The Incidence and Significance of Bovine Herpesvirus (infectious bovine rhinotracheitis) Antibodies in the Sera of Aborting Cattle

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RESUME

Dans cet article on rapporte les résultats d'épreuves de neutralisation du virus herpès bovin (IBR-IPV) faites avec les sérums de 463 animaux ayant avorté mais dont la cause de l'avortement n'a pas été diagnostiquée et 331 animaux témoins dont l'histoire ne révèle aucun avortement.

Cent trente et un (28.3%) des animaux avortés et cent cinq (31.7%) des témoins furent trouvés porteurs d'anticors du virus. Il fut impossible de déterminer quelqu'incidence de saison ou de gestation toutefois une variation dans l'incidence annuelle fut apparente.

A la lumière de ces résultats, il apparaît improbable que ce virus soit responsable d'une grande partie des cas d'avortements bovins non diagnostiqués dans le Québec et l'Ontario d'où proviennent les animaux éprouvés. Les problèmes suscités dans l'établissement d'un diagnostic d'avortement du au virus herpès bovin, sont discutés brièvement.

ABSTRACT

The results of bovine herpesvirus (IBR-IPV) neutralization tests conducted on the sera of 463 animals with a prior history of undiagnosed abortion and 331 control animals with no history of abortion are reported. One hundred and thirty-one (28.3 per cent) of the aborting and 105 (31.7 per cent) of the control animals, were found to harbour antibody to this virus. No significant seasonal or gestational incidence could be determined but some variation in the annual incidence was apparent. On the basis of these results it appears unlikely that this virus is responsible for a significant proportion of the undiagnosed cases of bovine abortion in Quebec and Ontario, where the animals tested were located. The problems involved in substantiating a diagnosis of abortion due to bovine herpesvirus are briefly discussed.

Introduction

A recent review (1) of virus infections associated with bovine abortion and infertility points out that Infectious Bovine Rhinotracheitis-Infectious Pustular Vulvo-Vaginitis (IBR-IPV) virus is the cause of a wide range of clinical manifestations in cattle. These include rhinitis, infectious pustular vulvo-vaginitis, abortion, balano-posthitis, infertility, conjunctivitis, encephalitis of calves and experimental mastitis. Recently the term bovine herpesvirus has been used to describe the agent causing these different syndromes (2). This nomenclature will be used throughout this paper to describe the agent currently designated as IBR-IPV virus.

Several reports from the United States (3,4,5,6,7) in recent years have indicated that bovine herpesvirus may be an important cause of abortion in cattle. In 1961, it was reported (8) that 8.13 per cent of animals and 18.9 per cent of herds in Southern Ontario showed antibodies in significant titre to this virus. In 1962, it was found (9) that the sera of 37 per cent of 1,000 cattle from 500 herds in Alberta contained bovine herpesvirus antibody. Isolations of this agent have been reported in Canada (10,11) from outbreaks of respiratory disease in cattle.

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TABLE I. — Individual and Herd Incidence of Herpesvirus Antibody

Classification		Total tested	Positive Number	Positive Per Cent
(a) Animals	Aborted	463	131	28.3
	Control	331	105	31.7
(b) Herds with one or more animals positive.	Aborted	190	76	40.0
	Control	74	35	47.3
(c) Herds with all animals tested positive.	Aborted	190	31	16.3
	Control	74	9	12.2

Its importance as a cause of abortion was not discussed although one report (10) mentioned the occurrence of two abortions in one herd.

The purpose of this paper is to report the incidence of herpesvirus antibodies in the sera of cattle with a history of prior abortion; to compare the results with those obtained in a group of normal controls; to comment on gross and histological changes in fetal tissues and to discuss briefly the significance of these findings.

