

Herd factors associated with the seroprevalences of four major respiratory pathogens in slaughter pigs from farrow-to-finish pig herds

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Abstract – The objective of this study was to investigate sero-epidemiological aspects of *Mycoplasma hyopneumoniae* (Mh), influenza H₁N₁ and H₃N₂ viruses and Aujeszky disease virus (ADV) in fattening pigs from 150 randomly selected farrow-to-finish pig herds. Different herd factors were examined as potential risk indicators for the percentage of pigs with antibodies against the 4 pathogens. The median within-herd seroprevalences of the pathogens were: Mh 76%, H₁N₁ 100%, H₃N₂ 40% and ADV 53%. There was a positive association between the seroprevalences of both influenza viruses, and a negative association between the seroprevalences of ADV and H₁N₁. The percentage of pigs seropositive for Mh increased with the purchase of gilts and with the season (slaughter date in March–April). The within-herd seroprevalences of both influenza viruses were higher in the case of a higher density of pig herds in the municipality. A higher number of fattening pigs per pen additionally increased the risk of being seropositive for H₃N₂. The percentage of pigs with anti-gE-antibodies against the wild type ADV increased with higher airspace stocking density in the finishing unit, increasing herd size, increasing number of pig herds in the municipality and slaughter date in March–April. Increased seroprevalences for these 4 respiratory pathogens were mostly associated with pig density in the herd and its vicinity, the winter period, and with the purchase of gilts. Purchase of gilts, number of fattening pigs per pen and airspace stocking density are risk factors that can be managed directly by farmers striving to attain a high respiratory health status of pigs.

pig / respiratory infections / seroprevalence / epidemiology

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Résumé – Facteurs de risque associés à la séroprévalence des 4 principaux agents pathogènes respiratoires chez le porc charcutier provenant d'élevages naisseurs-engraisseurs. L'objectif de ce travail est d'étudier les aspects séro-épidémiologiques vis-à-vis de *Mycoplasma hyopneumoniae* (Mh), des virus H₁N₁ et H₃N₂ de la grippe porcine et du virus de la maladie d'Aujeszky (VMA) chez des porcs charcutiers provenant de 150 élevages naisseurs-engraisseurs. Les caractéristiques d'élevages ont été étudiées comme étant des indicateurs de risque potentiel de séroprévalence des 4 agents pathogènes. Les séroprévalences médianes à l'échelle des élevages étaient: Mh 76%, H₁N₁ 100%, H₃N₂ 40% et VMA 53%. Une relation positive était observée entre les séroprévalences des deux virus grippaux. En revanche, une relation négative était mise en évidence entre la séroprévalence de la grippe (virus H₁N₁) et celle du VMA. Le pourcentage de porcs séropositifs vis-à-vis de Mh était plus élevé dans les porcheries où les éleveurs avaient introduit de nouvelles cochettes pour la reproduction et lorsque les porcs étaient abattus en mars-avril. La séroprévalence des deux virus grippaux était plus élevée dans les localités à forte densité d'élevages porcins et le risque de séroconversion vis-à-vis du virus H₃N₂ de la grippe porcine augmentait avec le nombre de porcs dans les unités d'engraissement. Le risque de trouver des animaux porteurs d'anticorps anti-gE dirigés contre les souches sauvages du VMA augmentait aussi avec le nombre de porcs présents dans les unités d'engraissement, la taille des élevages, le nombre de porcs dans la localité et les abattages en mars-avril. La séroprévalence vis-à-vis de ces 4 agents pathogènes respiratoires était le plus souvent associée avec la densité de porcs dans les élevages et dans le voisinage, la saison hivernale et après l'acquisition de cochettes pour la reproduction. L'introduction de nouvelles cochettes dans les porcheries, le nombre de porcs par case dans les unités d'engraissement et la densité de population sont des facteurs de risque qui peuvent être contrôlés directement par les exploitants qui veulent atteindre un niveau sanitaire élevé vis-à-vis des maladies respiratoires du porc.

porc / infections respiratoires / séroprévalence / épidémiologie

1. INTRODUCTION

With the intensification in pig production, veterinary services that are provided have moved from the so-called first-aid practice into planned prevention and control programs. A successful application of these programs largely depends upon a thorough understanding of the epidemiology of diseases. This is especially true for the prevention of respiratory disorders because they may be multifactorial [21, 34].

