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POPULATION DYNAMICS OF SWINE INFLUENZA VIRUS IN FARROW-TO-FINISH AND SPECIALISED FINISHING HERDS IN THE NETHERLANDS

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Influenza virus infections with subtypes H1N1, H3N2 and H1N2 are very common in domestic pigs in Europe. Data on possible differences of population dynamics in finishing pigs in farrow-to-finish herds and in specialised finishing herds are, however, scarce. The presence of sows and weaned piglets on the same premises may, however, affect the exposure of finishing pigs to influenza viruses. In a longitudinal study on 14 farrow-to-finish herds and 15 finishing herds, groups of pigs were followed by repeatedly testing the same animals for antibodies against all three influenza virus subtypes (H1N1, H3N2 and H1N2). At the end of the finishing period, the seroprevalences in farrow-to-finish and specialised finishing herds were 44.3% and 62.0% respectively for H1N1, 6.6% and 19.3% respectively for H3N2, and 57.2% and 25.6% respectively for H1N2. For all three subtypes, the incidence of influenza virus infections was highest at the beginning of the finishing period in farrow-to-finish herds, while the incidence of influenza virus infections was highest at the end of the finishing period in finishing herds. Respiratory disease, probably related to the influenza infections, was observed in five of these herds only, but also occurred at the beginning of the finishing period in farrow-to-finish herds and at the end of the finishing period in finishing herds. The observed differences of population dynamics of influenza virus may affect choice and timing of intervention measures.

Keywords: swine influenza; H1N1; H3N2; H1N2; incidence; herd type; population dynamics

1. Introduction

Influenza virus infections in swine are very common. In several studies in European countries, seroprevalences for the swine influenza strains H1N1 and H3N2 were found to be in the range of 20-80% in finishing pigs at the end of the finishing period and in sows (Masurel et al., 1983; Haesebrouck and Pensaert, 1986b; Yus et al., 1989; Elbers et al.,

1990; Teuffert et al., 1991; Elbers et al., 1992; Groschup et al., 1993; Ewald et al., 1994; Brown et al., 1995b; Maes et al., 1999; Maldonado et al., 2006). A more recent subtype, H1N2, seems to originate from the UK where it was found for the first time in 1994 (Brown et al., 1995a). H1N2 was subsequently reported from Belgium (Van Reeth et al., 2000), Italy and France (Marozin et al., 2002), Germany (Schrader and Suss, 2003), and Spain (Maldonado et al., 2006). Many, if not all of these strains are related to the original H1N2 strain from the UK (Marozin et al., 2002). Seroprevalence studies in Belgium (Van Reeth et al., 2000) and Spain (Maldonado et al., 2006) resulted in high seroprevalences of approximately 70% and 50% respectively for H1N2, but both studies were carried out in sows.

Studies in finishing pigs so far give overall estimates of the seroprevalence at the end of the finishing period. However, it is not clear at what moment during the finishing period these infections take place, nor do they differentiate between finishing pigs in specialised finishing herds and finishing pigs in farrow-to-finish herds. The presence of sows and piglets on the same premises as the finishing pigs may, however, affect the population dynamics of swine influenza in these herd types, and thus also affect the choice and timing of intervention measures, like vaccination or zoosanitary measures.

Longitudinal studies were therefore carried out to be able to compare the seroprevalences and incidences of swine influenza virus infections at different ages in finishing pigs in both herd types. This allowed us also to determine whether there are differences in the population dynamics of swine influenza virus infections in finishing pigs in farrow-to-finish herds versus finishing pigs in specialised finishing herds.

2. Materials and methods

2.1. Selection of herds

In the Netherlands, there are three regions with a high pig density (figure 1). In these regions the average pig density is more than 600 pigs per square kilometre. In contrast, the

83 average pig density in the remaining part of the Netherlands is below 100 pigs per square
84 kilometre. From the three pig dense regions all herds with >400 finishing pigs were selected
85 for possible participation in the study. This selection included 27% of the Dutch swine herds,
86 housing 69% of the Dutch finishing pig population.
87 In each category (farrow-to-finish and finishing herds) 64 herds were randomly selected.
88 A written request for participation in a longitudinal serological survey was sent to these
89 farmers. Vaccination against swine influenza was not allowed. The first 15 in each category
90 returning a positive response were included in the study. One farrow-to-finish herd that
91 applied for the survey withdrew shortly before the survey actually started and could not be
92 replaced on such short notice. Thus, 29 herds finally participated. In eleven of the finishing
93 herds the investigated pigs originated from only one breeding herd, in three finishing herds
94 the pigs originated from two breeding herds and in one finishing herd the pigs originated
95 from three breeding herds.