Materials and Methods

Sera

(i) Aborting animals. Blood samples were collected from 463 animals within four weeks of the act of abortion. They were harvested over an eight year period (1957-65) during the course of studies on the cause of abortion in cattle. All of these samples were negative to routine serological tests for Brucellosis (*Br. abortus*) and Leptospirosis (*L. pomona* and *L. hardjo*). In some cases additional bacteriological and histological examinations had been conducted on submitted fetal tissues and placentae with negative or inconclusive results. These animals, predominantly of dairy breed, were located on 190 farms in the provinces of Ontario and Quebec.

(ii) Control Animals. Three hundred and thirty-one samples were selected at random from cattle located in the same area which were being subjected to routine brucellosis testing during a three month period (September to November 1964). These sera were taken from 74 herds with no recent history of abortion and were negative when tested with *Br. abortus* rapid plate and tube agglutination antigens.

Tissue Culture Technique

Primary cultures of bovine embryo kidney cells were trypsinized and grown in 16 x 150 mm. disposable glass tubes. The cultures were maintained in a growth medium consisting of 0.5 per cent lactalbumin hydrolyzate (enzymatic), 0.1 per cent proteose peptone #3 (Difco), 19 per cent newborn calf serum and 0.001 per cent cysteine hydrochloride in Hank's balanced salt solution. The calf serum used was tested for the absence of bovine herpesvirus antibody.

At the time of inoculation, usually five or six days after seeding, the growth medium was removed from the cultures

TABLE II. — Distribution of Herpesvirus Antibody in Aborting Cattle

Stage of Gestation Months	Total tested	Positive Number	Positive Per Cent	Time of Year Month	Total tested	Positive Number	Positive Per Cent
2	3	1	33.3	Jan	55	17	30.8
				Feb	38	8	21.0
3	22	6	22.2	Mar	27	10	37.0
				Apr	27	7	25.9
4	35	13	37.1	May	24	8	33.3
				June	21	4	19.0
5	86	21	24.4	July	18	4	22.2
				Aug	31	9	29.0
6	60	21	35.0	Sept	43	15	34.8
				Oct	48	11	22.9
7	54	14	25.9	Nov	55	15	24.2
				Dec	76	23	30.3
8	69	19	27.5				
TOTALS	329	95	28.8		463	131	28.3

TABLE III. — Annual Incidence of Herpesvirus Antibody in Aborting Cattle

(1957-1965)

Year	Total tested	Positive Number	Animals Per Cent
1957.....	27	2	7.4
58.....	20	3	15.0
59.....	36	6	16.7
60.....	43	16	37.2
61.....	57	13	22.8
62.....	112	34	30.4
63.....	87	43	49.4
64.....	76	13	17.1
65.....	5	1	20.0
TOTALS.	463	131	28.3

and replaced by an equal volume (one ml.) of inoculum in which the serum being tested was diluted in medium 119 supplemented with 0.5 per cent lactalbumin hydrolyzate.

VIRUS NEUTRALIZATION TEST

All sera were inactivated in a water bath at 63°C. for 30 minutes. They were then tested at a final dilution of 1:4 against a dilution of herpesvirus containing 100 tissue culture infective doses (TCID). The test was prepared as follows: 0.5 ml. of each inactivated serum was mixed with 0.5 ml. of medium 199 fortified with 200 units of penicillin and 200 mg. of streptomycin per ml. One ml. of tissue culture fluid containing a calculated dose of 200 TCID of virus was added to the serum dilution. The serum-virus mixture was incubated for two hours at 37°C. then one ml. was inoculated into each of two cultures tubes.

After four days incubation at 37°C. the cultures were examined microscopically. Any sign of cytopathic effect in either tube was regarded as evidence of a serum sample devoid of antibody. Those samples in which both tubes were free of virus growth were considered positive. The sera were thus designated positive or negative on the basis that they did, or did not, possess antibody at 1:4 dilution.

