Serological Survey for Aujeszky's Disease in Native Sows of Canada

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SUMMARY

A serological survey was initiated to uncover subclinical foci of infection with pseudorabies virus. A total of 2819 serum samples, collected from sows during February to November 1977, were found negative for antibodies to pseudorabies virus.

RÉSUMÉ

Enquête sérologique sur la maladie d'Aujeszky chez des truies au Canada

Les auteurs rapportent les résultats d'une étude entreprise dans le but de découvrir l'existence de foyers d'infection par le virus de la pseudorage chez des truies, au Canada. Les 2819 échantillons, prelevés entre février et novembre 1977, se sont avérés négatifs.

INTRODUCTION

Three sources of evidence suggest that Aujeszky's disease is unlikely to be endemic in Canada: previous investigations on the viral etiology of porcine encephalitides have not detected Aujeszky's disease virus (6); no evidence of infection has been found among diagnostic specimens submitted to the Health of Animals laboratory in Ottawa (4); veterinarians have not diagnosed pseudorabies. However, no systematic attempt has been made to determine if pseudorabies virus is present in Canadian swine.

This paper reports the results of a national survey designed to uncover subclinical foci of infection, as indicated by the presence of antibodies against pseudorabies virus in sow serums.

MATERIALS AND METHODS

Virus

The pseudorabies virus was purchased from the American Type Culture Collection[1] and passaged once in primary porcine kidney cells. The infected cell culture fluids were harvested at 48 hours postinfection, after two cycles of freezing and thawing. The cell debris was sedimented at 1500 x g and the supernatant fluids were filtered through 0.45 μ Millipore filter[2]. Sufficient fetal bovine serum was added to the virus suspension to make a final concentration of 10% and the mixture was frozen in liquid nitrogen and stored in a mechanical freezer at -70° C.

Virus-neutralization tests

The virus neutralization (VN) tests were conducted according to the protocol prepared by the Pseudorabies Diagnostic Standardization Committee of the American Association of Veterinary Laboratory Diagnosticians[3] using the IB-RS-2 porcine cell line[4] and the Aujeszky's disease virus.

Immunodiffusion antigen

The pseudorabies antigen was prepared according to the method of Gutekunst *et al* (7) from infected primary pig kidney cell cultures. Control antigen was prepared in parallel from noninfected cells.

Immunodiffusion test

The tests were performed in 100 x 11 mm plastic Petri dishes, in the system used for equine infectious anemia diagnosis and described by Boulanger *et al* (1). The diameter of the wells, the distance between the wells and the disposition of the reagents were as described by Gutekunst *et al* (7).

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[1]American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852.

[2]Millipore Corporation, Bedford, Massachusetts 10730.

[3]Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa 50011.

[4]Supplied by Dr. W.G. Chapman. The Aminal Virus Research Institute, Pirbright, Woking, Surrey GU24 DNF England.

TABLE I

SAMPLING OF SOW SERUMS FOR THE PSEUDORABIES SURVEY

Provinces	Sampling Period in 1977 From Day/Month	To Day/Month	Number of Serums Tested	Number of Farms Sampled	Number of Places where Farms were Sampled	Tests Used in Number of Serums VN[a] Tube	VN[a] Micro	ID[a]
Newfoundland	1/3	6/ 6	100	12	11	14	86	0
Nova Scotia	9/2	13/ 5	100	57	33	34	66	0
Prince Edward Island	8/2	3/ 5	100	85	32	52	48	0
New Brunswick	9/2	28/ 7	100	55	36	43	57	0
Quebec	11/3	19/10	162	107	70	65	97	0
Quebec	20/9	21/11	395[b]	94	67	395	0	0
Quebec	11/3	11/10	457[c]	—	—	0	0	457
Ontario	3/2	23/11	528	365	196	481	47	528
Manitoba	5/2	25/ 5	200	160	93	198	2	0
Saskatchewan	3/3	8/11	179	115	89	0	0	179
Saskatchewan	3/3	8/11	103	—	—	0	0	103
Alberta	4/2	30/ 9	303	182	112	291	12	0
British Columbia	18/2	?/7	35	18	10	32	3	0
British Columbia	18/2	?/7	57	—	—	57	0	0
			2819	1250	749	1662	418	1267

[a]VN = Virus neutralization. ID = Immunodiffusion

[b]Serums collected directly on breeding farms

[c]Number of serums without the address of the original owner

Sampling procedure

It was decided that the most efficient method of obtaining information on the prevalence of pseudorabies in the Canadian swine population was to take, as target population, the culled sows passing through swine slaughterhouses subjected to federal meat inspection and participating in the Swine Brucellosis Testing Program. This program had been initiated twelve years ago as a convenient means to monitor swine brucellosis in the country and was assumed to be adequate for estimating the prevalence of a disease expected to be uniformly low or zero. A sample survey of this population was expected to be comprehensive, both geographically (all across Canada) and historically (the sows, being old, would have had ample opportunity to be infected by the virus).