96 *2.2. Sampling*

97 All farms were visited three (finishing herds) or four (farrow-to-finish herds) times during
98 the months of January to May 1999. In each herd one compartment was followed in a
99 longitudinal study and blood samples were collected during each visit.
100 In finishing herds one compartment where the piglets were 12 weeks old was sampled
101 during the first visit. On average, these piglets arrived 2-3 weeks before on the farm. Pigs
102 were tagged individually during the first sampling to allow for resampling of the same pigs
103 during subsequent visits. The second blood samples were taken four weeks later (age of 16
104 weeks) and a final sample was taken within one week before the first pigs from that
105 compartment were delivered to the slaughter house (on average at the age of 22 weeks).
106 Piglets within the sampled compartment always originated from one breeding herd. During
107 the finishing period no other pigs were added to that compartment.
108 In farrow-to-finish herds one compartment where the piglets were 8 weeks old was
109 sampled during the first visit. Pigs were tagged individually during the first sampling to allow

for resampling of the same pigs during subsequent visits. The second series of blood samples was taken four weeks later (age of 12 weeks), the third another four weeks later (age of 16 weeks) and the final series of samples was taken within one week before the first pigs from that compartment were delivered to the slaughter house (on average at the age of 22 weeks). Tagged piglets were kept together in the same compartment until slaughter, but while being transferred to the finishing facilities, piglets from two compartments were sometimes mixed. However, during the finishing period no other pigs were added to that compartment anymore.

The sample size for each compartment was calculated so that with an estimated seroprevalence of 50% and a confidence of 0.95 the margin of error was less than 20%. This resulted in sample sizes of 16-24 pigs per compartment using the formula for simple random sampling (Thrusfield, 1995). Within a compartment an equal number of pigs were sampled per pen, as far as the total number of samples allowed for this. Within each pen, pigs were selected at random (haphazardly).

All farmers were asked to record all clinical signs and medications in the compartment under study.

2.3. Serological examination

All sera were tested in a hemagglutination inhibition (HI) test (Kendal et al., 1982) for antibodies against influenza virus strains A/swine/Neth/Best/96 (H1N1), A/swine/Neth/St Oedenrode/96 (H3N2) and A/swine/Gent/7625/99 (H1N2). Sera were pre-treated with chicken erythrocytes and cholera filtrate to remove non-specific hemagglutinating factors and non-specific inhibitors of hemagglutination. Four hemagglutinating units (HAU) were used in each test.

A/swine/Neth/Best/96 (H1N1) and A/swine/Neth/St Oedenrode/96 were isolated in the Netherlands and are representative for influenza strains circulating in the Netherlands (Loeffen et al., 1999). A/swine/Gent/7625/99 (H1N2) is a Belgian strain, representative for H1N2 strains circulating among pigs in Belgium, which in turn is expected to be

representative for H1N2 strains all over Western Europe (Marozin et al., 2002). Although H1N1 and H1N2 strains both possess a hemagglutinin H1, these are of different origin (Brown et al., 1998) and little or no cross reactivity was found in serological tests after experimental infections (Van Reeth et al., 2000; Van Reeth et al., 2004; Van Reeth et al., 2006).

Sera were tested in serial twofold dilutions, starting at 1:9. Titres ≥18 were considered positive. A fourfold rise in titres in consecutive samples was considered evidence of an influenza virus infection somewhere during that period (taking into account approximately one week for titres to develop after an influenza virus infection). In the presence of maternal antibodies, titres of the second sera were not compared to the titres of the first sera, but to expected titres of the second sera if these were due to maternal antibodies only. To do this, the decay of maternal antibodies, having a half-life of approximately 12 days, was taken into consideration, extrapolating titres from the first sera (Loeffen et al., 2003).

2.4. Statistical analysis

Statistical analyses for the differences between farrow-to-finish herds (FFH) and specialised finishing herds (FH) were performed per strain and per time interval. The incidence, cumulative incidence and prevalence data were expressed as fractions per farm. The incidence being the number of seroconversions since the previous sampling, the cumulative incidence the total number of seroconversions until the moment of sampling, and the prevalence the fraction of seropositive pigs at each sampling. The test statistic (t) for the null hypothesis of no difference between the herd types was:

$$t = a - b\,.$$

wherein a and b are the fractions averaged over FFH and FH farms respectively. The null-distribution was obtained by random permutation of the labels FFH and FH over the farms.

