Abortion Produced Experimentally in Cattle with an Agent of the Psittacosis-Lymphogranuloma-Venereum Group of Viruses

by Paul Boulanger and G. L. Bannister[1]

The psittacosis-lymphogranuloma-venereum (P.L.V.) group of viruses has been implicated as the cause of clinical manifestations in a wide variety of domestic animals and men. For many years attention was focused principally on the human disease and on the psittacine birds that serve as carriers of the human infection. In 1940, McNutt (1) described a sporadic bovine encephalomyelitis, which when studied further in 1953 by Menges (2) and by Wenner (3) proved to be due to an agent belonging to the P.L.V. group of virus. Related organisms were demonstrated in a pneumonitis of cats by Baker (4) in 1942, in enzootic abortion in ewes by Stamp (5) in 1950, in an enteritis of calves by York and Baker (6) in 1951 and in a pneumonitis of sheep by McKercher (7) in 1952. More recently other reports of the infection have appeared in the Japanese and French literature.

This paper presents observations made in cattle during the course of preparation of immune sera to be used as controls in the complement-fixation test.

MATERIALS AND METHODS

Source and Characteristics of the Virus

The Psittacosis-lymphogranuloma-venereum group of viruses contains a heat-stable antigen that is common to the entire group (8). Therefore an antigen prepared with one strain of virus cross reacts in complement-fixation tests with sera of other members of the group. With this property in mind, we adopted for the preparation of antigens and antisera the virus of "Enzootic Abortion of Ewes" (EAE) obtained in 1957 through the courtesy of Dr. J. F. Stamp, Moredun Institute, Edinburgh, Scotland. This strain of virus grew rapidly in embryonated chicken eggs and could be handled with relative safety.

Infection of Experimental Animals

Milking cattle, three to five months pregnant, were inoculated through the teat canal with this viral agent in an attempt to produce serum antibodies. Previous work performed in this Institute by Mitchell, Walker and Bannister (9-11) has shown that this route of inoculation resulted in high antibody production in the blood serum and the milk-whey of cattle injected with the influenza, the Newcastle disease or mumps virus.

In the first and second experiments cows B22105 and 707338 were inoculated with saline suspension of infected chick embryo yolk-sac membranes. In the third experiment cow 25350 was inoculated with infected milk from animal 707338 collected at the height of mastitis. The fourth experiment was composed of three cows, 276672, 65302 and 91-459 that were placed in the same cubicles as the infected animals to determine the spread of infection through contact.

Demonstration of the Virus

Smears were made from milk or placental tissue, stained by Machiavello's method and examined with the microscope or the material was inoculated into the yolk-sac of chick embryos as outlined in one of our preceding papers (12).

Complement-Fixation Methods

Each serum and milk-whey was ti-

[1] Animal Pathology Laboratories, Health of Animals Division. Canada Department of Agriculture, Animal Diseases Research Institute, Hull, Quebec.

trated by a two-fold serial dilution-method, using 0.1 ml. of serum dilution, 0.1 ml. of three 50 per cent haemolytic units of guinea pig complement and 0.1 ml. of antigen. The period of fixation was 18 hours at approximately 90°C, followed by 30 minutes at 37°C, after the addition of 0.2 ml. of a 2.5% suspension of maximally- sensitized sheep red blood cells, the density of which was adjusted with the aid of a Klett-Summerson photoelectric colorimeter.

The antigen for the complement-fixation test was prepared from yolk-sacs from embryonated eggs inoculated with freshly harvested well adapted egg E. A.E. virus. Pools from heavily infected yolk sacs were homogenized in a Virtis homogenizer and phenolized to a 5 per cent concentration. After standing in the refrigerator for a week, the phenolized suspension was homogenized with four volumes of beef heart broth of pH7. The course particles were removed after slow centrifugation or standing. The supernatant after heating in boiling water for 30 minutes, constituted the antigen. Normal control antigens were prepared in a similar way from yolk-sac membranes of non-inoculated embryonated eggs.

The milk on the day of collection was coagulated by the addition of a drop of rennin. After centrifugation the supernatant whey was kept frozen until the test was performed. The blood serum was also kept frozen prior to test.

