The Detection of Antibody to Infectious Bovine Rhinotracheitis Virus in Ontario Cattle.

by Andrew S. Greig*

Infectious bovine rhinotracheitis is an infectious disease of cattle caused by a virus first reported in California (1). It is responsible primarily for the production of acute inflammatory changes in the mucous membranes of the upper respiratory tract. The virus is readily cultivated in tissue cultures of bovine cells where it produces visible cytopathic effects leading to death of the cells (2). The cytopathic effect in tissue culture is specifically neutralized by serum from immune or convalescent animals.

Infectious bovine rhinotracheitis, as a clinical disease, has not been reported in Ontario cattle. The disease, in fact, does not appear clinically to any extent in Eastern North America. A report, however, by Gillespie et al (3), indicated that antibodies to the disease occurred in 12% of pooled serums from 38 New York State cattle herds. In addition, a single serum collected in 1941 from a New Jersey calf contained detectable neutralizing antibodies.

This report deals with the detection of neutralizing antibodies to the virus of bovine rhinotracheitis in sera collected randomly from Ontario cattle. Although it is not a survey, an attempt was made to obtain a distribution of samples from across the southern portion of the province.

Materials and Methods

Sera. The sera tested were taken from those submitted to the Animal Pathology Laboratories in the area herd testing Brucellosis program. They were selected as far as possible on a random basis, except that only clear, non-hemolysed samples were chosen, and an attempt was made to spread the sampling across the southern part of the province on a county basis. No attempt was made to obtain any information relative to the health of the herds or their Brucellosis status. Records were kept, however, of the origins of the samples, and the unused portions of sera were frozen and stored. Two, 3 or 4 samples of sera were obtained from each herd tested. Prior to testing, all sera were heated in a water bath at 56°C for 30 minutes.

Area tested. The area tested comprised the 42 counties of Southern Ontario with the northern limits provided by the northern boundaries of the counties of Simcoe, Ontario, Victoria, Peterborough, Hastings and Renfrew. No efforts were made to relate the number of farms tested to the cattle population of any geographical area.

Virus. The virus used in the test originated in the United States and was kindly supplied by Dr. C. J. York, Research Department, Pitman-Moore Co., Indianapolis, Ind. It was cultivated in cultures of bovine embryo kidney (BEK). The fluid harvested from infected cultures was centrifuged at 2,000 RPM for 10 minutes to remove the cellular debris, the supernatant fluid was distributed in 2 ml. amounts in glass vials, heat-sealed and frozen at -20°C. A titration was performed on each batch of virus used in the study.

Tissue Culture. All tests were done on cultures prepared from a cell line of bovine embryo kidney (BEK) cells originating in this laboratory (4). For virus batch production, monolayers of BEK cells were prepared in T-60 (Kontes) flasks, while in neutralization tests 18 x 150 mm. hard glass tubes were employed in roller drums. The virus grew well on the BEK cells and produced cytopathic effects readily visible microscopically. Infected cells first became rounded, then refractile and opaque, and finally fell off the glass.

Neutralization test. Each serum sample was initially tested for antibodies at a dilu-

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TABLE 1

Results in 42 counties of Southern Ontario of serum sampling for neutralizing antibodies to infectious bovine rhinotracheitis

County	No. of farms tested	Positive Farms	No. of samples tested	Positive Samples
Brant	13	2	42	3
Bruce	3		12	
Carleton	10	4	30	4
Dufferin	2		8	
Dundas	11	3	33	4
Durham	10	3	30	3
Elgin	15	5	49	6
Essex	2		8	
Frontenac	11	2	33	3
Glengarry	12	7	36	9
Gray	3	1	8	2
Grenville	10		30	
Haldimand	12	1	38	1
Halton	5		20	
Hastings	12	2	36	2
Huron	8	3	32	6
Kent	6	2	20	3
Lanark	16	2	48	2
Lambton	12		43	
Leeds	11		33	
Lennox and Addington	11	4	33	7
Lincoln	8	1	31	1
Middlesex	22	3	88	3
Norfolk	11		32	
Northumberland	10	2	30	2
Ontario	12	3	38	5
Oxford	11		34	
Peel	3		12	
Perth	12	1	48	2
Peterborough	7		21	
Prescott	11	6	33	11
Prince Edward	7	1	21	3
Renfrew	10	2	30	2
Russell	9	6	27	10
Simcoe	8		32	
Stormont	9	1	9	1
Victoria	17	5	51	5
Waterloo	9	2	36	4
Welland	12		36	
Wellington	9	4	36	5
Wentworth	15	1	49	1
York	15	1	49	1
TOTALS	422	80	1365	111

tion of 1:4. Later, a group of those sera which showed antibodies at a 1:4 dilution were retested at dilutions up to 1:128.

