

Short Communication

Trend analysis suggested a change in subspecies among *Mycobacterium avium* isolated from pigs in Belgium, 1967–2013

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Mycobacterium avium is one of the most frequently isolated non-tuberculous mycobacteria, responsible of human and porcine infections. The species *M avium* consists of four subspecies; *M avium* subspecies *avium* (MAA), *M avium* subspecies *silvaticum* (MAS), *M avium* subspecies *paratuberculosis* (MAP) and *M avium* subspecies *hominissuis* (MAH) (Thorel and others 1990, Mijs and others 2002, Turenne and others 2007). Given its pathogenicity for both human beings and pigs, MAH represents an increasing public health concern (Pate and others 2011, Iwamoto and others 2012, Muwonge and others 2014). In pigs, these mycobacteria provoke granulomatous lesions mainly in lymph nodes

of the digestive tract, potentially reducing the value of carcasses (Alvarez and others 2011).

Recently, the authors reported on the predominance of MAH among Belgian patients (98.9 per cent) and slaughter pigs (100 per cent) infected by *M avium* (Vluggen and others 2016). Genotyping of these isolates revealed a large genetic diversity and the absence of a link between genotypes and the place of residence (human) or the farm of origin (pigs) suggesting an environmental source of infection (Vluggen and others 2016).

As porcine *M avium* isolates collected in Belgium during an earlier period of time (1967–1968 and 1992–1996) were available in the Belgian Co-ordinated collections of micro-organisms (BCCM)/Institute of Tropical Medicine (ITM) collection, the authors performed subspecies determination and genotyping on this panel in order to compare the results with those from the more recent isolates sampled in 2012–2013 (Vluggen and others 2016), to investigate the evolution of *M avium* subspecies distribution over time in infected domestic pigs.

All 37 porcine *M avium* isolates available from the collection maintained at BCCM/ITM (Belgium) were used for this study. This panel comprised isolates sampled, isolated and provided by the Belgian veterinary centre Het Centrum voor Onderzoek in Diergeneeskunde en Agrochemie – Centre d'Étude et de Recherches Vétérinaires et Agrochimiques (CODA-CERVA) during the periods 1967–1968 (n=12) and 1992–1996 (n=25).

The *M avium* species identification of the selected strains was confirmed by PCR detection of the insertion sequence (IS) 1245 (Guerrero and others 1995, Vluggen and others 2016) and the *M avium* subspecies characterisation was determined by analysing the presence/absence of IS901 (Godfroid and others 2005, Vluggen and others 2016). Genotyping of the *M avium* isolates was performed by multispacer sequence typing (MST) as described previously (Cayrou and others 2010, Vluggen and others 2016). The MST types were determined by consulting the MST database (Méditerranée infection 2015). Results were compared with those recently reported on a panel of current Belgian porcine *M avium* isolates (n=52) (Vluggen and others 2016).

Based on the presence of IS1245 (specific for *M avium* strains) and the presence/absence of IS901 (present in MAA/MAS but absent in MAH/MAP), 11 isolates among the 37 analysed were identified as MAH and 26 as MAA/MAS (Table 1).

Overall, 10 different MST types were identified; 5 among MAH and 5 among MAA (Table 1), with MST type 12 being the most prevalent among MAH (6/11) and MST types 2 and 3 the most prevalent in MAA isolates (14/26 and 6/26, respectively). The authors identified four new MST types which were recorded in the MST database developed by the 'Université de la Méditerranée' as MST type 117, 118, new 1 and new 2.

Comparison of MST types from porcine isolates obtained in the 1960s–1990s with those sampled in 2012–2013, revealed the presence of both MAA (n=26, 70.3 per cent) and MAH (n=11, 29.7 per cent) subspecies in the old panels versus only MAH isolates in the recent panel, and a change is observed in MST type over time (Table 2).

The present study was undertaken to determine the evolution of *M avium* subspecies infecting Belgian pigs over time. Unlike the omnipresence of the subspecies *hominissuis* reported among Belgian pigs infected by *M avium* in 2012–2013, the authors observed in their older panels of isolates the presence of both species, MAA and MAH. Moreover, the MST type changed over time.