The present study was conducted to get insight into sero-epidemiological aspects of four important respiratory pathogens in pigs, namely *Mycoplasma hyopneumoniae* (Mh), influenza H₁N₁ and H₃N₂ viruses and Aujeszky disease virus (ADV). Previous studies focusing on the epidemiology of Mh have been conducted to estimate the seroprevalence of Mh in pig herds [19], and to discern risk factors associated with reinfection of SPF herds with Mh [16, 22, 38, 42]. No information is available, however, about risk factors influencing the proportion of

Mh seropositive pigs per herd. Epidemiological characteristics of porcine influenza viruses have been investigated mainly from a descriptive point of view [17], or else, the emphasis was put on public health aspects [47]. Few studies [11, 12, 13] have so far investigated factors involved in the spread of influenza viruses within and between pig herds. The spread of ADV within and between pig herds has been the subject of many studies in Europe [11, 12, 15, 23, 39] and North-America [2, 4, 31, 46]. However, the results of these studies are not always consistent.

Because pig herds are commonly infected with different respiratory pathogens, it is imperative to investigate the epidemiology of these infections simultaneously in the same study population. Such an approach also allows to compare the epidemiological characteristics of different pathogens, and it guarantees that possible differences between the pathogens are not related to differences in study design. Many of the aforementioned studies have been carried out by

evaluating unconditional associations between risk factors and disease, or have considered only a limited number of risk factors. In this respect, a multivariate study constitutes a very useful tool to examine a wide range of factors, and to investigate the effects of an individual factor while other factors are held constant.

The present cross-sectional study was conducted to investigate sero-epidemiological characteristics of the aforementioned pathogens simultaneously in 150 randomly selected Belgian farrow-to-finish (FTF) pig herds. The specific aims of the study were (1) to identify risk indicators for the proportion of pigs seropositive for these four respiratory pathogens at slaughter, and (2) to determine the interrelationships of these seroprevalences.

2. MATERIALS AND METHODS

2.1. General features of the study population

The herds were located in the north-western part of Belgium. This is an area with a surface of 11% of the country, and which contains 54% of the Belgian pig population that is, approximately 2.7 million pigs. The study included FTF herds only. In all study herds, ADV vaccination was practised according to the mandatory vaccination scheme that is sows and boars were vacci-

nated two times per year with an inactivated vaccine or three times per year with an attenuated vaccine; gilts at least three times before first mating or insemination; fattening pigs (FP) at least once at the start of the fattening period. Vaccination against porcine influenza was practised in 23% of the herds. None of the herds had practised vaccination against Mh at least one year before the study was initiated.

2.2. Selection of herds

One hundred and fifty FTF pig herds were selected at random from the national registry [36]. A random selection was used to obtain representative data from the population herds. Herds in the area were stratified by herd size into three categories: 50 to 100 sows, 101 to 200 sows, and more than 200 sows. Small herds (< 50 sows) were excluded because they are unlikely to survive economically in the future. The number of selected herds per herd-size category was proportional to the number of sows in each herd-size category (Tab. I). This selection procedure avoided overrepresentation of the numerous herds with 50 to 100 sows.

First, a letter was mailed to the selected farmers introducing the study and its purpose. Next, they were contacted by phone and asked to collaborate. Thirteen percent ($n = 22$) of the selected herd owners did not

Table I. The number of sows and the number of pig herds per herd-size category in the geographic area and in the study.

	Herd-size category			Total
	50-100 sows	101-200 sows	>200 sows	
Number of pig herds in the geographic area	1 132	799	198	2 129
Number of sows in the geographic area	88 296	115 056	62 766	266 118
Number of pig herds in the study	50	65	35	150
Percentage of pig herds in the geographic area in the study	4.4	8.1	17.7	7.0

participate for the following reasons: slaughter of FP abroad ($n = 5$), distribution of slaughter pigs to several slaughterhouses ($n = 6$), end of activity ($n = 2$), no FTF herd ($n = 2$), herd size too small ($n = 2$), or refusal to participate ($n = 5$). Random sampling was repeated in corresponding strata to replace these 22 herds.

2.3. Collection of herd data

The data were obtained by the first author through inspections of the pigs and the pig units, and through face-to-face interviews of the pig farmers. A questionnaire with precise definitions of the data to be recorded and pertaining to potential risk indicators for respiratory disease was used (Tabs. II and III). The on-farm data were considered to be of sufficient quality because standardised definitions were used for the topics in the questionnaire, and because the data were obtained by one person. Information pertained to herd size, month of slaughter, pig and pig herd density in the municipality,

management practices, housing conditions, disease prevention procedures and hygienic measures. All these data were contemporary for the pigs that were examined at slaughter.