RESULTS

Experiment 1

In this experiment the cow (B22105) two days after inoculation developed a severe self limiting mastitis which has been described in a previous paper (12). The virus was demonstrated in

Fig. 1

Placenta of aborted cow (B22105) showing haemorrhages and necrosis

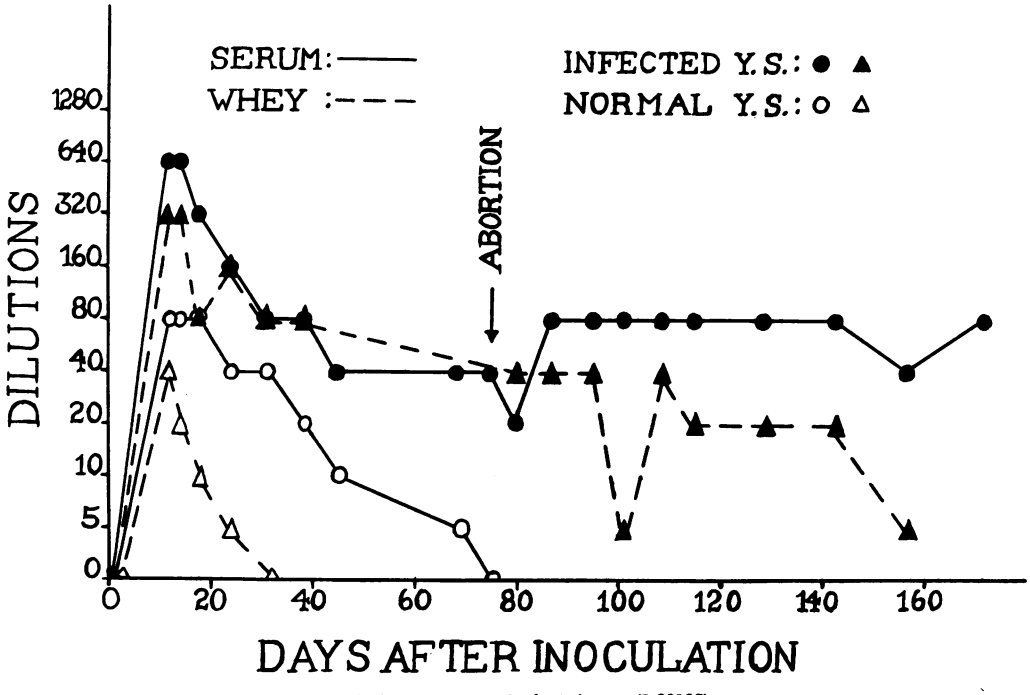
her milk for the first 10 post-injection days. The appearance of the milk had returned to normal on the 12th day. Afterward this animal remained clinically normal until the 75th post inoculation day when she aborted a premature foetus. The presence of elementary bodies in the foetal membranes was demonstrated by direct microscopic examination, by chick embryo inoculation and by intramuscular inoculation of another cow (22192). This second animal, initially negative, reacted positively seven days later in the complement-fixation test.

The placenta of the aborted cow (Fig. I) showed lesions which on macroscopic examination could not be differentiated from those produced by *Brucella abortus* infection. There was necrosis of the cotyledons and chorion. Some cotyledons were extremely hemorrhagic and others had a yellow straw-coloured appearance. The serum and whey titres of this animal on the 12th day after inoculation were 1:640 and 1:320 respectively with the infected antigen (Fig. II). The corresponding titres with the normal control egg antigen were 1:80 and 1:40 indicating a response to the egg material as well as to the virus. The antibodies for the normal egg protein had disappeared from the whey by the 26th day and from the serum by the 75th day at which time the animal aborted. Both the serum and the whey, had titres of 1:40 with the viral antigen on that day. Five days after abortion the serum titre had fallen to 1:20 but had risen to 1:80 by the 12th day and remained at this level for more than three months afterward.