Results

One hundred and thirty one (28.3 per cent) of the 463 sera from aborted animals and 105 (31.7 per cent) of the 331 controls proved positive. In 31 (16.3 per cent) of the 190 herds which contained the 463 aborting animals and nine (12.2 per cent) of the 74 control herds, all animals tested proved positive. Seventy six (40 per cent) of the herds with a history of abortion and 35 (47.3 per cent) of the control herds contained one or more positive animals. These results are summarized in Table I. It can be seen that no significant difference was apparent in the incidence of reactors, either on a herd or an individual basis, between control animals and those with a history of abortion.

In 329 of the 463 aborting animals, the stage of gestation at which abortion occurred was known and analysis revealed that in the 95 (28.8 per cent) which were positive there did not appear to be any significant relationship between the stage of gestation at which abortion occurred and the presence of herpesvirus antibodies. The percentage of positives ranged from a low of 22.2 during the third month of pregnancy to a high of 37.1 in the fourth month. These findings are outlined in Table II.

An analysis of the month-to-month occurrence of abortion in the 463 aborting animals revealed that the percentage of positive animals varied from a low of 19 (four animals) in June to a high of 34.8 (15 animals) in September. These results are also presented in Table II and it can be seen that no significant seasonal distribution is evident.

Analysis of the annual incidence of reactions obtained in the aborting animals during the years 1957-65 (Table III) shows some variations. The lowest incidence (7.4 per cent) was recorded in 1957 and the highest (49.4 per cent) in 1963. It is of interest that the incidence in 1964 was 17.1 per cent whereas of the control sera which were collected during that year 31.7 per cent gave positive reactions.

In May 1965 an isolation of bovine herpesvirus was made from the spleen of a fetus aborted at 155 days gestation. The herd of origin had been under surveillance since 1958 and had a history of a continuing mid-term abortion problem which is apparently of non-infectious etiology (12). Nine cows which had aborted during the years 1960-64 are included in the results of this study and only two of these showed herpesvirus antibody. The owner reported that the same animal, which aborted in May 1965 when the virus isolate was obtained, had aborted at 8 months of pregnancy in July of 1964 and had required four services before conceiving. Histological changes of pathological sig-

nificance were not apparent in the fetal tissues.

Discussion

The results obtained in this study indicate that bovine herpesvirus cannot be incriminated as the cause of a significant number of cases of abortion in which no other etiological agent can be implicated in those regions of Ontario and Quebec where the cattle tested were located. In fact the incidence of serologically positive animals in the group with a history of abortion was slightly lower than that found in the control group. The percentage figures obtained (28.3 and 31.7) were substantially higher than the 8.13 per cent reported in 1961 in S. Ontario (8) but were slightly lower than the 37 per cent found in Alberta cattle in 1962 (9). In a recent study on viral causes of abortion in Ohio (7), an incidence of 36.6 per cent serologically positive animals was reported in herds where abortion was a problem, however no controls were tested. It would certainly appear unjustifiable to incriminate this virus on serological evidence alone when over 30 per cent of the normal cattle population harbour antibody. Serum-virus neutralization test results, without further supporting evidence, can only be considered significant when paired serum samples, collected immediately after the act of abortion, show a markedly increased titre in the convalescent sample. The role of vaccination in the production of positive reactions should not be overlooked. Although a complete history was not available in all herds included in this study it is known that in many of them IBR vaccination was not practised.

It is impossible to draw any definite diagnostic conclusions from clinical evidence alone when abortion is the only manifestation of disease. Although abortions have been produced experimentally by inoculation of bovine herpesvirus during the first trimester of pregnancy (13), field observations indicate that the majority of naturally occurring cases take place in the third trimester (6). However, as it has been postulated (3) that bovine herpesvirus may in the future be associated with other syndromes than those so far described in cattle, it is all the more important that careful consideration be given to laboratory test results in relation to the clinical picture, before making a definite diagnosis.