Herd size was defined based on the number of sows on the premises. The 12 months during which the study was conducted were grouped in bi-monthly periods using slaughter date. Almost all pure-bred sows were Landrace sows; the remaining were Yorkshire and Large White sows. Cross-breeds, that is at least two-way crosses, were commercial hybrid sows or sows derived from own on-farm selection. In most cases, purchased gilts were bought at the age of 7 months. Herds purchasing gilts from more than one herd comprised herds that bought breeding pigs from multiple sources or from one source per purchase but that used more than one source. A compartment was defined as a subdivision of a building with its own ventilation system. All-in/all-out (AIAO) was considered to take place if the compartment (or the unit) was filled up the same day, was sold in one

Table II. Potential risk indicators for respiratory disease on farrow-to-finish pig herds. Descriptive data of continuous variables.

Potential risk indicator	N ^a	Min.	1° Quartile	Median	3° Quartile	Max.
Herd size	150	50	100	138	201	600
Pig density in the municipalities ($n = 56$)						
number of pigs per km ²	150	72	699	953	2 073	3 582
number of pig herds per km ²	150	0.5	1.8	2.3	3.3	4.7
PASD ^b in the finishing unit (m ² per pig)	150	0.43	0.66	0.69	0.75	1.50
ASSD ^c in the finishing unit (m ³ per pig)	150	1.20	2.20	2.50	2.86	5.00
Number of pigs per compartment in the finishing unit	147	75	120	280	400	1 000
Number of pigs per pen in the finishing unit	147	5.0	12.0	13.0	15.0	23.0
Number of compartments in the finishing unit	150	1.00	1.00	1.00	4.00	16.00

^a Number of herds for which data were available.

^b PASD: pen area stocking density.

^c ASSD: airspace stocking density.

Table III. Potential risk indicators for respiratory disease on farrow-to-finish pig herds. Descriptive data of categorical variables.

Potential risk indicators	N ^a	Categories (% of herds in each class)
GENERAL FACTORS		
Month of slaughter	150	January-February (19), March-April (16), May-June (11), July-August (14), September-October (21), November-December (19)
FACTORS RELATED TO THE SOWS		
Breed	150	pure-bred (30), cross-bred (33), both (37)
Purchase of gilts	150	yes (46), no (54)
Number of herds from which gilts are purchased	150	none (54), one (17), ≥ 2 (29)
Percent of gilts purchased	150	0% (54), 1-60% (20), >60% (26)
ADV vaccination scheme	146	at the end of pregnancy (3), all sows simultaneously (85), other schemes (12)
ADV vaccine	148	inactivated (69), attenuated (31)
Type of ADV vaccine strain	144	Bartha (34), Phylaxia (9), NIA3 (57)
FACTORS RELATED TO THE GROWING/FINISHING PIGS		
All-in/all-out policy	150	not practised (63), in the nursery unit only (24), in all stages of production (13)
Presence of growing unit	150	yes (59), no (41)
Ventilation system in finishing unit	150	natural, direct air-entry (46), mechanical, direct air-entry (21), mechanical, indirect air-entry (33)
Compartmentalisation	150	in all units (31), in nursery unit only (45), not practised (24)
Type of floor in finishing unit	150	fully slatted (8), partially slatted (92)
Preventive medication in the growing/ finishing unit	150	yes (11), no (89)
Deworming	150	once during lifetime (74), more than once during lifetime (26)
ADV vaccination scheme	148	single (86), double (14)
ADV vaccine strain	141	Bartha (67), Alfort (15), Begonia (18)
Porcine influenza vaccination scheme	149	not practised (77), single (16), double (7)
Hygiene within the unit	150	good (11), moderate (21), bad (68)
Hygiene outside the unit	149	good (16), moderate (68), bad (16)

^a Number of herds for which data were available.

or two times, and when a complete depopulation had taken place prior to restocking. Preventive medication of healthy pigs implied routine use of broad-spectrum antibiotics during 1 to 4 weeks in the feed or in the drinking water against respiratory or enteric diseases. Hygiene measures within the unit referred to cleansing followed by disinfection or a stand-empty period of minimum three days between two

production groups. Hygiene measures outside the unit referred to biosecurity measures, that is prevention of disease entry by lorries carrying pigs, feed, or manure, presence of a sanitary room, use of unit boots, clothing, and protective head-gear. The hygiene within and outside the herds was considered to be good, moderate or bad when these hygiene measures were always, sometimes or never practised.

2.4. Slaughterhouse inspection

The FP were slaughtered at the age of approximately 6 months when they reached 105-110 kg liveweight. Slaughter took place within three weeks after the lead author visited the herd. From the 60 to 150 pigs sent to the slaughterhouse, 25 pigs per herd were blood sampled. Samples were taken at fixed intervals, starting with the first pig. The interval was determined by dividing the total number of FP delivered by 25. The systematic sampling scheme was not considered to have biased the serological data of the batch that was sent to slaughter. Although it cannot be ruled out that cluster effects may have occurred within a herd by investigating only one group of pigs per herd, we insisted that the herd owner send a representative group of pigs to slaughter, that is a group without many poor performers or good performers.

