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An Epizootic of Swine Influenza in Ontario

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SUMMARY

A swine influenza virus (H_1N_1) was isolated for the first time in Ontario from pigs one week to one and one-half years old during an epizootic which occurred between January and May 1981. Each herd outbreak was characterized by the sudden onset of marked respiratory distress, usually affecting the entire herd, accompanied by paroxysmal coughing, anorexia, prostration and temperatures as high as 41.5°C and lasting for five to seven days. Morbidity was nearly 100%; mortality was less than 1%.

Hematology, bacteriology and postmortem studies were conducted on 18 pigs from 11 farms. A lymphopenia and acute hematological inflammatory cellular responses characterized by neutrophilia with a left shift, hyperfibrinogenemia and a decreased plasma protein: fibrinogen ratio were found in 50% of the pigs. The cranial lobes of the lung were collapsed and red due to a bilateral cranioventral pneumonia which affected the cranial, middle, accessory and cranioventral aspects of the caudal lobes. Histologically, there was a necrotizing bronchitis and bronchiolitis with a neutrophilic cellular exudate. *Pasteurella multocida* was the species of bacterium most frequently isolated from the lung; however, mixed cultures of *P. multocida* frequently combined with *Corynebacterium pyogenes* and other species were usually identified in the lung and other organs of pigs submitted dead.

RÉSUMÉ

Une épizootie d'influenza porcin, en Ontario

Les auteurs ont isolé le virus H_1N_1 de l'influenza du porc, pour la première fois, en Ontario, chez des porcs âgés d'une semaine à un an et demi, au cours d'une épizootie qui se manifesta entre les mois de janvier et mai 1981. Dans chacun des troupeaux, la maladie se caractérisa par l'apparition soudaine de difficultés respiratoires qui affectaient ordinairement tout le troupeau; elles s'accompagnaient d'une toux quinteuse, d'anorexie et de prostration, ainsi que d'une hyperthermie qui atteignit 41,5°C et dura de cinq à sept jours. La morbidité s'éleva à presque 100%, tandis que la mortalité demeura en deçà de 1%.

Les auteurs effectuèrent des épreuves hématologiques et bactériologiques, ainsi que la nécropsie de 18 porcs qui provenaient de 11 fermes. Ils constatèrent que 50% d'entre eux affichaient une lymphopénie et une neutrophilie avec virage à gauche, ainsi qu'une hyperfibrinogénémie et une baisse du rapport: protéines plasmatiques-fibrinogène. Les lobes pulmonaires caudaux étaient affaissés et rouges, à cause d'une pneumonie cranio-ventrale bilatérale qui affectait les lobes craniaux, les lobes moyen et accessoire, ainsi que la partie cranio-ventrale des lobes caudaux. L'histopathologie révéla la présence de bronchite et de bronchiolite nécrotiques, ainsi que celle d'un exsudat de neutrophiles. *Pasteurella multocida* représentait la bactérie isolée le plus souvent des poumons; on la retrouva toutefois associée à *Corynebacterium pyogenes* ou à d'autres microbes, dans les poumons et dans divers organes des porcs soumis au laboratoire après leur mort.

INTRODUCTION

Swine influenza (SI) is an acute, infectious, respiratory disease affecting all ages of swine (1,2). The disease is caused by a type A influenza virus, spreads rapidly throughout a herd and is most frequently encountered in the fall and winter months (1,2). It is characterized by the sudden onset of paroxysmal coughing, dyspnea, anorexia, prostration and fever which lasts for five to seven days. Recovery is rapid. Morbidity approaches 100% and, unless intercurrent diseases are present, mortality is negligible.

Swine influenza is enzootic in the midwestern and north central United States where mild outbreaks occur every fall (1). In Canada, there has been serological evidence of exposure of pigs to the virus in all provinces (3,4), but only one outbreak of the disease has been reported and it was limited to one premises in Manitoba in 1967 (5).

The spontaneous outbreak of SI in epizootic proportions in Ontario during the winter and spring of 1981 is reported in this paper. Concurrent with this Ontario outbreak, an SI epizootic had occurred in the neighboring province of Quebec (6), and a few cases

have been reported in Manitoba (G. Spearman, personal communication, 1981). Outbreaks were not detected in the adjoining states of New York (J.M. King, personal communication, 1981) and Michigan (R.F. Taylor, personal communication, 1981). The hematological data reported in this study are unique; hematological responses have not been previously reported in pigs with either experimental or natural swine influenza infection.

