

Prevalence of Antibodies to Swine Influenza Virus, Porcine Adenovirus Type 4 and *Haemophilus pleuropneumoniae* in Quebec Pig Farms with Respiratory Problems

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ABSTRACT

The prevalence of antibodies to three etiological agents involved in swine pneumonia was determined in Quebec pig farms which had experienced problems of mild to severe respiratory problems. Of the 350 sera collected from adult pigs, 67.0%, 18.3% and 46.6% had antibody titers to *Haemophilus pleuropneumoniae* type 1 and/or 5, to porcine adenovirus type 4 and to swine influenza virus, respectively. Comparatively, the serological prevalence rates for *Haemophilus pleuropneumoniae*, porcine adenovirus and swine influenza virus were 43.7%, 0% and 3.3% in farms not experiencing respiratory problems.

Key words: Antibody prevalence, *Haemophilus pleuropneumoniae*, adenovirus, influenza, swine.

RÉSUMÉ

Prévalence d'anticorps contre le virus de l'influenza du porc, l'adénovirus porcin du type #4 et *Haemophilus pleuropneumoniae*, au sein de troupeaux du Québec aux prises avec des problèmes respiratoires

Cette étude visait à déterminer la prévalence d'anticorps à l'endroit de trois agents étiologiques impliqués dans la pneumonie porcine, au sein de troupeaux du Québec qui avaient éprouvé des problèmes dus à une maladie respiratoire bénigne ou plus grave. Des 350 échantillons de sérum prélevés chez des sujets adultes, 67% possédaient des anticorps contre *Haemophilus pleuropneumoniae* des types #1 et/ou #5; 18,3%, contre l'adénovirus

porcin du type #4; 46,6%, contre le virus de l'influenza du porc. Par ailleurs, les taux de prévalence contre les trois agents précités s'établissaient respectivement à 43,7%, 0% et 3,3%, dans les troupeaux exempts de troubles respiratoires.

Mots clés: prévalence sérologique, *Haemophilus pleuropneumoniae*, adénovirus, influenza, porcs.

INTRODUCTION

During the last five years, respiratory problems have been reported frequently in Quebec pig farms. The incidence of pneumonia caused by *Haemophilus pleuropneumoniae* appears to be increasing throughout the country (1-5) and swine influenza is still a common infection in Quebec and Ontario (6,7).

In its acute form, swine influenza is characterized by the rapid onset of marked respiratory distress, usually affecting the entire herd, accompanied by paroxysmal coughing, fever, anorexia, prostration and abrupt recovery after five to seven days. Mortality is usually less than 1% (6,7,8). Pneumonia caused by *Haemophilus pleuropneumoniae* is usually more drastic. The onset is sudden and the affected pigs show a more severe respiratory distress, cyanosis and have difficulty moving. Frequently, a blood-stained frothy discharge from the nose and mouth is present. It affects predominantly growing pigs of two to six months of age with rapid spread within the herd. There is a high mor-

bidity and the case fatality rate may approach 25% (1,2).

A porcine adenovirus, identified as serotype 4, has been isolated from the lungs and other tissues of swine with respiratory problems in European countries (10,11,12). Experimental infections have demonstrated that this virus produced lesions in several organs including lungs, kidney and brain (10,11). A pneumonia of greater severity has been reproduced by simultaneous inoculation with *Mycoplasma hyopneumoniae* (13). High serological prevalences have been reported for adenovirus in England and Germany (13,14,15). Based on histopathological findings, its presence has been suggested in Saskatchewan in 1971 during an outbreak of fibrinous pleuropneumonia associated with *Haemophilus* (2).

The purpose of the present study was to determine the serological prevalences of these three agents in Quebec pig farms which had experienced or not experienced respiratory problems last two years.

MATERIALS AND METHODS

Serum Samples

Feeder swine sera were obtained from samples submitted to the Veterinary Diagnostic Laboratory, University of Montreal, for *Haemophilus pleuropneumoniae* testing during 1982-1983. Only sera from farms located less than 150 km from Saint-Hyacinthe, Quebec, were considered for this study. The sera were then randomly selected for testing in close pro-

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portion to the number of feeder swine on the farms. Of approximately 4,000 samples submitted, 350 samples from 11 farms with known respiratory problems were tested. As controls, sera were collected from three farms that had not experienced respiratory problems in 1983.