2.5. Serological testing

Antibodies were measured on 25 (Mh, ADV) or 10 (both influenza viruses) blood samples per herd with commonly used serological tests. The 10 samples used to detect antibodies against both influenza viruses were randomly selected from the 25 blood samples.

The presence of antibodies against Mh was determined with the DAKO[®] Mh ELISA (DAKO, Glostrup, Denmark) [14]. The DAKO[®] Mh ELISA detects antibodies to a Mh specific epitope localised on a 74-kDa protein. Sera with OD-values < 50% and $\geq 65\%$ of the OD_{buffer control} were considered positive or negative, respectively. Intermediate OD-values were considered doubtful and classified as negative in the statistical analyses. A standard haemagglutination-inhibition test (HI-test) was used to detect antibodies against influenza H₁N₁ and H₃N₂ viruses [33]. HI-titers were expressed as the inverse of the highest serum dilution that yielded complete inhibition of

haemagglutination activity. HI-titers ≥ 4 and ≥ 20 were considered positive for influenza H₁N₁ and H₃N₂ viruses, respectively. Only sera of herds where FP were not vaccinated against influenza were analysed (n = 115). The presence of anti-gE-antibodies against ADV wild virus was determined with the Ingelvac[®]Aujeszky ELISA (Svanova Diagnostics, Uppsala, Sweden) [3]. This test allows differentiation of a vaccinated pig from an infected pig. Sera with OD-values < 45% and $\geq 55\%$ of the OD_{negative control} were considered positive or negative, respectively. Intermediate OD-values were considered doubtful and classified as negative in the statistical analyses.

The 4 serological tests have a high specificity and sensitivity [3, 33, 35]. None of these tests discriminated between different isotype classes of immunoglobulins. Antibody titres raised against the four pathogens during the fattening period, that is in pigs from approximately 3 months until 6-7 months, were considered to be detectable until slaughter age.

2.6. Statistical analyses

The 95% confidence intervals of the within-herd seroprevalences were calculated as described by Wayne [45]. Associations between Mh, influenza H₁N₁ and H₃N₂, and ADV seroprevalences were tested using logistic regression analysis (SAS 6.12, GLIMMIX, 1997) [37]. The herd was included as a random effect [24]. Associations between the seroprevalences of two different agents were expressed by the odds ratio (OR) and they were analysed in two directions, with either agent as the dependent and independent variable.

Logistic regression analysis (SAS 6.12, PROC GENMOD, 1997) [37] was also used to assess the associations, at the herd-level, between the proportion of seropositive pigs (dependent variable) and potential risk indicators (independent variables). Overdispersion due to the non-independence between

pigs of the same herd [29] was taken into account by including a scale parameter in the logistic model [10]. A forward stepwise procedure was used to select the variables which were significantly ($P < 0.05$; two-sided) associated with the different seroprevalences [32]. At each step, the variable with the lowest P -value was added to the model. This procedure was continued until no additional variables were significant. If any design variable of a categorical variable was significant, then any other associated design variables were retained in the model. First-order interaction terms between significant independent variables were also tested in the intermediate and final models, and included if they were significant ($P < 0.05$; two-sided). Squared terms of significant continuous variables were analysed in order to assess the assumption of linearity in the logit of the seroprevalence within the range of observed values [20]. In order to assess the goodness of fit of the final models [30], root mean squared errors (RMSE) were calculated according to the following

$$\text{formula: } \sqrt{\frac{1}{n} \sum (P_{\text{observed}} - P_{\text{fitted}})^2} \text{ with } n$$

denoting the number of herds, and P denoting the herd seroprevalence. Potential values for RMSE range between 0% and 100% with these endpoints corresponding to perfect predictability or complete lack of predictability by the model, respectively. ORs

and 95% confidence intervals were calculated from the final logistic regression models.

