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Prevalence of *Actinobacillus pleuropneumoniae* serovars in England and Wales

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Actinobacillus pleuropneumoniae is the causative agent of porcine pleuropneumonia, an economically important endemic bacterial disease of pigs worldwide (Bossé and others 2002). Epidemiologically, serotyping is the gold standard method, with *A. pleuropneumoniae* being classified into 16 different serovars (1 to 4, 5a, 5b, 6 to 15) based on the presence of surface carbohydrates, principally of the capsule. The prevalence of different serovars varies from country to country (Dubreuil and others 2000). Classically, the serovar of an isolate is determined immunologically using polyclonal serum obtained from animals (typically rabbits) that have been immunised with killed whole cells of a reference strain of the relevant serovar. Such immunological-based methods have limitations, in particular, cross-reactivity between serovars. Cross-reactivity has been reported between serovars 1, 9 and 11,

between serovars 4 and 7 and between serovars 3, 6 and 8 (Zhou and others 2008). To circumvent this cross-reactivity, investigators have devised multiplex PCRs for the simultaneous identification of serovars 2, 5 and 6 (Jessing and others 2003), 1, 2 and 8 (Schuchert and others 2004) and 1, 7 and 12 (Angen and others 2008). In addition, the authors have devised a multiplex PCR that determines whether an isolate is serovar 3, 6 or 8 (Zhou and others 2008). In previous *A pleuropneumoniae* immunological-based (slide agglutination) serotyping studies carried out in the UK (McDowell and Ball 1994, Anon 2004), serovars 3, 6 and 8 were prevalent. The aim of the present study was to determine the prevalence of *A pleuropneumoniae* serovars among isolates from England and Wales using the capsulation gene-based PCR, rather than the potentially problematic immunological-based method used in previous studies.

A total of 378 bacterial isolates, collected from England and Wales between 1995 and 2007, were obtained from the Veterinary Laboratories Agency. Initially, the isolates were subjected to the serovar 3, 6 and 8-*apxIVA* PCR (Zhou and others 2008). Thereafter, isolates that were not of serovar 3, 6 or 8 were subjected to additional capsule gene-based PCRs to determine whether they were serovar 2, 7 or 12 (Hüssy and others 2004, Angen and others 2008). Appropriate *A pleuropneumoniae* reference strains were used as positive and negative controls.

A comparison of the prevalence of different serovars in the present and the two previous UK studies is shown in Table 1. As in previous studies, there were small numbers of isolates of serovars 2 and 12. Notably, rather than being the most prevalent serovar, as was found in the 2004 study (which investigated isolates collected between 1995 and 2004), serovar 3 isolates were rare. Immunologically, serovar 3 predominated, but using capsule-based PCR, serovar 8 was the most prevalent, followed by serovars 6 and 7. The results reflect the disparity between immunological and PCR-based serotyping. This is illustrated in Table 2, in which there is a direct comparison of 66 isolates that had been screened immunologically in the previous UK serovar prevalence study (Anon 2004) and by PCR in the present study. Thirty-four of these isolates were serovar 3 immunologically. However, by PCR, only one of the 34 isolates was serovar 3, two were serovar 6 and the remaining 31 were serovar 8. Thus, immunological serotyping overestimates serovar 3 and underestimates serovars 6 and 8.

The prevalence of *A pleuropneumoniae* serovars contrasts with that of other European countries. For example, in Norway, Sweden and Switzerland (Sørensen and others 2006) and Denmark (Klausen and others 2007), serovar 2 predominates, and serovar 9 is the most common in the Czech Republic (Kucerova and others 2005). One possibility investigated was that the serovar prevalence had altered over time. However, serovar 8 was the most common (based on PCR-based serotyping) in both 2000 to 2004 (108 of 122 isolates [88.5 per cent]) and 2005 to 2007 (38 of 46 isolates [82.6 per cent]). The remaining isolates were collected before 2000.

In a review of seroprevalences around the world, 25 of 27 countries reported the presence of serovars 3, 6 or 8 (Dubreuil and others 2000): immunological-based methods were used to identify these serovars in all cases. Serovar 3 was reported as one of the most prevalent serovars in Belgium, Brazil, Hungary, Ireland and the UK. It is likely that the true prevalence

of serovar 3 is less, and that of serovars 6 and 8 higher, than reported in that study. Since bacterin vaccines are produced using isolates that are representative of the serovar(s) prevalent in a given country, the present results have implications for the choice of vaccine material. The results suggest that serovars 8, 7 and 6 should form the basis of bacterin vaccines developed for use in the UK, and that the inclusion of serovar 3 would be of little value.

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TABLE 1:
Serovar distribution of *Actinobacillus pleuropneumoniae* isolates as a percentage in UK-based studies in 1994 (McDowell and Ball 1994), 2004 (Anon 2004) and 2009 (the present study)

Percentage of isolates (number of isolates present in year of study)			
Serovar	1994 [*]	2004 [*]	2009 [†]
2	9.8 (5)	5.2 (5)	3.4 (13)
3	17.6 (9)	52.1 (50)	0.5 (2)
6	27.5 (14)	6.2 (6)	10.1 (38)
7	5.9 (3)	7.3 (7)	5.3 (20)
8	35.3 (18)	27.1 (26)	78.0 (295)
9	2.0 (1)	-	-
10	2.0 (1)	-	-
12	-	2.1 (2)	2.7 (10)

^{*} Serotyping by slide agglutination using polyclonal rabbit sera raised against *A. pleuropneumoniae* reference strains

[†] Serotyping using capsule-based PCR

TABLE 2:
Direct comparison of the serovars of 66 isolates of *Actinobacillus pleuropneumoniae* that were analysed both immunologically in 2004 (see Table 1) and by PCR in this study

Percentage of isolates present in year of study (number of isolates)		
Serovar	Immunological	PCR
2	6.1 (4)	3.0 (2)
3	51.5 (34)	1.5 (1)
6	3.0 (2)	3.0 (2)
7	7.6 (5)	9.1 (6)
8	30.3 (20)	81.9 (54)
12	1.5 (1)	1.5 (1)
Total	100 (66)	100 (66)