In each case the serum-virus neutralization test was set up by mixing 1 ml. of serum with 1 ml. of tissue culture maintenance medium and 2 ml. of virus suspension containing 200 tissue culture 50% infectious doses ($TCID_{50}$) of virus per ml. The 1:4 dilution of serum represented the final dilution after the 1 ml. of medium and 2 ml. of virus dilution were added to 1 ml. of serum. The serum-virus mixtures were incubated at 37°C for 2 hours prior to inoculation of tissue cultures. After incubation, the mixtures were cooled to room temperature. The medium was poured off two tissue culture tubes and replaced with 1 ml. of a serum virus mixture and set in a roller drum in the incubator at 37°C. The test could be read in 4 days time and rarely was held for as long as 6 days. The results were interpreted on the absence or presence of cytopathic effects. In cases where 1 tube showed cytopathic effects and the other did not, the sample was considered negative for antibodies. In the serum titrations the end point was taken as the dilu-

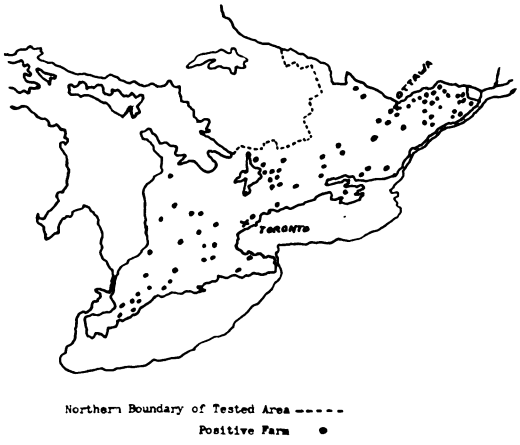

FIG. 1 — Distribution of Positive Farms in Southern Ontario.

tion which showed the absence of cytopathic effects in both inoculated tubes.

Results

During the course of this work, 1,365 serum samples from 422 farms in Southern Ontario were tested for neutralizing antibodies to infectious bovine rhinotracheitis. Of these, 111 samples showed serum antibody in dilutions of 1:4 or greater. The 111 samples originated from 80 different premises. In other words, by the methods used 8.13% of the serum samples, representing 18.9% of the farms tested, showed positive results. The details of the results by counties are shown in Table I. The geographical distribution is shown in Figure I.

The highest concentration of positive farms appeared in the Ottawa valley in the counties of Glengarry, Prescott and Russell, where in each case over half the farms tested had positive animals. In only 6 counties where over 10 farms were tested, were no positive animals encountered. These included Leeds and Grenville in Eastern Ontario and Lambton, Norfolk, Oxford and Welland in Western Ontario. In another 8 counties showing no positive samples, the numbers tested, being below ten were too few to make fair comparisons with most of the counties tested.

Among the 80 positive farms, 4 had all the samples tested positive, 22 had 2 of 3 or 2 of 4 samples positive and 54 had 1 out of 3 or 4 samples positive.

Sixty of the samples which showed positive results at a dilution of 1:4 were retested at dilutions up to 1:128. The results, shown graphically in Figure 2, demonstrate that the endpoints of the sera titrations form a curve with the peak at a dilution of 1:8.

Discussion

Considering that IBR is not recognized as a clinical disease in the province, the results of this test show a surprisingly high percentage of animals with neutralizing titres among Ontario cattle. It is particularly remarkable to consider that almost one-fifth of the farms tested showed the presence of positive animals. Undoubtedly the smallness of the sampling size per farm tended to miss herds with only a few reactors, placing the calculated percentages on the conservative side.