3. RESULTS

The within-herd seroprevalences and 95% confidence intervals are presented in Table IV. The median within-herd seroprevalences were 76% for Mh, 100% for H_1N_1 , 40% for H_3N_2 , and 53% for ADV. The mean seroprevalences of Mh in the bi-monthly periods were: J-F 68, M-A 82, M-J 75, J-A 68, S-O 72, N-D 68. The seasonal variation for both influenza viruses was not significantly different between the bi-monthly periods. The mean seroprevalences of ADV in the bi-monthly periods were: J-F 40, M-A 71, M-J 44, J-A 36, S-O 47, N-D 57. The minimum and maximum within-herd seroprevalences for each pathogen were 0% and 100%, respectively. The distributions of within-herd seroprevalences of Mh, H_1N_1 and H_3N_2 , and ADV are shown in Figure 1. The percentages of herds in which no seropositive pigs could be detected were: 1% for Mh, 4% for H_1N_1 , 20% for H_3N_2 , and 24% for ADV. The percentages of herds in which all investigated pigs were seropositive for the different pathogens were: Mh 7%, H_1N_1 77%, H_3N_2 32%, and ADV 31%. The distribution of the within-herd seroprevalences of Mh was

Table IV. Within-herd seroprevalences of *Mycoplasma hyopneumoniae* (Mh), influenza H_1N_1 and H_3N_2 viruses, and Aujeszky disease virus (ADV) in slaughter pigs from 150 farrow-to-finish pig herds.

	Within-herd seroprevalences			
	Mh	H_1N_1	H_3N_2	ADV
Minimum	0 [0-12] ^a	0 [0-27]	0 [0-27]	0 [0-12]
Median	76 [60-89]	100 [73-100]	40 [15-73]	53 [32-70]
Maximum	100 [88-100]	100 [73-100]	100 [73-100]	100 [88-100]

^a Between brackets: 95% confidence intervals.

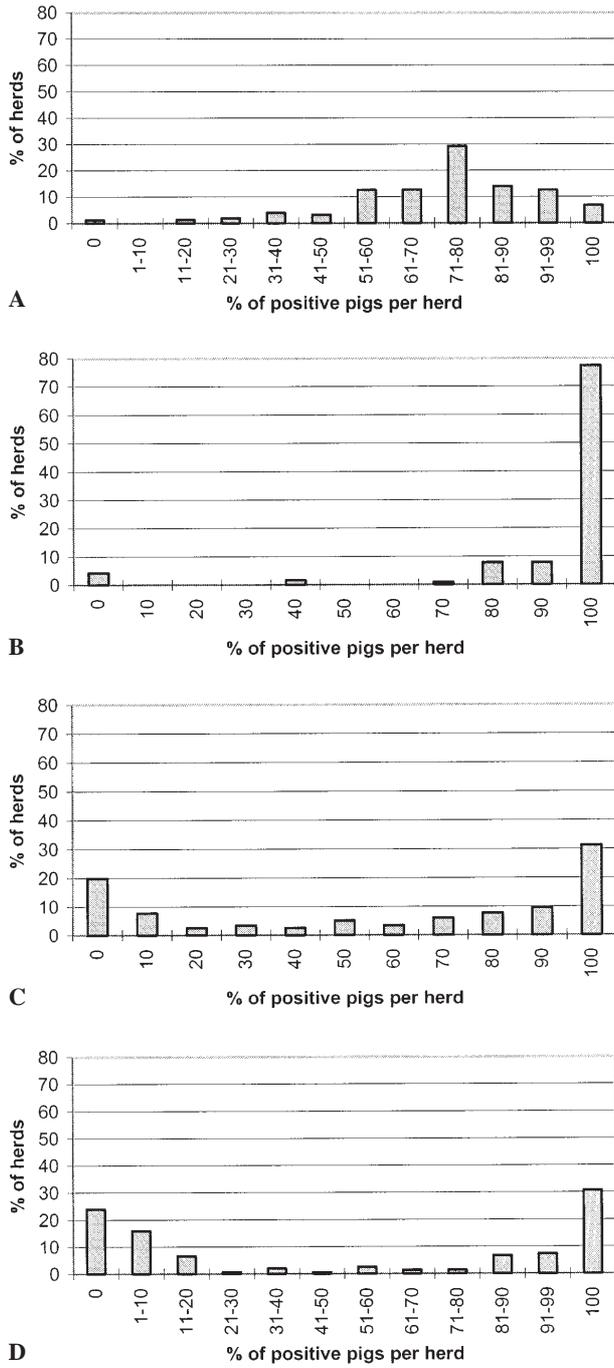


Figure 1. The distribution of the within-herd seroprevalence of *Mycoplasma hyopneumoniae* (A), influenza H₁N₁ virus (B), influenza H₃N₂ virus (C), and Aujeszky disease virus (D).

skewed with a larger tail towards the lower seroprevalences, whereas the within-herd seroprevalences of the influenza H₁N₁ virus showed less variation because almost all FP were seropositive. The distribution of the within-herd seroprevalences of ADV, and to a lesser extent of the influenza H₃N₂ virus, showed a bimodal distribution with only 13% and 21% of the herds in the range of 20 to 80% seroprevalence, respectively.