MATERIALS AND METHODS

This study was conducted on 18 pigs (13 submitted alive, five submitted dead) ranging in age from one week to one and one-half years, that were submitted to the Veterinary Services Laboratory, Ontario Ministry of Agriculture and Food, Huron Park, Ontario. The pigs came from 11 farms on dates between January 30 and May 27, 1981. Lungs from a further 12 pigs from nine other farms from other parts of Ontario were submitted to the Virology Section, Veterinary Services Laboratory, Guelph, Ontario. Ten pigs from one additional herd were investigated serologically only.

History and Clinical Information

Histories and clinical signs were obtained by interviewing veterinarians and producers at the time of submission of specimens.

Hematology

Blood samples were obtained from the cranial vena cava or by intracardiac puncture from pigs submitted alive. Pigs were killed with an overdose of barbiturate.

Pathology

Necropsies were performed on all carcasses. Lung samples were fixed in 10% neutral buffered formalin, sectioned at 6 μm and stained with hematoxylin and eosin (H & E).

Bacteriology

Portions of lung and less frequently tonsil, spleen, kidney, trachea, nostril and intestine were streaked on 5% bovine blood agar plates and incubated for 24 hours at 37°C.

Virology

Lung tissue was ground and resuspended at a dilution of 1:5 in tryptose phosphate broth containing 1000 I.U. of penicillin and 400 μg of streptomycin. The tissue suspensions were centrifuged at 1500 rpm for five minutes and the supernatants passed through a 0.45 μm filter to remove bacteria. The filtrates were inoculated in 0.2 mL volumes into the allantoic cavity of nine to 11 day old embryonated eggs. After incubation at 37°C for seven days, allantoic fluids were harvested and blind passaged up to three times. Appropriate controls were maintained.

Microtiter hemagglutination (HA) tests were conducted on allantoic fluids collected from eggs which had died after 24 hours or at the termination of each passage. Serial twofold dilutions of the allantoic fluid in phosphate buffered saline and 0.5% chicken red blood cells were employed.

Microtiter hemagglutination inhibition (HI) tests were conducted on HA positive allantoic fluids using four HA units and twofold dilutions of an antiserum to A/swine/Illinois/1/63 (H_1N_1). Further HI identification studies were conducted on six isolates from three areas of the province. In these tests, antisera to A/swine/Wisconsin/49/76,[1] A/swine/Illinois/1/63 and A/swine/Ontario/5/81 were employed.

Neuraminidase inhibition (NI) tests were conducted on eight isolates using a technique described previously (7).

Serology

Acute and convalescent serum samples were obtained three weeks apart from 16 pigs on three farms; two of the farms were part of the gross studies; the third farm was not. All sera were heat inactivated at 56°C for 30 minutes and treated with kaolin (8) to remove nonspecific inhibitors prior to serological HI testing. Twofold dilutions of the sera were made starting at 1:8 and four HA units of A/swine/Manitoba/647/67 (5) were employed.

An antiserum to A/swine/Ontario/5/81 was produced in chickens inoculated intraperitoneally with 3 mL of allantoic fluid diluted 1:1 with Freund's incomplete adjuvant, and intramuscularly with 1 mL into the pectoral muscles on each side. The allantoic fluid had an HA titer of 1:160. The birds were bled three weeks postinoculation.