Experiment II

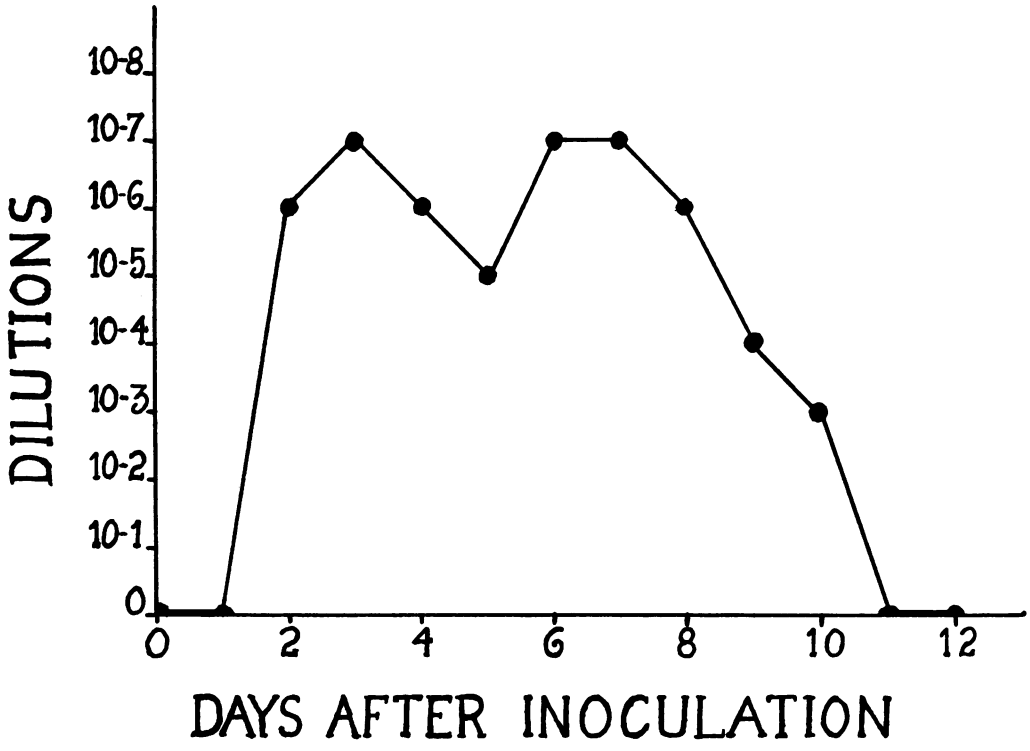
The clinical manifestations described in the first experiment were not entirely duplicated in the second trial. Two days after intramammary inoculation

Fig. II



Serological response of aborted cow (B.22105)

Fig. III



Infectivity of mastitis milk from cow 707338 for embryonated chick eggs.

with the same virus, the cow (707338) showed a very high temperature, 107°C. She refused feed and the right side of the udder, the only one inoculated, was hot and firm to palpation. The milk contained a few clots which deposited upon centrifugation. The 3rd day, the temperature of the animal was still high, 106°C. and there was evidence of severe mastitis in the inoculated side of the udder. On the 4th day the temperature was down to 104.6°C. the cow commenced to eat but some vaginal mucous discharge was present. On the 6th day this cow aborted two dead calves, possibly due to the high temperature reaction resulting from the severe udder inflammation.

The psittacosis agent could not be demonstrated in the placenta neither by direct microscopic examination nor by embryonated egg inoculation. The milk when inoculated into embryonating chicken eggs, reached a high titre, 10-7, on the 3rd, 6th and 7th days post injection (Fig. III) proving that the virus had multiplied in the mammary gland.

Antibodies were demonstrable, by means of the complement-fixation test in the blood serum of this animal on the 6th day after infection (Fig. IV). The titre reached a peak of 1:640 on the 9th day and was still at this level on the 14th day. However it had considerably decreased by the 36th day and almost entirely disappeared three months after infection. This, in contrast to the serological picture presented by the animal in the first experiment, was interpreted as a sign of transient infection confined to the mammary gland. The antibodies were demonstrated in the inoculated side of the udder, one day after they were present in the blood serum. Another day elapsed before they were present in the

milk whey of the non inoculated side, where they were of slightly lower titre.

Experiment III

In the last experiment, the cow (25-350), inoculated with infected mastitis milk from animal 707338, showed a very few small clots in her milk on the 7th day after inoculation. She remained clinically well throughout the experiment and calved normally. Complement-fixing antibodies appeared in the blood serum on the 8th day after inoculation and by the 12th day reached a titre of 1:640 which was comparable to the observation made in the second experiment (Fig. V). The antibodies were not demonstrable in the milk-whey until the 10th post-inoculation day, and did not reach as high a titre as in the previous experiment.