Influenza H₃N₂ virus seropositive pigs had a 2.29 times higher risk ($P < 0.05$) to be seropositive for the influenza H₁N₁ virus than those that were H₃N₂ seronegative. Conversely, influenza H₁N₁ virus seropositive pigs had a 2.06 times higher risk (NS) to be seropositive for the H₃N₂ virus. ADV seropositive pigs had a lower risk (OR = 0.35; $P < 0.05$) to be seropositive for the influenza H₁N₁ virus and vice versa (OR = 0.30; $P < 0.05$). The other associations between seroprevalences were not significant.

The risk indicators associated with the proportion of pigs seropositive for Mh, influenza H₁N₁ and H₃N₂ viruses, and ADV are listed in Table V. The seroprevalence of Mh increased with the purchase of gilts, and with slaughter dates in March-April. The seroprevalence of the influenza H₁N₁ virus was higher with higher densities of pig herds in the municipality. The percentage of pigs seropositive for the influenza H₃N₂ virus was higher with increasing numbers of pig herds in the municipality and with increasing numbers of FP per pen. The proportion of ADV seropositive pigs increased with higher airspace stocking densities in the finishing units, higher herd sizes, more pig herds in the municipality, and with slaughter dates in March-April.

The interaction terms and the squared terms of the continuous independent variables in the intermediate and final models were not significant. RMSE values for the final models were 19% for Mh, 38% for the influenza H₁N₁ and H₃N₂ viruses, and 37% for ADV.

4. DISCUSSION

The herds involved in this study are representative of the population of FTF pig herds with over 50 sows because they were selected at random within the selected region, and because the percentage of non-responders (13%) was fairly low [43]. The different number of blood samples for the respective pathogens that is 25 for Mh and ADV and 10 for both influenza viruses, was partly determined by practical and budget considerations. These numbers permitted to detect at least one seropositive pig at the 95% confidence level from a group of 150 pigs for minimum seroprevalences of 11% and 25%, respectively. Martin et al. [28] and Carpenter and Gardner [7] have illustrated some features that should be considered in interpreting individual results of an imperfect test performed on a sample from a herd. The latter authors showed that increasing the number of pigs tested per herd, results in increases in herd sensitivity, especially for seroprevalences below 80%. For seroprevalences more than 80%, increasing the number of pigs tested per herd results in decreases in herd sensitivity, especially when the sensitivity of the test is $< 90\%$. Applied to the seroprevalences of both influenza viruses in our study, increasing the number of tested pigs per herd would have resulted in an increase in herd sensitivity for the influenza H₃N₂ virus, but would have resulted in a slight decrease in herd sensitivity for the influenza H₁N₁ virus.

The positive association between the seroprevalences of influenza H₁N₁ and H₃N₂ viruses corroborates with the results of Elbers et al. [11]. It is not possible to draw conclusions about whether the positive association between both influenza viruses results from similar management practices or environmental conditions promoting the spread of both pathogens, or whether infection with one virus predisposes to infection with the other virus. Evidence for the latter hypothesis, however, is not available. Moreover, from combined experimental infec-

Table V. Risk indicators for the seroprevalences of *Mycoplasma hyopneumoniae*, influenza H₁N₁ and H₃N₂ viruses, and Aujeszky disease virus in slaughter pigs from 150 farrow-to-finish pig herds: parameters in the final logistic regression models, coefficients with standard errors, *P*-values and odds ratio with 95% confidence intervals (CI).

Final model for <i>Mycoplasma hyopneumoniae</i> (deviance 739; RMSE 19%)			
Parameters	Coefficients ± standard error	<i>P</i> -value	Odds ratio [95% CI]
intercept	0.5830 ± 0.1041	< 0.001	
purchase of gilts	0.6656 ± 0.1626	< 0.001	1.95 [1.41–2.68]
slaughter date in March-April	0.6080 ± 0.2442	0.013	1.84 [1.14–2.96]
scale parameter	2.1375 ± 0.0000	–	
Final model for the H ₁ N ₁ virus (deviance 419; RMSE 38%)			
Parameters	Coefficients ± standard error	<i>P</i> -value	Odds ratio [95% CI]
intercept	0.9973 ± 0.6105	0.102	–
number of pig herds in the municipality	0.6830 ± 0.2975	0.022	1.98 ^a [1.11–3.55]
scale parameter	2.3161 ± 0.0000	–	–
Final model for the H ₃ N ₂ virus (deviance 879; RMSE 38%)			
Parameters	Coefficients ± standard error	<i>P</i> -value	Odds ratio [95% CI]
intercept	–2.9361 ± 1.1135	0.008	
number of pig herds in the municipality	0.3800 ± 0.1614	0.019	1.46 ^a [1.07–2.01]
number of fattening pigs per pen	0.1844 ± 0.0791	0.020	1.20 ^b [1.03–1.40]
scale parameter	2.5633 ± 0.0000	–	–
Final model for Aujeszky disease virus (deviance 2914; RMSE 37%)			
Parameters	Coefficients ± standard error	<i>P</i> -value	Odds ratio [95% CI]
intercept	0.5601 ± 0.9542	0.5572	
airspace stocking density in the fattening unit	–1.3050 ± 0.3514	< 0.001	0.52 ^c [0.37–0.73]
herd size	0.0064 ± 0.0018	< 0.001	1.38 ^d [1.15–1.64]
number of pig herds in the municipality	0.6029 ± 0.1568	< 0.001	1.83 ^a [1.34–2.48]
slaughter date in March-April	1.1970 ± 0.4435	0.007	3.31 [1.39–7.90]
scale parameter	4.1865 ± 0.0000	–	–