The samples were collected in each of the ten provinces so that the numbers of serums tested was roughly proportional to provincial swine populations as published by Statistics Canada (3). However, to ensure adequate coverage within individual provinces, a minimum of 100 tested serums was required for each.

The collection occurred generally as planned except for Quebec and British Columbia. In Quebec, some samples were collected directly at large breeding farms by veterinarians of the Contagious Diseases Division. This method of collection was used in that province to supplement the sampling at the abattoirs, since in many instances insufficient information was available to check on the coverage achieved by the abattoir. In British Columbia, two thirds of the serums had to be collected in abattoirs subjected to provincial meat inspection.

Subsampling Procedure

Only a portion of the total number of serums collected was tested. The selection of serums for testing was done as follows: serums submitted to the laboratory were placed in racks, usually in sets of 25 tubes, in the order submitted. Each set of 25 tubes was randomly subsampled, at a 20% rate by selecting the first five numbers from a computer generated pseudorandom (PR) permutation of the numbers 1 through 25 (the PR number generator had previously been tested and found to be acceptable). The sixth and subsequent numbers in the permutations were to be used if the preceeding samples were contaminated, did not contain enough material or came from herds which had already been sampled adequately (not more than five sows per herd). The sampling rate of 20% provided an even flow of samples throughout the period surveyed. It was based on the assumption that

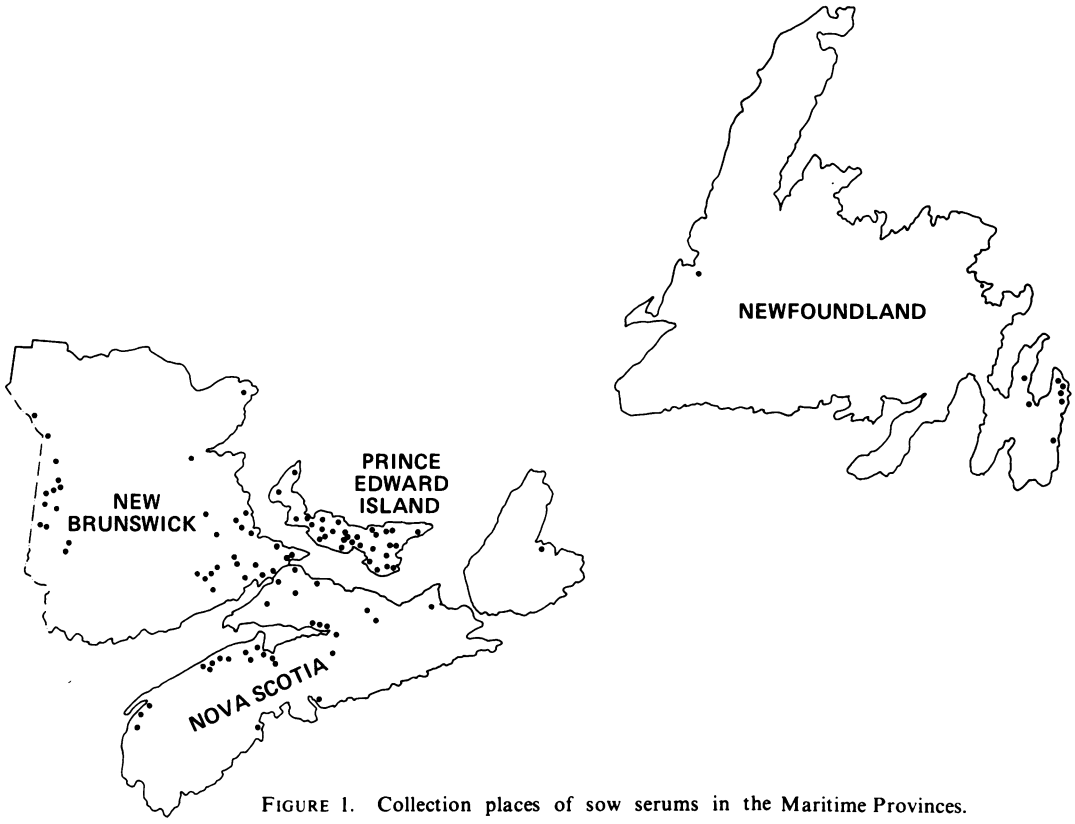

FIGURE 1. Collection places of sow serums in the Maritime Provinces.