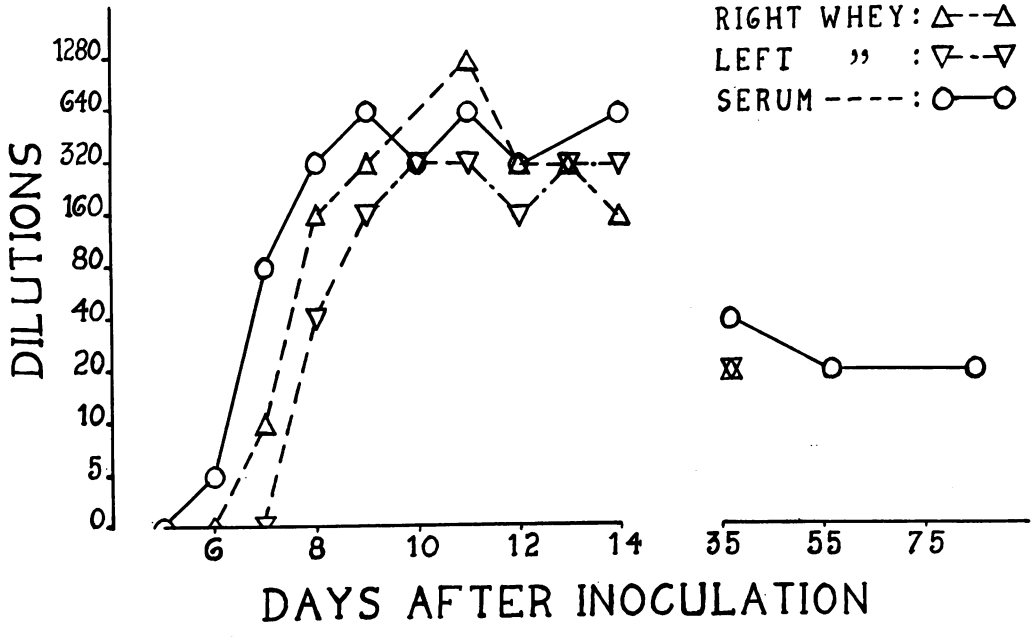
Experiment IV

The three contact animals, that were placed in the respective cubicles with the infected animals, remained clinically healthy throughout the experiment. Complement-fixation tests performed on their sera did not reveal the presence of antibodies indicating that the infection was not transmitted to these animals through contact.

DISCUSSION

Many abortions are seen in cattle in which no etiological agent such as *Brucella abortus, Vibrio foetus* or *Leptospira* can be demonstrated. The clinical history of some of these problematic herds indicate that often the abortions are preceded by an abnormal thick yellow milk which is sterile in the routine bacteriological tests for mastitis. In a few herds presenting symptoms of atypical mastitis, we have succeeded in demonstrating that *Leptospira pomona* was implicated as will be reported later. However in many cases the cause of abnormal milk and subsequent abor-

Fig. IV



Serological response of cow 707338

tion remains unknown.

In view of the wide distribution of the P.L.V. group of viruses in domestic animals of certain countries, it seemed worthwhile to consider the possibility of these agents being the etiological agent in abortion of cattle. Such a thought was expressed by Giroud (13) who demonstrated elementary bodies in the placenta of aborted cattle. We have demonstrated experimentally, in one animal, that both the viral agent and the antibodies can co-exist in a subject for a long period without the appearance of clinical manifestations. Apparently the infection flared up and resulted in abortion. In this case evidently the infectious agent, migrated from the mammary gland to colonize in the uterus. It seems a logical assumption that with favorable condition, such migration to the uterus, could take place from other organs such as the intestines or the lungs where this group of agents are known to cause diseases.