3. Results

3.1. Seroprevalence

For H1N2, at the end of the finishing period the seroprevalences in farrow-to-finish and specialised finishing herds were 57.2% and 25.6% respectively (table 1). The seroprevalence was in all age groups higher in farrow-to-finish herds than in specialised finishing herds (P=0.01, P<0.01 and P=0.03 for 12, 16 and 22 weeks of age respectively). For H1N1 and H3N2, at the end of the finishing period the seroprevalences in farrow-to-finish and specialised finishing herds were 44.3% and 62.0% respectively for H1N1 and 6.6% and 19.3% respectively for H3N2. The differences of the seroprevalences in farrow-to-finish herds and finishing herds were not statistically significant..

3.2. Incidence

For H1N1 the differences between both herd types are statistically significant between 16 and 22 weeks of age (P<0.01) and for H1N2 between 0 and 12 (P=0.05) and 12 and 16 (P=0.01) weeks of age (table 2). No significant differences (P<0.05) were seen for H3N2. The cumulative incidences are shown in table 3. They are essentially comparable to the seroprevalence, however, corrected for antibodies due to maternal immunity or antibodies from an infection that declined to a level below the detection limit of the test.

3.3. Clinical symptoms of respiratory disease

Twenty-four farmers (from 11 farrow-to-finish herds and 13 finishing herds) kept a record of clinical signs and medications. In eleven of them, an episode of respiratory disease was recorded: five in farrow-to-finish herds and six in finishing herds. The severity of the symptoms was variable, ranging from only coughing for a few days up to a combination of coughing, laboured breathing and decrease of feed intake. Five of these episodes coincided with an incidence of 80-95% of the pigs for one of the influenza subtypes in the same period. Two of these were in farrow-to-finish herds and both occurred between the age of 8 and 12

186 weeks (1x H1N2 and 1x H3N2). The other three occurred in finishing herds, all of them
187 between the age of 16 and 22 weeks (1x H1N1 and 2x H1N2).

188 **4. Discussion**

189 In this study we found that the timing of influenza infections in finishing pigs is quite
190 different in finishing pigs from farrow-to-finish herds compared to finishing pigs from
191 specialised finishing herds. In farrow-to-finish herds the incidence of influenza virus
192 infections was highest at the beginning of the finishing period, while in finishing herds the
193 incidence of influenza virus infections was highest at the end of the finishing period. This
194 pattern was observed for all three influenza virus subtypes. It can be speculated that the
195 exposure of finishing pigs to influenza virus is different in both herd types.

196 Sows and especially weaned piglets may be a reservoir for continuous circulation of
197 influenza viruses (Loeffen et al., 2003). Indirect exposure of finishing pigs to influenza virus
198 from sows or weaned piglets will be more easily achieved in farrow-to-finish herds than in
199 specialised finishing herds. Multiple virus introductions into a compartment of finishing pigs in
200 a farrow-to-finish herd could thus result in a high incidence at the beginning of the finishing
201 period. Large outbreaks are apparently rare at that time, possibly due to the combination of
202 decreasing numbers of piglets with maternal antibodies and increasing numbers of piglets
203 with antibodies due to an infection. Introductions later during the finishing period may then
204 die out quickly due to the already high seroprevalence. A compartment of pigs in a finishing
205 herd on the other hand may be subject to only a few virus introductions. On average this
206 may result in populations with more susceptible animals in the second half of the finishing
207 period, with any introduction at that time resulting in a large outbreak within that
208 compartment.

209 The presence of finishing pigs in a herd might also affect influenza virus infections in
210 sows and weaned piglets. Especially with subtype H1N2, at least 8.6% percent of the
211 weaned piglets seroconverted before they were 8 weeks old. This could also explain the
212 higher seroprevalence at the age of 12 weeks in farrow-to-finish herds, compared to

finishing herds. It may be that in the breeding herds that supplied the piglets to the specialised finishing herds, virus circulation in weaned piglets was lower than in weaned piglets on farrow-to-finish herds. This would suggest that the presence of finishing pigs may result in an additional exposure of weaned piglets to the virus and thus may affect virus circulation in weaned piglets. It's also possible that the relatively recent introduction of the H1N2 subtype in the population somehow causes this effect. This would, however, indicate that farrow-to-finish herds and specialised breeding herds are at a different risk for virus introduction. Given that the two herd types were not located in exactly the same place, these results have to interpreted with some care anyway.