Serological Tests

Antibodies to porcine adenovirus type 4 (PAV4) were titrated by the indirect fluorescent antibody technique using continuous porcine kidney cell cultures (PK-15) infected with the respective virus and performed as described previously (16,17). Reciprocal titers of ≤ 20 were considered as negative.

An isolate of porcine influenza virus antigenically similar to the A/Swine/Wisconsin/49/76 strain (kindly supplied by Dr. DiFranco, Institut Armand Frappier, Laval-des-Rapides, Quebec) was used for this study. This virus was inoculated in 0.2 mL volumes into the allantoic cavity of 9 to 11 day-old embryonated eggs. After incubation at 37°C for three to four days, allantoic fluids were harvested and blind passaged up to three times. Microtiter hemagglutination (HA) tests were conducted on allantoic fluids collected from eggs which had died from the infection. For this purpose,

serial twofold dilutions of the allantoic fluid done in phosphate buffered saline (PBS) pH 7.2 and 0.5% chicken red blood cells suspension were employed. Antibodies to the virus were determined by the hemagglutination-inhibition (HI) technique after the sera were treated with kaolin to remove nonspecific inhibitors (7). Twofold dilutions of the sera were made in PBS and four HA units of the virus were employed. Reciprocal titers of < 20 were considered as negative.

The antibodies to *Haemophilus pleuropneumoniae* were detected using the 2-mercaptoethanol tube agglutination test (18). Reference strains of serotypes 1 and 5 (kindly supplied by Dr. Gunnarsson, National Veterinary Institute, Uppsala, Sweden) were used for the antigen preparations. These organisms were grown in 10% CO₂ atmosphere on chocolate blood agar supplemented with Isovitalax (BBL Microbiology Systems, Mississauga, Ontario).

RESULTS

The results of this serological survey are reported in Table I. Of the 350 sera selected from the pig farms experiencing respiratory problems, 67.0% (235/350) had antibodies to at least one of the three agents studied. Overall, 46.8% (266/350), 18.3% (64/350) and

46.6% (161/350) were positive for *Haemophilus pleuropneumoniae* type 1 and/or 5, for porcine adenovirus type 4 and for swine influenza virus, respectively. Prevalence rates higher than 40% were observed for antibodies to swine influenza virus in 9 of the 11 farms tested. Only one of these farms (no. 2) was negative for antibody to porcine adenovirus type 4 and serological prevalence rates higher than 30% were noted in three farms (no. 7, 8 and 11). The prevalence rates for *Haemophilus pleuropneumoniae* varied from 0% (farm no. 4) to higher than 80% (farms no. 5, 7, 8 and 10). Nine farms had serological prevalence rates higher than 50% for this agent.

In all three farms without respiratory problems, the serological prevalence rates for *Haemophilus pleuropneumoniae* were higher than 40% and all the sera tested were negative for porcine adenovirus type 4 antibodies. Overall, only one of the thirty sera tested had a significant titer ($> 1/20$) for swine influenza virus.

The majority of the sera tested from those herds experiencing respiratory problems carried antibodies to more than one of the agents studied. Although all the animals in farms no. 3 and 4 were affected with a respiratory disease (morbidity of 100%), only 20.5% (10/49) and 18.0% (2/11) were

TABLE I
PREVALENCE OF ANTIBODIES TO *HAEMOPHILUS PLEUROPNEUMONIAE*, PORCINE ADENOVIRUS AND SWINE INFLUENZA VIRUS IN QUEBEC PIG FARMS WITH OR WITHOUT RESPIRATORY PROBLEMS