Gillespie (5) showed that the virus of IBR and that of infectious pustular vulvovaginitis (IPV or coital exanthema) of cattle are immunologically identical. This latter disease has been observed and the virus isolated from Ontario cattle (6) but, like IBR, the incidence of IPV in the province, at least from the dearth of clinical reports, would hardly account for the high percentage of serum reactions. The reactions, while mostly of low titre, were nevertheless of a high enough level to be considered indicative of the presence of antibody.

It is possible that IBR or IPV may either occur subclinically or in a very mild form but still be able to induce antibody development in the animal. Another possibility is that the virus or viruses involved may be associated with other conditions, as for

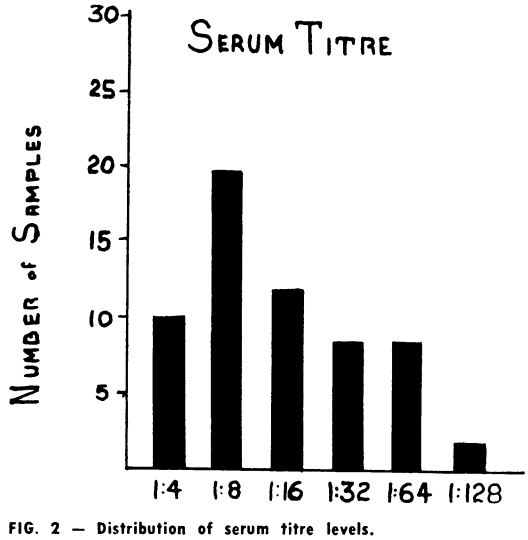

FIG. 2 — Distribution of serum titre levels.

example shipping fever, where the effects of the virus are obscured by the sequela of other etiological factors.

Summary

Serum-virus neutralization trials in tissue culture have demonstrated that 8.13% of the sera tested from Ontario cattle had antibodies to infectious bovine rhinotracheitis virus. These positive sera were obtained from 18.9% of the herds tested.

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A Rapid and Simple Method for the Demonstration of Acetone and Aceto-acetic Acid in the Urine

A dry reagent sufficiently sensitive for routine demonstration of acetone and aceto-acetic acid in urine has been developed. It is composed of 0.003 g of sodium nitroprusside, 0.130 g of dibasic sodium phosphate, and 0.041 g of glycine. With the help of an adjuvant the reagent is compressed into tablets. Urine containing 40-50 mg% of acetone gives a clearly positive reaction with this reagent. The test is performed in the following manner: the tablet is placed on a piece of filter paper and a drop of the examined urine is poured on it. If the urine contains more than 40 mg% of ketone bodies a change in colour of the tablet from light violet to purple red occurs. The intensity of the reaction depends upon the amount of ketone bodies. This method can also be used for the demonstration of ketone bodies in blood serum. The serum should be diluted with an equal part of distilled water. Serum containing 0.1% or more of acetone gives a clearly positive reaction. The method can also be used in the examination of milk.

B. Uhlik Veterinarski Arhiv (Journal of Veterinary Faculty, University of Zagreb) Vol. 30:190, 1960.

A Test for Rapid Detection of Changes in the Acidity of Milk in Inflammation of the Udder

A test is developed which detects differences in acidity of the milk deriving from various quarters of the same udder. In various conditions the pH of the milk from the affected quarter changes and this can be detected by means of the described test.

In mild and initial phases of conditions the pH of the milk switches to the alkaline zone and in more severe, to the acid one. The results obtained from various quarters should be compared. A negative result does not exclude the existence of a disease while a positive one does indicate the presence of a pathologic process.

The test is performed by an indicator mixture composed of 20 parts of 0.2% bromcresolpurple, 5 parts of 0.25% bromthymolblue and one part of 0.2% chlorphenolred. The indicator is placed on 4 areas of filter paper and on each area a drop of milk from a separate quarter is poured. The reaction is read after one minute. Healthy milk gives a bluish colour, while that with an increased alkalinity (pH 6.8 or more) a more or less intensive purple red. In the case of pH under 6.4 a dirty yellow or bright yellow appears. A suspicion of an udder affection is justified in all cases in which differences in pH between various quarters are found. *B. Uhlik Veterinarski Arhiv (Journal of Veterinary Faculty, University of Zagreb) Vol.* 30:200, 1960.