RESULTS

History and Clinical Findings

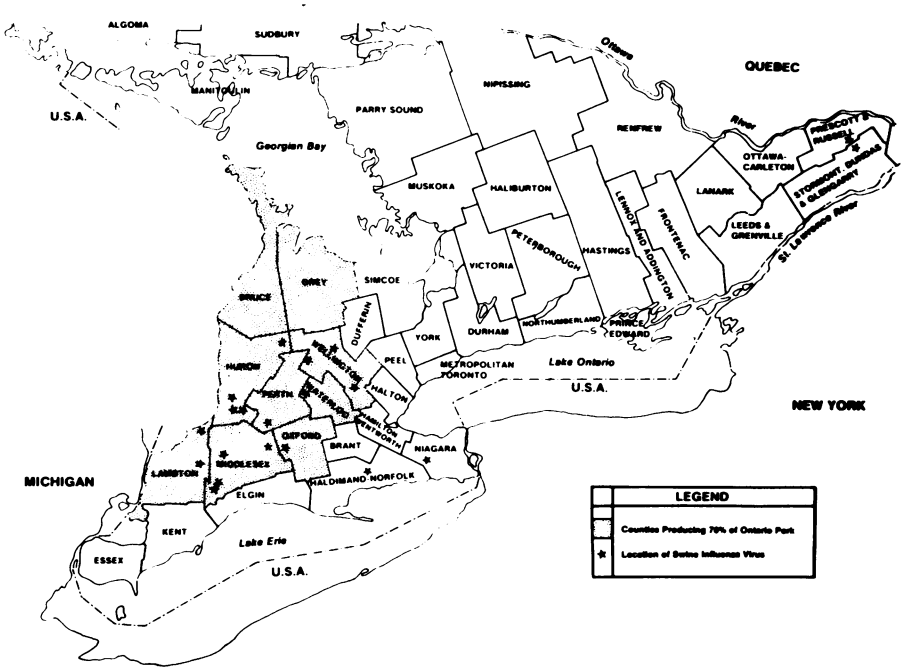
Submissions to the laboratory, from January 30, 1981 to May 27, 1981, came from eight breeding (farrow-to-finish) and three finishing herds. In one of the farrow-to-finish herds, only the pigs in the finishing barn were affected. Herd sizes ranged from 300 to 2000 animals. The province wide nature of the epizootic is outlined in Figure 1.

Clinical findings were similar, consisting of an abrupt onset of paroxysmal coughing, marked respiratory distress, anorexia, prostration and fever with body temperature up to 41.5°C. Animals remained acutely ill for five to seven days, then recovered almost as abruptly as they had become ill. The cough persisted for several days in some herds. The mortality rate was less than 1%.

The findings differed on three farms. In one finishing herd of 700 pigs there were no deaths but a second similar outbreak which was restricted to recent additions arriving the week before, occurred in mid June 1981. All pigs came and had always come from the same supplier. The supplier did not have any outbreaks on his farm. Laboratory work was not conducted during the second episode. On the second farm, a farrow-to-finish operation, the outbreak was restricted to the 700 pigs in the finishing barn. Deaths did not occur, but a second outbreak was reported in August 1981. Laboratory work was not conducted during the second episode. The one dead pig submitted from the third farm, a 500 pig finishing operation, had a dysentery just prior to death.

In all cases, medication, tetracycline in the feed (200 g/1000 kg) and/or chloramphenicol in the water (500 mg/L), was administered during the illness. Individual, prostrated pigs were also given an intramuscular injection of chloramphenicol (20 mg/kg) daily for two or three days.

[1]Provided by Dr. G. Dulac, Animal Diseases Research Institute, Nepean, Ontario.



Swine Influenza Virus Isolations in Southern Ontario, 1981

FIGURE 1. Map of Ontario. The counties in which the farms are located and laboratory diagnoses of swine influenza were established in 1981 are identified by stars.

Hematology

Hematology results from 12 of the 13 pigs submitted alive are presented in Table I. Ten of the animals exhibited varying degrees of acute inflammatory cell responses characterized by neutrophilia with a left shift (Pigs No. 9,13), absolute neutropenia with a degenerative left shift (Pigs No. 3,8,12) or just a responsive left shift (Pigs No. 5,6,7,10,11). These changes were usually associated with a hyperfibrinogenemia ($\geq$ 5 g/L) and a reduced plasma protein: fibrinogen ratio ($\leq$ 15:1); (Pigs No. 3,6,7,8,12,13). Eosinopenia (Pigs No. 2,3,5,6,7,8,9, 10,11,12) was almost universal. Absolute lymphopenia was also common (Pigs No. 3,5,8,9,10,12).

Pathology

One animal did not have gross lesions. All other pigs had varying degrees of bilateral cranioventral pneumonia affecting cranial, middle, accessory and cranioventral portions of caudal lung lobes (Figure 2). The pneumonic portions were red to purple, and slightly depressed. There was usually a sharp line of demarcation between normal and affected lung, but occasionally, affected lobules were scattered among normal lung tissue. A tenacious mucoid to mucopurulent exudate was present in trachea and bronchi, and lobules were demarcated by interlobular edema. The mediastinal and bronchial lymph nodes were enlarged and edematous.