The presence of oedematous placental membranes, hepatomagaly, subcapsular renal oedema and haemorrhage and generalized petechiation have been associated with abortion caused by this virus (6). It has even been suggested (7) that subcutaneous oedema, sero-sanguinous fluid in the pleural and peritoneal cavities and perirenal oedema and haemorrhage are significant lesions in fetuses infected by bovine herpesvirus. The latter findings are a common feature of aborted bovine fetuses and in our opinion cannot be considered pathognomonic of any single cause. In many cases they cannot be associated with the presence of an infectious agent.

Histologically, in fetuses aborted following experimental infection with herpesvirus (13), extensive focal necrosis of the hepatic parenchyma and the red pulp of the spleen was a striking feature together with lymphocytic infiltration of the hepatic triads and splenic trabeculae. Gross pathognomonic lesions were not observed. The failure to demonstrate intranuclear inclusion bodies may be due to the fact that fetal expulsion does not occur at the acute febrile stage but several weeks, or possibly months later. When fetal death occurs some time before the act of abortion post-mortem autolysis is usually advanced and precludes histological evaluation of tissue changes. This may also in many cases prevent the demonstration of any increase in the serum-virus neutralization titre in convalescent serum samples as a peak titre may have been reached prior to the time of fetal expulsion.

Virus isolation is a most important adjunct to confirming a diagnosis. However, as this virus is a member of the herpes group it is probable that it can survive within the living animal body for an indefinite period without necessarily causing any pathological damage. Our experience with the virus isolation made from the fetus described above, when considered in relation to the herd history leads us to suspect that virus isolation alone may be insufficient evidence on which to base a diagnosis of abortion due to this agent in the absence of any histological lesions.

It is important that clinical history, the results of serological, bacteriological and virological examinations and macroscopic and microscopic findings in fetal tissues and placentae, be considered before making

a final diagnosis of abortion due to bovine herpesvirus.

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News and Views

Record Year for University of Guelph

Th newest college at the University of Guelph, Wellington College of arts and science, is expected to become the largest, in terms of student registration, with the opening of the fall term in September. There will be approximately 1,700 students in the various classes at Wellington College, this year. The Ontario Agricultural College, always the largest in former years will register about 300 students fewer, as a total student body of some 4,370 students is listed on the Guelph campus. For the first time, the student body at Macdonald Institute is expected to increase considerably. With the fall enrolment there could be a student body of 600 young women. Still affected by limitations is handling large enrolments, the Ontario Veterinary College, the oldest of the four colleges at the university, will have a student body of approximately 270.

Another new high is expected to be reached in the school of graduate studies, now catering to master and doctoral degrees. It is reported that about 400 students from many parts of the world will enrol in this advanced study service. During the last year, there were approximately 3,500 students enrolled in the university.

Associate Dean at O.V.C.

President W. C. Winegard of the University of Guelph has announced the appointment of Dr. Dennis George Howell as Associate Dean (Research) at the Ontario Veterinary College. Dr. Howell, who will take up his position this summer, will co-ordinate the research program at O.V.C. Dr. Howell himself has been actively engaged in research projects, and has published a number of papers, particularly on brucellosis. For the past six years, Dr. Howell has been head of the Veterinary Department of Glaxo Laboratories Ltd., England. During this time he was a frequent visitor in veterinary schools and research laboratories in all parts of the world. A graduate of the Royal Veterinary College, University of London, in 1951, Dr. Howell later obtained both a Diploma in Bacteriology and a Ph.D.

Dr. Schofield Returns to Canada

Dr. F. W. Schofield of Korea visited Canada recently and paid a special visit to the Ontario Veterinary College. Dr. Schofield, an internationally known veterinary pathologist and author of many scientific papers, was for many years on the faculty of O.V.C., and was head of the Department of Pathology at the time of his retirement in 1955. After retirement, Dr. Schofield was on the faculty of Seoul National University and continued his special interest in the Orphanage at Mapo. Dr. Schofield has been honoured on many occasions, and in 1962 received an honourary degree from the University of Toronto.