Molecular epidemiology of two fowl cholera outbreaks on a free-range layer farm

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Short Title – Fowl cholera in free range chickens

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Abstract. Two outbreaks of fowl cholera on a multi-age free range egg farm were investigated. The outbreaks occurred in 1994 and 2002. A total of 22 strains of *P. multocida* were available for study – 11 from the 1994 outbreak and 11 from the 2002 outbreak. Lesions typical of acute fowl cholera were seen in the 1994 outbreak, whereas both acute and chronic FC occurred in the 2002 outbreak. The isolates were examined in an extended phenotypic typing methodology, by a *P. multocida*-specific PCR, by the Heddleston somatic serotyping scheme and by restriction endonuclease analysis (REA) typing using the enzyme *Hpa*II. All 22 strains had the same phenotypic properties, all were confirmed as *P. multocida* by PCR, all were Hedddleston serovar 4, and all had the same REA pattern. Our results indicate that these two outbreaks were caused by the same clone of *P. multocida* – despite the eight year time period between the outbreaks.

Fowl cholera is a common and widely distributed disease of poultry and is of major economic importance.[16] The disease is caused by the bacterium *Pasteurella multocida.*[16] Three subspecies within *P multocida* (*P multocida* subsp *multocida*, *P multocida* subsp *septica* and *P multocida* subsp *gallicida)* are now recognised.[15] Molecular techniques such as restriction endonuclease analysis (REA) and ribotyping have enabled workers to distinguish clones of *P multocida* as a method of studying outbreaks of fowl cholera in turkeys.[1,2,4,5,11] However, there are only limited reports of the use of these techniques for fowl cholera outbreaks associated with chickens.[3,13,14]

In this report, we describe the use of REA and conventional biochemical and serological typing to examine the molecular epidemiology of two acute outbreaks of fowl cholera, separated by eight years, on a free range layer farm.

Materials and Methods

Farm and outbreak details. The two outbreaks occurred on a free range egg farm located near the coast of Central Queensland. In 1994, the farm had five sheds housing a total of 8,000 chickens. By 2002, there were 15 sheds and a total of 50,000 layers. The birds were housed at night and allowed free movement around an open paddock during the day. A dam in the paddock supports a population of wild water-birds. From the first outbreak in 1994, a total of 11 isolates of *P. multocida* were obtained for study. The isolates were obtained from the pericardium, lung and liver of four birds except that for one bird the pericardium isolate was lost before any extensive characterisation was performed. In the second outbreak in 2002, a total of 11 isolates

were obtained from 11 birds. The isolates were obtained from liver (eight birds), heart (two birds) and thoracic cavity (one bird).

Phenotypic characterisation. The isolates were subjected to a phenotypic characterisation as follows. The Gram stain reaction of the organisms was determined using the procedure of Burke.[7] The presence of catalase, oxidase, ß-galactosidase and urease activity and the ability to produce indole were determined as described.[6] The ability to ferment the following carbohydrates was tested using a microplate fermentation method as previously described[1]:- L-arabinose, dulcitol, D-glucose, D-lactose, maltose, D-mannitol, D-sorbitol, D-sucrose, D-trehalose and D-xylose. On completion of the phenotypic characterisation, the isolates were assigned to a biovar of *P. multocida* as previously described. [8]

Serological characterisation. All isolates were serotyped by the Heddleston somatic serotyping scheme[10] in a gel diffusion test. The antisera used in this serotyping had been produced at this Institute using a previously described methodology. [10]

Molecular characterisation. All isolates were also examined by the *P. multocida*-specific PCR described previously.[12] REA was performed, using the restriction endonuclease enzyme used *Hpa*II, on all the isolates as previously described.[1]

Results

Field information. In the 1994 outbreak, disease was seen in adult laying birds (25 weeks old) from only one of the five houses. Dingoes had attacked this house, which contained 1,200 chickens, three nights before the first deaths began. After the

first four days of the outbreak, 200 chickens had died and 50 were clinically ill. Addition of oxytetracycline to the water on the fourth day of the outbreak resulted in a rapid response. The typical clinical signs seen were increased respiratory rate and yellow diarrhoea. Many chickens were prostrate with necks outstretched.

In 2002, another outbreak again occurred – again mainly affecting a single shed. After three days, around 150 chickens had died. Again, oxytetracycline was added to the water and a rapid response was seen. Two weeks after this flare up, the owner was away from the property for two days. The disease spread rapidly during this period – with around 1,500 chickens dying. Oxytetracycline was again added to the water and the deaths ceased. A full course of antibiotic treatment was completed. The typical clinical signs seen in this outbreak were prostrate birds with necks outstretched. There was no evidence of any nasal discharge or swollen wattles or combs.

Post-mortem observations. During the 1994 outbreak liver necrosis was the only gross abnormality, whereas a wider range of lesions was observed during the 2002 outbreak. These included liver necrosis; fibrinous peritonitis, pericarditis and perihepatitis; pneumonia and abscess formation. Histopathological examination of a range of tissues confirmed widespread fibrinopurulent inflammation and necrosis, often with intralesional bacteria.