The microscopic lesions were necrotizing bronchitis and a bronchiolitis (Figure 3). Airways were filled with neutrophils, smaller numbers of mononuclear cells, disintegrating, sloughed, epithelial cells, mucus and necrotic debris (Figure 3). Focal superficial necrosis progressing to transmural ulceration of bronchial and bronchiolar walls, usually leaving a ring of fibromuscular tissue in the most severe cases, was common (Figure 3). Less severely damaged and regenerating airways were lined by multiple layers of hyperplastic epithelium (Figure 4). Alveolar lumina contained a similar inflammatory cell exudate and alveolar walls had an

TABLE I
HEMATOLOGY RESULTS FROM 13 PIGS WITH SWINE INFLUENZA SUBMITTED ALIVE TO THE HURON PARK LABORATORY

Pig No.	Hb g/L	PCV L/L	RBC (X10^{12}/L)	MCV fL	WBC (x10^9/L)	Segs(%) (x10^6/L)	Bands(%) (x10^6/L)	Meta(%) myelocytes (x10^6/L)	Lymphs(%) (x10^6/L)	Monos(%) (x10^6/L)	Eos(%) (x10^6/L)	Baso(%) (x10^6/L)	Fibrinogen g/L	T/S Plasma Protein g/L	PP: Fib Ratio
1	105	0.31	5.86	56	17.1	10602(62)	171(1)	0	5643(33)	342(2)	171(1)	171(1)	3	65	21:1
2	114	0.34	6.62	53	17.1	8892(52)	171(1)	0	7695(45)	171(1)	0	171(1)	2	66	32:1
3	136	0.41	7.09	60	11.4	228(2)	6954(61)	1596(14)	2622(23)	0	0	0	7	88	12:1
4	Sample Clotted: Discarded														
5	145	0.43	7.81	57	11.4	7752(68)	228(2)	0	3420(30)	0	0	0	3	86	28:1
6	120	0.40	6.86	60	16.4	5904(36)	820(5)	656(4)	8856(54)	0	0	164(1)	6	75	13:1
7	120	0.38	6.42	61	15.6	4056(26)	780(5)	624(4)	10296(66)	0	0	0	5	69	13:1
8	143	0.42	7.28	60	6.6	924(14)	3102(47)	924(14)	1584(24)	66(1)	0	0	12	87	6:1
9	108	0.32	5.87	57	23.6	16520(70)	2124(9)	708(3)	4248(18)	0	0	0	3	73	23:1
10	105	0.33	5.46	62	5.9	2596(44)	236(4)	118(2)	2891(49)	59(1)	0	0	2	69	34:1
11	111	0.33	6.25	55	11.4	5130(45)	570(5)	456(4)	5244(46)	0	0	0	3	53	17:1
12	123	0.38	6.13	63	19.8	7722(39)	10296(52)	792(4)	990(5)	0	0	0	5	68	13:1
13	140	0.41	8.20	53	21.6	9720(45)	3888(18)	1296(6)	6480(30)	0	216(1)	0	7	91	12:1
Normal[a]	100-160	0.32-0.50	5.0-8.0	50-68	11.000-22.000	4000-7500 (28-47)	0-500(0-4)	0	5000-10000 (39-62)	300-1500 (2-10)	0-1500 (0.5-11)	0-300 (0-2)	N.A.	60-80	N.A.

[a]Normal values used are those established for the Teaching Hospital Clinical Pathology Laboratory, Ontario Veterinary College, Guelph, Ontario.
N.A. = Not available.

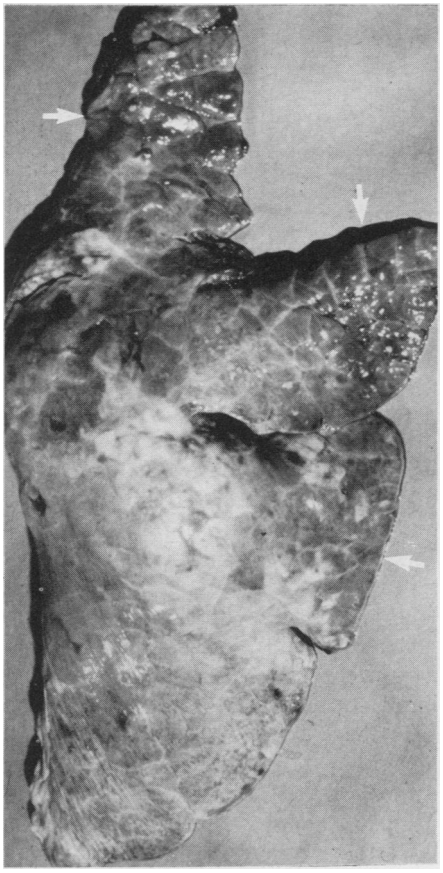


FIGURE 2. Consolidation of the cranioventral portions (arrows) of lung from a pig with swine influenza.