^a increase of one herd per km².

^b increase of one pig per pen.

^c increase of 0.5 m³.

^d increase of 50 sows.

tions with the porcine respiratory coronavirus (PRCV) and H₁N₁ virus, it appeared that PRCV causes an interfering rather than a synergistic effect against the replication of H₁N₁ [44]. The slightly different *P*-values

and ORs between H₁N₁ and H₃N₂ (2.29 and 2.06) in the present study are caused by different variations in herd-seroprevalences with respect to both influenza viruses. In contrast to our results, Elbers et al. [11]

found a slightly positive association (OR=1.2) between the seroprevalences of H₁N₁ virus and ADV. The study population of Elbers et al. [11] consisted of pig-finishing herds whereas in the present study, only FTF pig herds were included. The significant negative association between the seroprevalences of both viruses observed in our study may also be explained by the fact that HI-antibodies were not examined in herds (23%) where influenza vaccination was practised in the growing/finishing pigs. This procedure was preferred because the HI-test is not able to differentiate between infection and vaccination titres. Infections with ADV were more common in herds that vaccinated against influenza, there was a median within-herd seroprevalence of 74% versus 28% in herds that did not vaccinate against influenza, and it can be expected that infections with influenza viruses also occurred more frequently in these herds. By excluding these herds, a possible positive association between the seroprevalences of the H₁N₁ virus and ADV may have been missed.

Jorsal and Thomsen [22] found that the risk for reinfection of SPF herds with Mh was significantly increased by the number of herds from which pigs are purchased and not by the frequency of purchase. Other investigators [1, 48] demonstrated a positive association between purchase of growing pigs and lung lesions. Since the present study was conducted in FTF pig herds, the effect of purchasing gilts and not of growing pigs was investigated. The variables used to measure the purchase of gilts were highly correlated (data not shown) and all of them were, individually taken, significantly associated with the seroprevalence of Mh. Purchase of gilts expressed as a yes/no variable was the most significant risk indicator. Various hypotheses can be raised to explain the positive association between purchase of gilts and the increased proportion of Mh seropositive slaughter pigs. Gilts often harbour Mh organisms in the respiratory tract. Transport and changes in management and

housing conditions may decrease immunity in purchased gilts, and may lead to excretion of higher numbers of Mh when compared to gilts derived from own on-farm selection. Purchased gilts may also be responsible for the introduction of new, and possibly more pathogenic Mh strains [8] in the herd. Pigs slaughtered in March and April were at higher risk to be Mh seropositive compared to pigs slaughtered in other months. Although it cannot be ruled out that the worst herds were selected in March and April, the higher risk to be seropositive at slaughter in this period is probably due to the cold and wet weather during the winter [16]. Such conditions can promote the spread of Mh within and between herds. Furthermore, ventilation may be decreased during this period to maintain an optimal temperature in the units. Previous studies showed that the prevalence and severity of lung lesions [40], and the reinfection rate of SPF pig herds with Mh [38] were higher during the winter period.

Ewald et al. [13] found that a high regional pig density was a risk factor for herds to become infected with influenza H₁N₁ and H₃N₂ viruses. Similarly, we found that the proportion of seropositive pigs per herd increased with pig density in the municipality. A higher number of pigs or pig herds in the municipality may facilitate airborne transmission between herds, and may lead to increased contacts between herds [25]. An increase in the number of FP per pen was associated with a higher risk to be seropositive for the influenza H₃N₂ virus. This factor allows more opportunities for direct nose-to-nose contact or for aerosol spread of the virus between penmates. Furthermore, a large number of pigs per pen creates physiological stress, which in turn can alter the immune system and predispose pigs to infection.