	Farm No.	Number of Animals	Animals with Antibody (%)							Pneumonia in Farm	
			<i>Haemophilus</i>	Influenza	Adenovirus	Influenza <i>Haemophilus</i>	Influenza Adenovirus	<i>Haemophilus</i> Adenovirus	<i>Haemophilus</i> Influenza	% morbidity	% mortality
Farms with respiratory problems	1	60	76.7	40.0	26.7	25.0	0.0	11.7	15.0	40.0	10.0
	2	18	66.7	55.6	0.0	55.6	0.0	0.0	0.0	45.0	20.0
	3	49	12.3	8.2	4.1	4.1	0.0	0.0	0.0	100.0	4.0
	4	11	0.0	0.9	0.9	0.0	0.0	0.0	0.0	100.0	8.0
	5	25	96.0	84.0	24.0	60.0	0.0	0.0	24.0	23.0	8.0
	6	32	71.9	56.3	18.8	37.5	0.0	0.0	18.8	100.0	2.5
	7	18	100.0	44.5	38.9	27.8	0.0	22.2	16.7	50.0	20.0
	8	36	91.6	66.7	33.3	41.7	0.0	11.1	22.2	100.0	10.0
	9	57	56.2	45.7	5.3	40.4	0.0	3.5	1.8	100.0	15.0
	10	24	91.6	70.8	12.5	58.3	0.0	0.0	12.5	100.0	20.0
	11	20	50.0	40.0	40.0	5.0	0.0	5.0	35.0	50.0	7.5
All	350	64.8	46.6	18.3	32.3	0.0	4.9	14.4	73.4	11.2	
Farms without respiratory problems	12	10	50.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	13	10	40.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	14	10	40.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	All	30	43.7	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0

seropositive for one or more of the three agents studied, suggesting other possible etiologies.

To evaluate the relationship between prevalence of antibodies and severity of respiratory disease, the herd prevalence rates of antibodies to each specific agent were regressed against the mortality rates for the farms with respiratory problems. Regression of the prevalence rates of adenovirus antibodies against mortality resulted in a regression equation with a negative slope, whereas those for *Haemophilus pleuropneumoniae* and influenza virus were positive. However, none of the slope coefficients were statistically significant and the correlation coefficients for all three associations were less than 0.5.

DISCUSSION

The results of this serological study agree with previous studies which have demonstrated that pigs in Canada are highly exposed to *Haemophilus pleuropneumoniae* (1-5). Furthermore, epizootics of swine influenza have been reported recently in Quebec and Ontario (6,7). The high serological prevalence rates observed in this study suggest that this viral agent is still very common in pig herds in the St-Hyacinthe area.

To our knowledge, only circumstantial evidence of a porcine adenovirus associated with respiratory problems has been reported in Canada (2) and the virus has not yet been isolated. The demonstration of antibodies to the porcine adenovirus type 4 in the sera of the sick pigs tested in this study gives strong evidence as to its presence in Quebec pig herds. The overall serological prevalence was found to be considerably less than rates reported in England and Germany (13,14,15). The known pathogenicity of this virus for the respiratory system (10,11,13) suggests that it may have been implicated alone or in mixed infection with *Haemophilus pleuropneumoniae*, swine influenza virus, or with other possible etiological agents, in several cases of pneumonia in Quebec pig farms.

Except for pneumonia attributable solely to *Haemophilus pleuropneumoniae*, all pig pneumonias are probably of complex etiology including

viruses, mycoplasmas, *Pasteurella*, *Corynebacterium*, streptococci and other possible secondary agents. It is also well recognized that immune status and environmental conditions also greatly affect the severity of pneumonia in pigs. In the present study, all pig sera tested were selected from farms in the area of St-Hyacinthe and environmental conditions were quite similar on all farms. The majority of the sera collected in farms experiencing respiratory problems were positive for antibodies to at least two of the agents studied. With the exception of one influenza virus positive serum, only antibodies for *Haemophilus pleuropneumoniae* were detected from sera collected in farms without clinically evident respiratory disorders. The prevalence rates for *Haemophilus pleuropneumoniae* were quite similar in both groups of farms. Thus, it seems that the morbidity and mortality from respiratory disease were more likely to occur when a previous or subsequent infection with influenza virus or adenovirus was associated to the infection with *Haemophilus pleuropneumoniae*. Relationship between herd prevalence rates for the three organisms and the herd mortality rates could not be demonstrated statistically in the present study. Further studies are needed to understand the interactions between *Haemophilus pleuropneumoniae* and those viral agents.