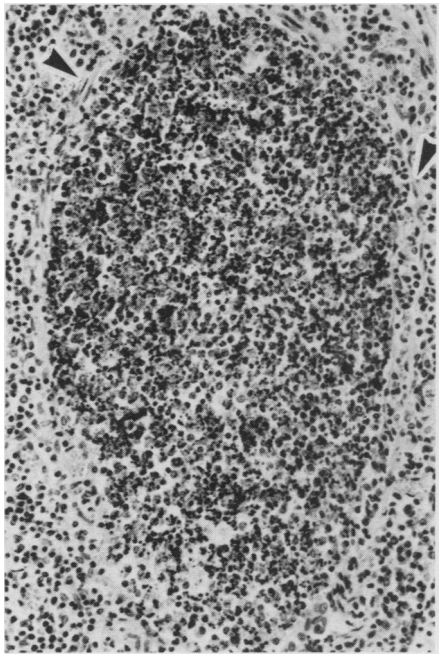


FIGURE 3. Necrotizing bronchiolitis. The lumen of the bronchiole is filled with inflammatory cells and necrotic debris. The entire bronchiolar wall has been destroyed leaving only a ring of fibromuscular tissue (arrows) outlining the limits of the bronchiole. H & E. X208.

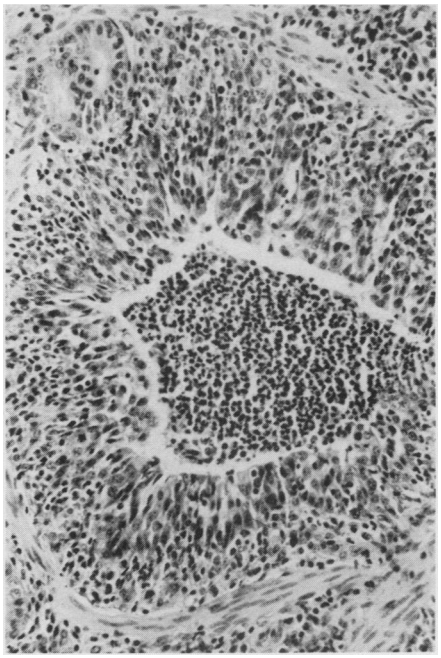


FIGURE 4. Hyperplasia of bronchiolar wall in lung of a pig recovering from swine influenza. The lumen is filled with cellular exudate. H & E. X208.

increased mononuclear cellular infiltration. In minimally affected lungs, alveolar lumina contained no exudate and the cellular infiltrate into alveolar walls was mild. Prominent perivascular, peribronchiolar and peribronchial lymphocytic cuffing were common. Micro- and larger abscesses in lung parenchyma and bronchioles were present in a few cases.

Bacteriology

Pasteurella multocida was cultured from the lung of seven of the 18 pigs. *Corynebacterium pyogenes* was also cultured from the lung of three of these seven pigs (two submitted dead; one alive). In these three pigs, both organisms were isolated from several of the other organs cultured, notably tonsil and spleen. *Pasteurella multocida* was isolated from both the lung and the spleen of one pig submitted alive. *Streptococcus suis* type II was isolated from the lung of one pig submitted alive.

Virology

Swine influenza virus (H_1N_1) was isolated from the lungs of pigs from 20 farms in southwestern, west central and eastern Ontario (Figure 1).

On allantoic inoculation of embryonated eggs with lung tissue suspensions, deaths occurred between 48 and 72 hours on the first passage and allantoic fluid HA titers of 1:20 to 1:320 were seen. All of the isolates were closely related to A/swine/Illinois/1/63 on HI testing.

The results of HI studies conducted on six of the isolates (two each from three areas of the province) are outlined in Table II.

All eight of the isolates studied (A/swine/Ontario/1/81 to A/swine/Ontario/8/81 inclusive) had an N_1 neuraminidase surface antigen.

Serology

Serology results are presented in Table III. A $\geqslant$ fourfold rise in titer was identified in 12 of the 16 pigs studied and a twofold rise in two others. An acute sample was not available from one pig, but the convalescent sample had a 1:16 titer. One pig did not have demonstrable titers in acute or convalescent sera.