Decreased airspace stocking densities in the finishing unit were associated with a decreased risk to be gE positive for ADV. An increased air volume per pig results in a lower concentration of infectious particles,

including ADV. This lower infection pressure leads to a decreased pig-to-pig transmission of ADV within the unit. Although the range in herd sizes (50-600 sows) was fairly small compared to other studies [18, 46], we did find a positive association between herd size and ADV seroprevalence. This is consistent with results obtained by Anderson et al. [2], Morrison et al. [31] and Christensen et al. [9], but it is in contrast with studies conducted by Weigel et al. [46]. ADV is introduced more often in large herds because they usually have more frequent contacts outside the herd than small herds. Furthermore, ADV may circulate persistently in a large herd because susceptible pigs are usually present, whereas the infection may fade out in small herds. Stegeman et al. [39] reported less ADV transmission in herds with 100 or more sows than in herds with less than 100 sows, but the authors ascribed the lower ADV seroprevalence in larger herds to confounding effects of better management procedures in these herds. The proportion of ADV seropositive pigs in the present study was higher in herds located in municipalities with a high pig herd density. This corroborates with results of previous studies [4, 27, 46]. Transmission experiments of ADV showed that a higher density of pigs increases ADV spread because of the higher number of contacts between the pigs [6]. In a pig-dense region, the contacts by area spread increase and as a result, ADV may circulate more easily. The type of transmission modes by which ADV was transferred between the herds, however, cannot be elucidated from the present study. Pigs slaughtered in March and April had the highest risk to be seropositive against ADV. The higher risk in March and April can be attributed to the same factors as those mentioned above for Mh. These factors promote survival of the virus within a herd and transmission of the virus between herds [5]. The prevalence of anti-gE antibodies was fairly high. This may be due to the fact that vaccination against ADV became mandatory in Belgium (Flanders)

only in 1993, that is approximately two years before the present study.

Within each independent categorical variable, two or more biological relevant categories have been made (Tab. III). For a limited number of independent categorical variables namely ADV vaccination scheme, type of floor in the finishing unit and porcine influenza vaccination scheme, the frequency rate was less than 10% in one of the categories. Although no trend could be observed between the different categories of these variables, this situation may have led to non-significant results.

The spread of the four pathogens was investigated by assessing the proportion of seropositive pigs in each herd. Serological testing has the characteristic that clinical as well as subclinical infections are detected. Consequently, the risk indicators found in this study are probably different from factors influencing the severity of clinical respiratory symptoms. Success at preventing disease occurrence may be greater by concentrating on occurrence and spread of infections, rather than focusing solely on clinical disease. Elbers et al. [11] demonstrated that infections with both influenza viruses and ADV are also economically important since pigs seropositive with respect to these agents showed a significant decrease in daily weight gain compared to seronegative pigs. In addition, Maes et al. [26] found that pig herds subclinically infected with EP achieved a significantly higher daily weight gain through vaccination against Mh. The percentage of seropositive pigs within the herds may be influenced by the spread of infections within and between herds.

Finally, some comments should be made with regard to the use of a cross-sectional study design and the causality of the risk indicators. First, the study took one year but only one group of pigs from each herd was investigated in the slaughterhouse. Consequently, within each herd, it was only possible to trace infections during a fairly short

period namely those that occurred approximately 4–5 months prior to slaughter. In addition, because cross-sectional studies measure events at a particular time, they suffer from the fact that cause and effect are sometimes difficult to separate. The risk indicators found in this study however, essentially fulfilled the criteria which are used to transpose observed associations into causal relations [41]. Second, unmeasured variables associated with the seroprevalences of the investigated pathogens may be potent sources of confounding [43]. In order to minimise a possible background noise mediated by such factors, a large number of variables known to be associated with respiratory disease were included in the analyses. The RMSE values of the final logistic models were fairly low (Tab. V). This corresponds to a fairly high to moderate predictability of the final models [30].

In conclusion, the present study pointed out for the first time that purchase of gilts, a high number of pigs per pen, and a high airspace stocking density in the fattening unit increased the risk for pigs to become seropositive for Mh, H₃N₂ and ADV, respectively. The results confirmed that the winter period, a high regional density of pig herds, and a large herd size constitute important risk factors for the spread of respiratory pathogens. Purchase of gilts, a high number of pigs per pen, and a high airspace stocking density are risk indicators that can be managed directly by farmers striving to attain or to sustain a high health status of the pigs. Density of pig herds in the municipality and herd size are less manageable by the farmers; these factors should be considered by the government in search for optimal control measures against these pathogens.

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