the number of sows slaughtered during the period would be similar to the numbers slaughtered during the corresponding period of the previous year. This method was chosen since the sampling rate could easily be adjusted while the project was being carried out to obtain more or fewer serums, as required. In practice, the rate was not changed except for Newfoundland, Saskatchewan and British Columbia where a considerably higher percentage of serums (100%, 50%, 100%) had to be accepted to reach the allocated numbers.

Since there could have been positive serums submitted to the laboratory but not included among the tested subsamples, it is worth calculating confidence intervals for the numbers of possible positive serums among the total number submitted from each province; these were obtained based on the hypergeometric probability distribution (5).

RESULTS

The serum samples, collected during February to November 1977, had no antibodies to Aujeszky's disease virus. Of the 2819 serums tested, 2424 were collected in slaughterhouses and 395 were collected on breeding farms in the Province of Quebec. Trace back of the samples obtained *via* the slaughterhouse was successful in 1807 serums. These samples were assigned to 1250 farms located in 749 places (2) (Table I and Figs. 1 to 3). Addresses of the original owners could not be found in 617 cases; 457 in Quebec, 103 in Saskatchewan and 57 in British Columbia.

Upper 99% confidence limits for the numbers of possible positive serums among those submitted to the laboratory are presented in Table II. It must be noted also that these confidence limits refer to the numbers submitted to the laboratory and not to the total population.

Except for the serums collected in the Maritime provinces and the 457 samples collected in the slaughterhouses in Quebec, most serums were tested satisfactorily with the micro VN test (Table I). Extreme hemolysis or cytotoxicity were the main reasons preventing the use of the micro VN test. At the beginning of the survey, the serums that could not be tested by the micro VN test were done in tubes on primary pig kidney cells. However, the limitations

TABLE II

UPPER 99% CONFIDENCE LIMITS FOR THE NUMBERS OF POSSIBLE POSITIVE SERUMS AMONG THE COMPLETE SAMPLES SUBMITTED TO THE LABORATORIES

Province	Serum Received in Labs	Serums Tested	Upper Confidence Limits[a]
Newfoundland	100	100	—
Nova Scotia	500	100	20
Prince Edward Island	500	100	20
New Brunswick	500	100	20
Quebec	810	162	20
Quebec	395[b]	395	—
Quebec	457[c]	457	—
Ontario	2640	528	21
Manitoba	1000	200	20
Saskatchewan	564	282	7
Alberta	1515	303	21
British Columbia	92	92	—

[a]Nearest whole number.
[b]Serums collected directly on breeding farms.
[c]Numbers of serums without the address of the original owner.

of the tube test for assaying large numbers of serums led to the adaptation of the immunodiffusion (ID) test.

The 528 serum samples from Ontario were tested by the micro VN test and found negative. Subsequently, each of these serums was also tested by the ID test and was again found negative. Because of this result, 739 serums were tested by the ID test only; 457 from Quebec and 282 from Saskatchewan (Table I).

DISCUSSION

From Figures 1 to 3, it is clear that the serums tested originated from many places in Canada and that the sample provided a wide coverage of the swine population. The negative findings of this survey indicate that Aujeszky's disease is unlikely to be currently endemic in Canada.

A major difficulty encountered with the VN test in this survey was the presence of cytotoxicity found in a large proportion of serums. In