TABLE II

HEMAGGLUTINATION INHIBITION TITERS OF SIX SWINE INFLUENZA VIRUS[a] ISOLATES FROM THREE AREAS IN ONTARIO

	Ontario Isolates					
	Southwestern		West Central		Eastern	
Antiserum	ONT/1	ONT/5	ONT/4	ONT/12	ONT/8	ONT/13
A/swine/Wisconsin/49/76 (pig origin)	1:64	1:64	1:64	1:64	1:64	1:64
A/swine/Wisconsin/49/76 (chicken origin)	1:64	1:64	1:32	1:64	1:32	1:64
A/swine/Illinois/1/63 (chicken origin)	1:128	1:64	1:64	1:128	1:64	1:64
A/swine/Ontario/5/81 (chicken origin)	1:128	1:256	1:256	1:128	1:256	1:128

[a]Microtiter technique with four HA units.

TABLE III
ACUTE AND CONVALESCENT HI TITERS TO SWINE INFLUENZA VIRUS (A/SWINE/ONTARIO/5/81) OF 16 PIGS FROM THREE FARMS

Farm No.	Serum No.	Acute	Convalescent
1	1	< 1:8	< 1:8
	2	< 1:8	1:64
	3	N.D.	1:16
2	1	< 1:8	1:256
	2	< 1:8	1:128
	3	< 1:8	1:256
3	1	1:32	1:64
	2	< 1:8	1:64
	3	< 1:8	1:64
	4	< 1:8	1:128
	5	1:64	1:128
	6	< 1:8	1:64
	7	< 1:8	1:128
	8	< 1:8	1:64
	9	1:64	1:256
	10	< 1:8	1:128

N.D. = Not Done.

DISCUSSION

The sudden onset of pronounced respiratory distress accompanied by paroxysms of coughing and anorexia, prostration and fever affecting entire herds in large numbers with few deaths and abrupt recovery after five to seven days is the classic description of swine influenza (1). Swine influenza infection was confirmed based on the gross lung lesions, histology, serology and virology findings (1,2).

Although the majority of cases came from the counties of southwestern Ontario with heavy pig populations, SI was identified from all areas of the province except the north and east central regions (Figure 1). With the lack of isolates from the east central region one could speculate that the SI in the eastern area may have represented a contiguous epidemiological zone with the concomitant Quebec outbreak (6), whereas the southwestern region was a separate epidemiological focus. Alternatively, the infection may have been present throughout the province and have gone unrecognized or uninvestigated in the east central region. The results of HI studies indicate that the isolates from the eastern and western regions of the province were similar with respect to their hemagglutinating surface antigens.

The gross lung lesions resembled those of mycoplasma (enzootic) pneumonia of pigs (MPP) and some of the microscopic findings of alveolar wall inflammatory cell infiltrates, and prominent perivascular and peribronchiolar lymphocytic cuffing resembled changes seen in MPP (9). The necrotizing bronchitis and bronchiolitis which were the most consistent histological findings in the lungs, are expected with SI (1,2) but not with MPP (9). The frequent isolation, from pneumonic lungs, of *P. multocida,* which is a common secondary bacterial invader in MPP (9), is however suggestive of MPP involvement in some of the pigs. It would not be unexpected for a few pigs to die during an SI outbreak especially those complicated with MPP and secondary bacteria in their lungs (1). The widespread use of broad spectrum antibiotics during this outbreak would seem justified only in those cases where concomitant diseases existed.

Reports of hematological studies in acute SI viral infections were not found. The hematological findings in most cases were consistent with acute inflammatory responses (10). Although the absolute lymphopenia so frequently seen may have been a function of stress (10), there are several reports of a decrease in the numbers of circulating T-lymphocytes and decreased T-cell blastogenesis in human beings after acute influenza virus infection (11,12,13) including inoculation with a swine influenza virus vaccine (12). Most of the lymphocytes in circulating peripheral blood of swine are T-cells (10).

The principal question raised with regard to this outbreak is whether the factors responsible for varying degrees of pathogenicity of orthomyxoviruses (14) could have accounted for this sudden, widespread outbreak of disease. The surface antigens of the isolates are similar to previous North American isolates of SI (H_1N_1). In this epizootic, the disease affected swine of all ages. In the 1979 survey 17% of Ontario swine had antibodies to the H_1 serotype of influenza (4). Since outbreaks of SI had not been reported previously, it seems reasonable to speculate that the strain present in Ontario in the winter of 1981 had an enhanced pathogenicity in comparison with the previous H_1 serotypes of SI prevalent in this area prior to 1981.

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