs had snout deformities, *P. multocida* could be isolated from 50-60% of 6- to 8-week-old pigs. Pedersen (1983), in his review of the cultural and serological diagnosis of atrophic rhinitis, made no reference to the sensitivity of nasal swabbing. Because serological tests are not available for the detection of *P. multocida* in pigs, nasal swabbing and culturing onto a selective medium is currently the only practical method for detecting toxigenic isolates in live pigs. The mouse inoculation test, which is a more sensitive procedure, is unacceptable because of the large numbers of mice required for routine testing (Pedersen 1983).

Detection of toxin-producing *P. multocida* was strongly associated with clinical signs of atrophic rhinitis in the herd. This finding is consistent with evidence from the UK (Rutter 1983; Rutter *et al* 1984) and Denmark (Pedersen 1983) which indicated that toxigenic *P. multocida* could be isolated from herds with progressive disease but not from herds without a history of atrophic rhinitis. Rutter (1987) also observed that as the prevalence of clinical signs increased so did the prevalence of infection in weaner pigs. To maximise the chance of detecting infected pigs in a herd, veterinarians swabbed pigs that had snout abnormalities, were sneezing, or had nasal discharges. If infection prevalence was low and pigs did not show signs of disease, much larger samples would have been required. For example, at 1% prevalence, 229 weaner pigs would need to have been sampled in a large herd to be 90% confident of detecting at least one infected animal. Such intensive sampling would have been impractical except in a small number of herds.

In the present study the isolation rate of toxigenic *P. multocida* of 22.7% from pigs suspected clinically of having atrophic rhinitis was less than isolation rates from field cases reported by Rutter *et al* (1984) or the rate of 46% calculated from the data of Sawata *et al* (1984). The reasons for the lower rates are speculative but include clearance of toxigenic isolates from the nasal cavities of affected pigs and suboptimal handling of swabs between collection and identification of colonies

at laboratories. Evaluation of infection status on the basis of a single isolate per swab rather than multiple isolates may also have contributed to differences.

Our study showed a significant crude association between purchase of more than 5 pigs from herd SA and isolation of toxigenic *P. multocida*. Of 3 herds in this category, the 2 that purchased the greatest number of breeding pigs (9 and 13) from herd SA, were classed as infected. The probability of introducing infected pigs is a function of prevalence of infection in the source herd and the number of introductions. For example, if 10% of pigs in herd SA were infected, the probabilities of these 2 herds not introducing infection ($(1-P)^n$) were 0.39 and 0.25, respectively.

Owners of both infected herds in the high risk group also introduced more than 50 pigs from other sources in the 2 years prior to the study but this factor was unlikely to be causal. All other herds except one, which supplied pigs to the 3 positive herds in the 2 years prior to the survey were sampled in a similar fashion and found free from clinical or cultural evidence of infection. Samples were not collected from one source, a large breeding company, but the consulting veterinarian indicated that the herd was free from infection (B Munro, personal communication 1988). Therefore, we believe that introduction of more than 5 pigs from herd SA was causally associated with the risk of isolation of toxigenic *P. multocida* and that the observed statistical association between introduction of more than 50 pigs from other sources and isolation was a spurious one.

Evaluation of factors other than introductions was limited by the small number of herds from which toxigenic isolates were recovered and the small number of herds in some exposure categories. Smith (1983) reported that management and housing factors such as poor ventilation, continuous throughput in farrowing and weaner accommodation, and high stocking densities were important risk factors for atrophic rhinitis. Such evaluations will be more appropriate should atrophic rhinitis become more prevalent in NSW.

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Investigations of an enteric infection of cockatoos caused by an enterovirus-like agent

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SUMMARY: An enteric infection in cockatoos associated with a 30nm diameter enterovirus-like agent seen in faeces and intestinal epithelial cells is described. The disease is characterised by intractable, profuse, mucoid diarrhoea, weight loss, dehydration and death. Lesions in the intestine consist of villous atrophy, villous fusion, enterocyte hyperplasia and, in some cases, chronic inflammation. Affected birds so far examined have concurrent psittacine beak and feather disease.

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Introduction

Enteric disease associated with the presence of virus particles in faeces is becoming more widely recognised in birds. To date, infections have been reported predominantly in gallinaceous birds. These include rotaviruses in chickens (McNulty *et al* 1983; Meulemans *et al* 1985), turkeys (Saif *et al* 1985; Yason and Schat 1986) and pheasants (Gough *et al* 1985; Reynolds *et al* 1987); rotavirus-like particles in turkeys (Saif *et al* 1985) and pheasants (Reynolds *et al* 1987); astrovirus in turkeys (Saif *et al* 1985; Reynolds and Saif 1986); parvoviruses in chickens (Kisary 1985) and turkeys (Trampal *et al* 1983); calicivirus in chickens (Wyeth *et al* 1981) and guinea fowl (Gough and Spackman 1981); entero-like virus in chickens (McNulty *et al* 1984; Spackman *et al* 1984) and turkeys (Saif *et al* 1985); reovirus in turkeys (Goodwin *et al* 1985); adenovirus in turkeys (Saif *et al* 1985); coronavirus in turkeys (Pomerey 1984) and enteric virus-like particles 40-55nm in diameter in chickens (Frazier *et al* 1986).

This paper describes the clinical signs and lesions of an enteric disease in galahs (*Cacatua roseicapilla*) and a sulphur-crested cockatoo (*C. galerita*) associated with the presence of virus particles in the faeces.

Materials and Methods

Examination of Naturally Affected Birds

The clinical and pathological description is based on 17 cases.