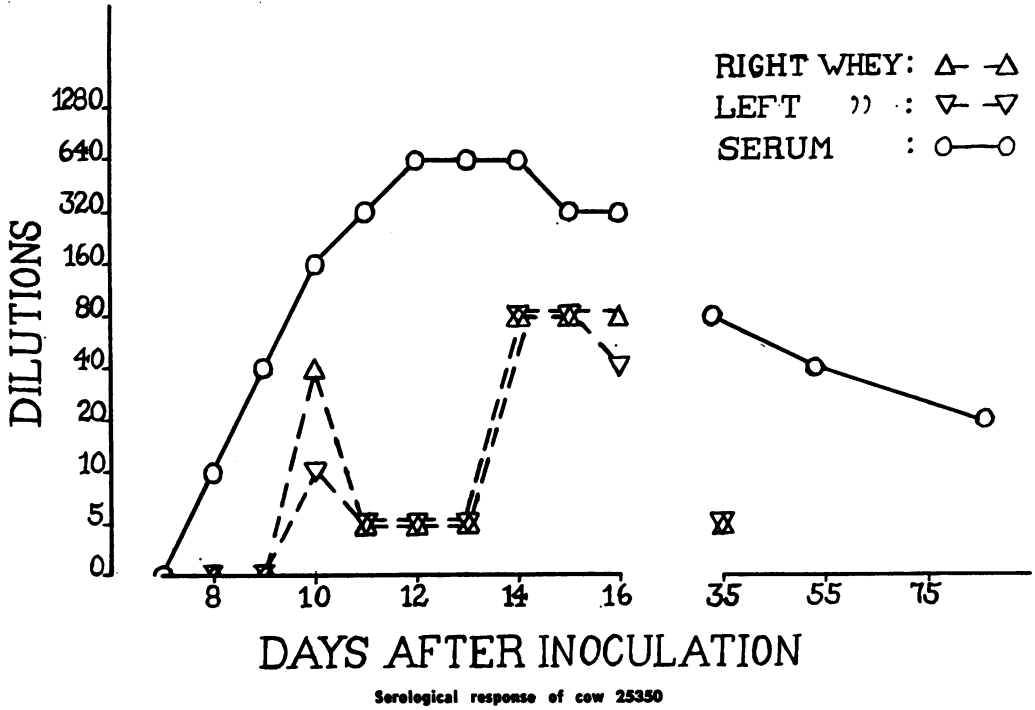
SUMMARY

Intramammary experimental inoculation of a pregnant milking cow with an agent of the psittacosis-lymphogranuloma-venereum group of viruses resulted in a severe mastitis followed by abortion seventy-five days after inoculation. However in two other similarly exposed animals, the udder infection occurred as revealed by the high serological response in the blood serum but the infection did not progress to the uterus. Three control animals placed in contact with the infected subjects remained normal clinically and serologically indicating that the infection was not transmitted readily by contact.

RESUME

Une mammite suivit d'avortement

Fig. V



Serological response of cow 25350

soixante-quinze jours plus tard, a été produite expérimentallement chez une vache laitière en gestation à la suite de l'inoculation intramammaire dans le canal galactophore d'un agent du groupe de la psittacose-lymphogranulomatose-venereum. Toutefois chez deux autres vaches laitières en gestation, bien que l'infection de la mamelle ait eu lieu tel que démontré par l'épreuve sérologique du sang, la maladie ne s'est pas propagée à l'utérus comme dans le premier cas. Trois vaches mises en contact des sujets infectés sont demeurées normales à l'examen clinique et sérologique indiquant que l'infection ne s'est pas transmise par simple contact.

ACKNOWLEDGEMENTS

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Réunion de deux Sociétés de Médecine Vétérinaires à Magog, P.Q.

Ces jours derniers, les Sociétés de Médecines vétérinaires — région de Qubec et région de Mntréal — tenaient une réunion conjointe au Club du Mont Orford, à Magog, dans les Contons de l'Est.

Cette réunion, dont l'organisation avait été confiée aux docteurs Gustave Faniel, M.V. et R.-B. Charpentier, M.V. de Magog, fut un réel succès. Elle groupait de nombreux vétérinaires ainsi que leurs épouses, venus de divers coins de la province.

Le programme avait été tracé de façon à joindre l'utile à l'agréable. Dans l'après-midi, les amateurs de golf purent pratiquer leur sport favori. Il y eut ensuite un coquetel, une courtoisie de la Cité de Magog, suivi d'un banquet.

La réunion scientifique comportait une conférence du docteur L.-A. Gendreau, de Sherbrooke, qui fit une revue complète de la mammite bovine et des problèmes qu'elle suscite. Puis, un forum animé par le docteur Martin Trépanier, Chef des Cliniques à l'Ecole de Médecine vétérinaire, à St-Hyacinthe, souleva de nombreuses discussions.

Pendant les conférences, les dames ont pu assister à une démonstration d'orgue Hammond, faite par les Studios d'orgue Hammond, de Sherbrooke.

Une danse termina la soirée.

La Cie ROGAR, de St-Hyacinthe, a facilité la tenue de cette réunion par l'apport d'une contribution substantielle.