Even though serological differentiation between H1N1 and H1N2 is possible in experimental sera (Van Reeth et al., 2004; Van Reeth et al., 2006), nothing is known about differentiation in field sera. In the field multiple infections can be expected, and variations occur in haemagglutinin, even within one subtype (De Jong et al., 2001). Seroprevalence and incidence of H1N1 and H1N2 might as a result be overestimated. However, if the serological results of these two subtypes are compared at the age of 22 weeks, the agreement beyond chance is very low (Kappa value 0.078). Under the assumption that both subtypes occur independently, this suggests a very low level of cross-reactivity.

The seroprevalence of H1N1 is comparable to the levels that were found in finishing pigs in 1980 (44%) (Masurel et al., 1983) and 1987 (62%) (Elbers et al., 1990) in the Netherlands. The seroprevalence found for H3N2 is, however, much lower than the ones found in those same studies (68% in 1980; 33% in 1987). All other seroprevalence studies carried out in Europe in the past two decades also showed much higher seroprevalences (Haesebrouck and Pensaert, 1986b; Yus et al., 1989; Teuffert et al., 1991; Groschup et al., 1993; Ewald et al., 1994). The reason for the low seroprevalence therefore remains unknown and it remains to be seen whether this is a structural decrease or just an uncommonly low seroprevalence during a single season. The seroprevalence of H1N2 is not comparable to published results from other countries, because they were carried out in sows (Van Reeth et al., 2000; Maldonado et al., 2006).

While the population dynamics of influenza virus infections is different between farrow-to-finish and finishing herds, it would be equally important to determine whether the occurrence of clinical symptoms, and subsequent economic losses, are also different between both herd types. Unfortunately, also with respect to this, no information can be found in existing literature. Some studies that tried to determine the importance of influenza virus infections as a cause for respiratory disease, found that approximately 50% of the respiratory outbreaks were caused by influenza virus infections (Loeffen et al., 1999; Barigazzi and Donatelli, 2003; Choi et al., 2003). There was however no differentiation in herd type, or time of infection. In this study, eleven episodes of respiratory disease were noticed, almost equally distributed over both herd types. Only five of them coincided with a seroconversion against one of the influenza subtypes of at least 80% of the pigs in the same period, also equally distributed over both herd types. These five episodes are the ones most likely related to the influenza virus infections, which is also consistent with the 50% of respiratory disease caused by influenza virus infections (Loeffen et al., 1999). Although this is not enough to allow for any statistical analysis, it is noticeable that the two episodes of respiratory disease in farrow-to-finish herds occurred between the age of 8 and 12 weeks, while the three episodes in finishing herds occurred between the age of 16 and 22 weeks. Finishing pigs in both herd types may be equally subject to respiratory disease, but at a different moment during the finishing period. All three subtypes may have been involved in the occurrence of clinical symptoms.

Differences in time of influenza virus infections in both herd types may ask for a different approach in preventive measures, whether these are zoosanitary measures or vaccination. If infections, and subsequent economic losses, in specialised finishing herds occur mainly at the end of the finishing period, it would be possible to wait with vaccination, until maternal antibodies have mostly disappeared. Interference of vaccination with maternal antibodies (Wesley and Lager, 2006) would thus not be a real problem and full and equal protection may be reached. It might even be considered to vaccinate only once at such a time. In general, single vaccinations are considered to be insufficient for full protection. However,

partial protection was shown after single vaccination, that may be enough to sufficiently reduce damages due to influenza virus infections under the right circumstances (Haesebrouck and Pensaert, 1986a).

In farrow-to-finish herds on the other hand, there seems to be no optimum with respect to the time of vaccination. Before all maternal antibodies disappear in a population, most of the influenza virus infections, and possibly also the economic losses due to the infection, already occur. Vaccination at a young age, and probably multiple vaccinations to circumvent the negative effect of maternal antibodies, may be indicated.

With respect to zoosanitary intervention measures, the possibilities for influenza seem somewhat limited. Zoosanitary measures may focus on reduction of damages (either due to the primary infection or due to secondary infections). Mainly climate control and general hygiene seem to be relevant zoosanitary measures for this purpose. Zoosanitary measures may also focus on preventing infection by keeping the virus out. Given the ease with which influenza viruses may spread, hygienic measures for this purpose probably have to be rather strict, involving air filtration and strict hygiene protocols for anything and anyone entering the premises. In farrow-to-finish herds this may be more difficult to achieve, also being more expensive because it involved a broader range of age groups, than in specialized finishing herds, given that most infections seem to occur at a later stage in the latter herd type.

While these conclusions and recommendations may apply to swine herds in general, variations in individual herds may occur. It is therefore advisable that, where possible, results from investigations in an individual herd are also taken into account before putting together a strategy of vaccination or any other kind of intervention.