In Europe, various mycobacteria have been recently reported from pigs at slaughterhouses, with 11 per cent of the isolates belonging to the *M avium* complex (MAC) (EFSA 2011). Mycobacteria from MAC are ubiquitous bacteria that can be found in water, food, soil and other environmental samples and

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TABLE 1: Distribution of *Mycobacterium avium* subspecies and their MST of 37 Belgian porcine isolates sampled in the years 1967–1968 and 1992–1996

Subspecies identification based on the IS profiles and MST types	IS1245/IS901 profile	Isolates sampled in 1967–1968	Isolates sampled in 1992–1996	Total
<i>M avium</i> subspecies <i>hominissuis</i>	+/-	2 2 MST type 12	9 4 MST type 12 2 MST type 13 1 MST type 15 1 MST type 118 (new) 1 MST New 2	11
<i>M avium</i> subspecies <i>avium/silvaticum</i>	+/+	10 3 MST type 2 4 MST type 3 3 MST type 117 (new)	16 11 MST type 2 2 MST type 3 2 MST type 4 1 MST New 1	26
Total		12	25	37

–, absence of the fragment; +, presence of the fragment; IS, insertion sequence; MST, multispacer sequence typing

TABLE 2: MST types identified among Belgian porcine *Mycobacterium avium* isolates in this study (1967–1968 and 1992–1996 sample) in comparison with the study from [Vluggen and others \(2016\)](#) (1) (2012–2013 sample)

<i>M avium</i> subspecies	MST type	1967–1968 panel	1992–96 panel	2012–2013 panel	Total
MAA	2	3	11		14
	3	4	2		6
	4		2		2
	117 (new)	3			3
MAH	New 1		1		1
	12	2	4	28	34
	13		2		2
	15		1		1
	118 (new)		1		1
	New 2		1		1
	20			2	2
	22			7	7
	30			1	1
	51			2	2
	85			3	3
	91			1	1
	102			2	2
103			1	1	
104			3	3	
109			1	1	
110			1	1	
Total		12	25	52	89

MAA, *Mycobacterium avium* subspecies *avium*; MAH, *Mycobacterium avium* subspecies *hominissuis*; MST, multispacer sequence typing

are considered opportunistic pathogens for numerous animal species, mainly birds and pigs, as well as for human beings ([Radomski and others 2010](#)). In particular, MAA is the aetiological agent of tuberculosis in birds, including wood pigeons ([Mijs and others 2002](#)). One hypothesis to explain the decrease of MAA in domestic pigs over time is the increase of biosafety measures designed to reduce the risk of introduction, establishment and spread of animal diseases, and their gradual implementation in the pig sector after the epidemic of classical swine fever in the beginning of the 1990s ([Arrêté royal 1953](#), [Vanthemsche and Sagerman 1994](#)). These measures probably induced the decrease of entrance of birds in the piggeries.

In The Netherlands, a decrease in prevalence of MAA infections was observed after the arrest of compost use in piggeries ([Komijn and others 2007](#)). However, in Belgium, the compost is still being used ([Arrêté royal 2003](#)).

Strains with particular IS profiles (absence of IS1245 usually present in *M avium* strains or false PCR detection of IS901 provoked by the presence of ISMav6) have been reported in different

studies realised on contemporary strains ([Ichikawa and others 2009](#), [Vluggen and others 2016](#)). No such strains were found among the 1960's sample. Although limited by the small sample size of the present study, this observation suggests that these variants may have appeared more recently.

During the authors' 2012–2013 study, MST 12 was the most frequently isolated MAH genotype, among Belgian porcine samples, as well as the human samples ([Vluggen and others 2016](#)), suggesting a common source of infection ([Vluggen and others 2016](#)). However, in total 11 different MST types were identified among pigs, underlining the large genetic diversity of MAH isolates.

The main limitations of the present report are the small number of *M avium* isolates available in the collection and the lack of information concerning their origin. Although it is known that the strains were isolated from Belgian pigs and analysed in different Belgian laboratories, information about the farms of origin or the reason for sample collection are not available.

In conclusion, the authors report here a temporal difference in the subspecies distribution among porcine *M avium* isolates between the years 1967–1968/1992–1996 and years 2012–2013 in Belgium. MAH seems to have relatively increased at the expense of MAA, and MST types changed over time, although MST12 remained predominant among MAH isolates. The disappearance of MAA isolates could be the result of increased biosafety measures gradually put in place in the pig sector since the 1990s. Analysis of a larger and well characterised sample would be required to confirm the observations reported here.

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