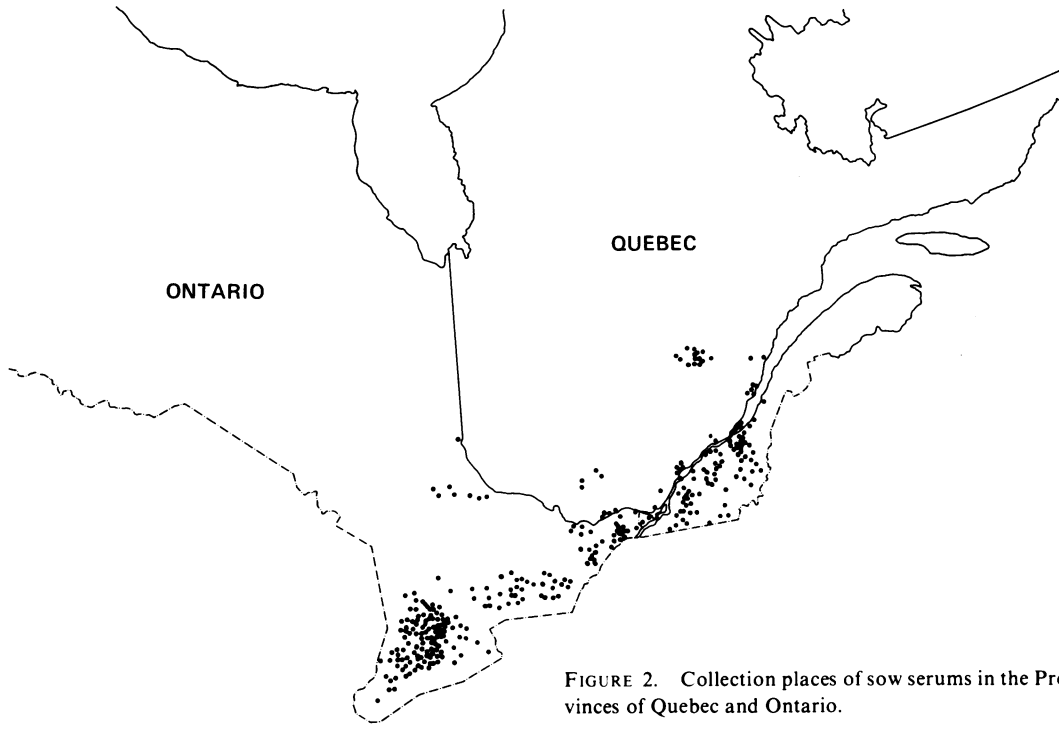

FIGURE 2. Collection places of sow serums in the Provinces of Quebec and Ontario.

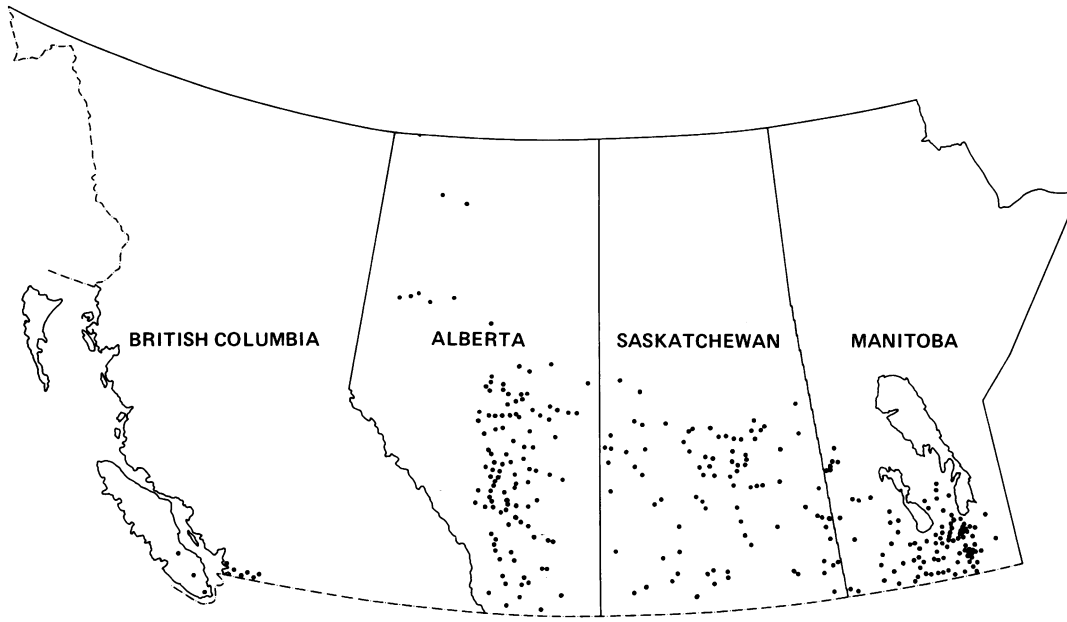

FIGURE 3. Collection places of sow serums in the Prairie Provinces and British Columbia.

an effort to overcome the problems associated with serums of poor quality, the ID test was adapted from the method of Gutekunst *et al* (7). The ID test was used successfully for 1267 serum samples and nonspecific reactions were not encountered. The test was specific inasmuch as reactions appeared only between the positive antigen and the homologous antiserums used as controls. In future surveys for pseudorabies, involving serums collected in abattoirs, the ID test should be considered as a replacement for the more cumbersome VN test.

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