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Table 1: Seroprevalences for swine influenza subtypes H1N1, H3N2, and H1N2, in farrow-to-finish herds and finishing herds. Any significant (P<0.05) differences between farrow-to-finish and finishing herds (pair wise comparisons for the same influenza virus subtype over the same period) are indicated with a shaded background. Any trends towards significance (P<0.1) are indicated by a square.

Table 2: Incidences for swine influenza subtypes H1N1, H3N2, and H1N2, in farrow-to-finish herds and finishing herds. Incidences were calculated based on the number of seroconversions per period; in farrow-to-finish herds the periods of 0-8 and 8-12 weeks are added to each other to be able to compare them to the period of 0-12 weeks in finishing pigs. Legend as in table 1.

Table 3: Cumulative incidences for swine influenza subtypes H1N1, H3N2, and H1N2, in farrow-to-finish herds and finishing herds. Incidences were calculated based on the number of seroconversions per period. Legend as in table 1.

Figure 1: Location of the three most pig-dense regions in the Netherlands and the location of the herds participating in the longitudinal study (farrow-to-finish herds (∇) and finishing herds (Δ))

Table 1

Herd type	Age (weeks)	Number of herds	H1N1			H3N2			H1N2		
			Number of samples	Prevalence (%)	95%CI	Number of samples	Prevalence (%)	95%CI	Number of samples	Prevalence (%)	95%CI
Farrow-to-finish	8	14	290	21.7	11.1-32.3	290	21.7	12.9-30.6	290	53.8	38.4-69.2
	12	14	287	20.9	5.9-35.9	287	15.7	2.4-29.0	287	48.8	28.9-68.7
	16	14	286	44.4	22.3-66.5	286	8.7	0.0-20.1	286	60.8	42.7-79.0
	22	14	287	44.3	23.1-65.4	287	6.6	0.0-15.7	283	57.2	38.2-76.3
Finishing	12	15	322	22.0	8.1-36.0	322	35.7	19.7-51.8	322	18.6	6.6-30.6
	16	15	321	22.1	8.7-35.6	321	20.2	7.0-33.5	320	10.3	0.0-21.1
	22	15	321	62.0	44.6-79.4	321	19.3	3.7-34.9	320	25.6	7.6-43.7

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Table 2

Herd type	Period (weeks of age)	Number of herds	H1N1			H3N2			H1N2		
			Number of samples	Incidence (%)	95%CI	Number of samples	Incidence (%)	95%CI	Number of samples	Incidence (%)	95%CI
Farrow-to-finish	0-8	14	290	-	-	290	-	-	290	8.6	0.0-19.0
	8-12	14	287	15.7	2.7-28.6	287	10.1	0.0-23.3	287	24.4	8.5-40.3
	12-16	14	286	31.1	8.1-54.1	286	1.4	0.0-2.9	286	25.9	7.9-43.9
	16-22	14	285	2.5	0.0-7.1	285	1.1	0.0-2.6	281	1.4	0.3-2.6
Finishing	0-12	15	320	6.9	0.0-15.7	320	4.1	0.0-8.5	320	5.0	0.0-14.5
	12-16	15	320	13.8	4.8-22.7	320	10.3	0.9-19.7	319	2.8	0.0-5.9
	16-22	15	308	41.2	22.1-60.4	308	8.8	0.0-21.1	307	18.6	1.4-35.7

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Table 3

Herd type	Period (weeks of age)	Number of herds	H1N1			H3N2			H1N2		
			Number of samples	Incidence (%)	95%CI	Number of samples	Incidence (%)	95%CI	Number of samples	Incidence (%)	95%CI
Farrow-to-finish	0-8	14	290	-	-	290	-	-	290	8.6	0.00-19.0
	0-12	14	287	15.7	2.7-28.6	287	10.1	0.0-23.3	287	33.1	12.7-53.4
	0-16	14	286	46.9	24.2-69.5	286	11.5	0.0-24.6	286	59.1	40.0-78.2
	0-22	14	285	49.5	26.6-72.3	285	12.6	0.0-25.8	281	60.5	41.4-79.6
Finishing	0-12	15	320	6.9	0.0-15.7	320	4.1	0.0-8.5	320	5.0	0.0-14.5
	0-16	15	320	20.6	8.6-32.6	320	14.4	3.3-25.4	319	7.8	0.0-17.5
	0-22	15	308	62.7	45.4-79.9	308	23.4	7.2-39.5	307	26.7	8.6-44.9

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