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. 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 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):

\[ n = \frac{3.84^2(1-P/E^2)}{E^2} \]

where 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 herds 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.
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 clinically 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 (Cowen 1974). Capsular type and toxigenicity of \( P.\) multocida isolates to bovine turbinate cells were determined (Eamens et al. 1988).

**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. Non-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.

**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 3).

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**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 (9-833)                            | 42 (1-600)     |
| No. from herd S.A.                    |                |                 |                    |
| 0                                     | 42 (1-600)     |
| 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.
housed in the same shed than those in which there was either partial or total segregation of age groups into separate sheds. Purchased more than 50 pigs from herds other than SA. Under sows, lactating sows, weaners, growers and finishers) were isolated (p $\leq$ 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 2

| No. Sampled | Herds | Pigs | Type D | Non-toxigenic | Type A | 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.

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### TABLE 3

| Pig introduction practices and isolation of toxigenic P. multocida from New South Wales pig herds, March — July 1987 |
|---------------------------------------------------------------|
| Factor | Number of herds | p* |
|-----------------------------|--------------|----|
| 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§ | | |
| 5-8 | 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 x 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.
‡ Maxumin number of sources was 6.

### Discussion

The apparent herd prevalence 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 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 andWeaner 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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References

Cowen ST (1974) - In: Cowen and Steel's Manual for the Identification of Medical Bacteria. 2nd ed. London: Cambridge University Press, p 78

DiGiacono RF and Koepsell TD (1986) - *J Am Vet Med Assoc* 190: 22

Eamens GJ, Kirkland PD, Turner MJ, Gardner IA, White MP and Hornizk CL (1988) - *Aust Vet J* 65: 120

Fleiss JL (1981) - Statistical methods for rates and proportions. 2nd ed. New York: Wiley and Sons, p 14

Pedersen KB (1983) - *Cultural and serological diagnosis of atrophic rhinitis in pigs*. Agriculture - Atrophic Rhinitis. Seminar in the CEC program of Coordination of Research on Animal Pathology, Copenhagen, 25-26 May, edited by KB Pedersen and NC Nielsen. Commission of European Communities, Brussels, p 22

Pedersen KB and Barford K (1981) - *Nord Vet Med* 33: 513

Rutter JM (1983) - *Res Vet Sci* 34: 287

Rutter JM (1987) - *Pig News Inform* 8: 385

Rutter JM and Rojas X (1982) - *Vet Rec* 110: 531

Rutter JM, Taylor R.I, Crighton WG, Robertson IB and Benson JA (1984) - *Vet Rec* 115: 615

Sawata A, Nakai T, Tuji M and Kume K (1984) - *Jap J Vet Sci* 46: 141

Schoss P (1983) - *Clinical diagnosis of atrophic rhinitis*, Agriculture - Atrophic Rhinitis. Seminar in the CEC program of Coordination of Research on Animal Pathology, Copenhagen May 25-26, edited by KB Pedersen and NC Nielsen. Commission of European Communities, Brussels, p 13

Smith WJ (1983) - *Infectious atrophic rhinitis — non-infectious determinants*, Agriculture — Atrophic Rhinitis. Seminar in the CEC program of Coordination of Research on Animal Pathology, Copenhagen May 25-26, edited by KB Pedersen and NC Nielsen. Commission of European Communities, Brussels, p 151

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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); astroviruses in turkeys (Saif et al 1985; Reynolds and Saif 1986); paroviruses 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 (Pomeroy 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 galerita*) 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.