In the 1994 outbreak, an organism subsequently shown to *P. multocida* was recovered in pure culture from the liver, lung and pericardium of all four birds examined. In the 2002 outbreak, two submissions of birds were examined. In the first submission, isolates subsequently shown to be *P. multocida* were obtained from the liver and other

tissues of all six birds examined. In the second submission, which occurred after the commencement of antibiotic treatment, two of the seven birds examined yielded no bacteria on culture. The remaining five birds yielded growth typical of *P. multocida* from mutiple organs – with only one culture per bird being subjected to full characterisation.

Phenotypic characterisation. All 22 isolates examined in detail were Gram negative rods that produced indole, were oxidase and catalase positive, ß-galactosidase and urease negative, and fermented glucose, mannitol, sorbitol, sucrose, and xylose but not arabinose, dulcitol, lactose, maltose or trehalose. On the basis of these properties, all the isolates were identified as *P. multocida* subsp *multocida* and were identified as belonging to biovar 3 as previously defined.[8]

Serologic characterisation. All isolates were found to be Heddleston serovar 4.

Genotypic characterisation. All 22 isolates were positive in the *P. multocida* PCR and all isolates had the same REA profile (see examples in Fig. 1).

Discussion

We have previously shown that REA patterns, generated using *Hpa*II, is a useful technique for studying the epidemiology of fowl cholera outbreaks in turkeys.[1] Other studies have also reported that *Hpa*II REA patterns are a useful means of differentiating avian isolates of *P. multocida* and identifying clones of *P. multocida*. [3,13,14]

Despite using a range of phenotypic, serotypic, and genotypic tests including REA analysis, we could find no difference between the strains associated with the 1994 and the 2002 outbreaks. This is despite the fact that we looked at multiple birds and at multiple organs from within some birds. It would appear that a single clone of *P. multocida* caused these two outbreaks of fowl cholera.

Christensen *et al.*[3] reported that two contemporaneous outbreaks of fowl cholera in chickens in Denmark were caused by a single clone (as defined by REA and phenotypic characterisation). Recently, Muhairwa *et al.*[13] have reported that three of seven chicken flocks examined contained healthy birds colonised with *P. multocida*. Each of these three flocks was colonised by a single clone of *P. multocida*, as defined by phenotypic testing, with two of the farms, one a brown layer flock with a history of fowl cholera and the other a fowl cholera vaccinated broiler parent flock, sharing the same phenotypic clone of *P. multocida*. None of the isolates from these chicken flocks studied by Muhairwa *et al.*[13] were examined by REA.

In fowl cholera outbreaks of turkeys, a single clone of *P. multocida*, as defined by REA and/or ribotyping, is often associated with outbreaks within a single property.[1,5] The finding of a single clone within a property is not universal as one previous study has shown the presence of multiple REA types within one of the three turkey farms studied.[5]

The only previous molecular study of fowl cholera outbreaks over time involved a Muscovy duck farm.[13] This farm was a multi-age farm with a yearly clean-up period in which no birds were present on the property.[13] The property had outbreaks of fowl cholera in 1996, 1997 but not in 1998.[13] Muhairawa *et al.* [13] found the 1996 and 1997

outbreak strains as well as the 1998 carrier strains to all have different REA patterns – although within each year group, only a single REA pattern was found. The finding of different REA clones over time may be due to the yearly clean out – a period during which no birds were present on the property. In contrast, the farm we investigated did not have any time during which no birds were present on the property over the eight years separating the outbreaks we investigated.

Our work does not provide any evidence on the source of the clone of *P. multocida* associated with these two outbreaks. It is possible that there is an outside reservoir that periodically results in entry of *P. multocida*. Alternatively, it is possible that this strain of *P. multocida* is now endemic in birds on this property. The finding that healthy chickens can be carriers[13] suggests that the clone of *P. multocida* may be resident within birds on this property. If the clone is now resident within the birds, then the multi-age nature of the operation makes it most likely that the clinical cases of fowl cholera will continue to occur over time. Under these circumstances, it would seem that the only control option is to depopulate, thoroughly clean the premise and re-populate with birds free of *P. multocida*. This depopulation-repopulation approach has been recommended by others[9].

In summary, this report shows that a single clone of *P. multocida* caused the two outbreaks of fowl cholera on this farm, despite the outbreaks being separated by eight years.

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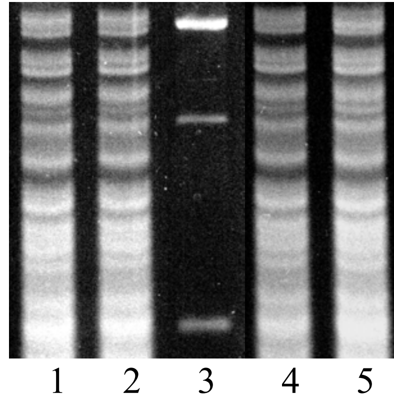
Figure 1. Example of *Hpa*II REA patterns. Lanes 1 and 2 – 1994 outbreak strains, Lane 3 – molecular weight marker, Lane 4 and 5 – 2002 outbreak strains.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