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REFERENCES

1. SANFORD SE, JOSEPHSON GKA. Porcine *Haemophilus pleuropneumoniae* epizootic in Southwestern Ontario: clinical, microbiological, pathological and some epidemiological findings. *Can J Comp Med* 1981; 45: 2-7.
2. SCHIEFER B, MOFFAT RE, GREENFIELD J, AGAR L, MAJKA JA. Porcine *Haemophilus parahemolyticus* pneumonia in Saskatchewan I. Natural occurrence and findings. *Can J Comp Med* 1974; 38: 99-104.
3. LOMBIN LH, ROSENDAL S, MITCHELL WR. Evaluation of the complement fixation test for the diagnosis of pleuropneumonia of swine caused by *Haemophilus pleuropneumoniae*. *Can J Comp Med* 1982; 46: 109-114.
4. GREENWAY JA. *Haemophilus pneumoniae* in B.C. swine. *Can Vet J* 1981; 22: 20-21.
5. MITTAL KR, HIGGINS R, LARIVIÈRE S. Evaluation of slide agglutination and ring precipitation tests for capsular serotyping of *Haemophilus pleuropneumoniae*. *J. Clin Microbiol* 1982; 15: 1019-1023.
6. MORIN M, PHANEUF JB, SAUVAGEAU R, DIFRANCO E, MARSOLAIS G, BOUDREAU A. An epizootic of swine influenza in Quebec. *Can Vet J* 1981; 22: 204-205.
7. SANFORD SE, JOSEPHSON GKA, KEY DW. An epizootic of swine influenza in Ontario. *Can Vet J* 1983; 24: 167-171.
8. EASTERDAY BC. Swine influenza. In: Leman AD, Glock RD, Mengeling WL, Penny RHC, Scholl E, Straw B, eds. *Diseases of swine*, 5th ed. Ames, Iowa: The Iowa State University Press, 1981: 184-194.
9. GAGNON AN, BOUDREAU A, MARSOLAIS G, LUSSIER G, MAROIS P. Maladies porcines à étiologie virale dans la province de Québec. I. Influenza. *Can Vet J* 1974; 15: 312-315.
10. KASZA L. Isolation of an adenovirus from the brain of a pig. *Am J Vet Res* 1966; 27: 751-758.
11. SHADDUCK JA, KOESTNER A, KASZA L. The lesions of porcine adenoviral infection in germ-free and pathogen-free pigs. *Pathol Vet* 1967; 4: 537-552.
12. RASMUSSEN PG. Porcine adenoviruses. Isolation and cytopathogenic examination of four serological types. *Acta Vet Scand* 1969; 10: 10-17.
13. KASZA L, HODGES RT, BETTS AO, TREXLER PC. Pneumonia in gnotobiotic pigs produced by simultaneous inoculation of a swine adenovirus and *Mycoplasma hyopneumoniae*. *Vet Rec* 1969; 84: 262-267.
14. DARBYSHIRE JH. Adenovirus antibodies in the sera of pigs. *Vet Rec* 1967; 81: 118-121.
15. BIBRACH B. Untersuchungen über die serologische Einordnung von 9 in Bayern aus Schweinen isolierten Adenovirusstämmen. *Zentralbl Veterinärmed* 1969; 16B: 327-334.
16. DEA S, ELAZHARY MASY. Prevalence of antibodies to porcine adenovirus in Quebec swine by indirect fluorescent antibody test. *Am J Vet Res* 1984; 45: 2109-2112.
17. ELAZHARY MASY, DEA S. Indirect fluorescent antibody test for serological diagnosis of porcine adenovirus. Abstract of the 64th Conference of Research Workers in Animal Diseases, Chicago, 1983: 50.
18. MITTAL KR, HIGGINS R, LARIVIÈRE S, LEBLANC D. A 2-mercaptoethanol tube agglutination test for diagnosis of *Haemophilus pleuropneumoniae* infection in pigs. *Am J Vet Res* 1984; 45: 715-719.
19. NIELSEN R. Serological and immunological studies of pleuropneumonia of swine caused by *Haemophilus parahemolyticus*. *Acta Vet Scand* 1974; 15: 80-89.