Rinderpest Studies

Attenuation of the Rabbit Adapted Strain of Rinderpest Virus

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From early in 1943 to February 28, 1946, a project on rinderpest was conducted at the War Disease Control Station by the Dominion Department of National Defence and the United States Department of War. This was under the supervision of a joint Canadian-United States Commission. The project was taken over and conducted for six months by the Division of Animal Pathology of the Dominion Department of Agriculture. The majority of researches undertaken during the period in which the project was under the Joint Commission have been reported in a special issue of the American Journal of Veterinary Research (1). Baker (2) in one of these studies reported the adaptation of a bovine strain of rinderpest virus to rabbits, by five successive passages alternating between calves and rabbits. This resulted in the establishment of the virus in rabbits so that serial passages could be maintained by the intravenous route of inoculation. Once adapted it regularly produced a sharp rise in temperature 36 to 48 hours following inoculation. Rabbits killed at the peak of temperature showed congestion of the gastric and intestinal mucosa of varying intensity and usually an enlargement of Peyer's patches. Death rarely occurred and concurrent with the return of temperature to normal, recovery was prompt and complete. By February 1946, this lapinized strain of rinderpest virus after 65 serial passages through rabbits remained virulent for cattle, thus demonstrating that no attenuation of the virus had resulted from the rabbit passages.

Attempts to propagate the infected rabbit spleen in developing chick embryo were unsuccessful, presumably because the concentration of virus in the spleen was insufficient to reach the threshold of infection in embryos. However, using an inoculum of virulent spleen from calf No. 826 a new series of egg passages was successfully carried out. Calf No. 826 developed typical rinderpest infection following the subcutantous inoculation of virulent spleen from a rabbit of the 5th serial transfer after five alternating calf-rabbit passages had been made. Spleen tissue from calf No. 826 in dilutions as high as 10^{-5} regularly produced a febrile reaction with subsequent pathological lesions in rabbits whereas this failed to occur by the injection of rabbits with the regular bovine strain of rinderpest virus even in large doses by any routes of inoculation. Incidentally, the infective spleen from this calf was the source of virus from which the 65th serial rabbit passage resulted. On the basis of these facts, it was believed changes occurred during the alternating calf-rabbit passages which resulted in the adaptation of the virus to rabbits. Consequently, the strain from calf No. 826 is referred to as "lapinized."

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Using the technique described by Jenkins and Shope (3) for adaptation of the virus in the developing chick embryo, it was found, after 7 serial passages on the chorio-allantoic membrane of 10-day incubated eggs, that the allantoic and amniotic fluids did not, at first, contain an amount of virus sufficient to establish infection in the yolk-sac of 7-day incubated eggs. The complement fixation test as described by Cooper (4) demonstrated a gradual increase of virus in the egg fluid of each passage and eventually the use of this fluid as inoculin was successful. All serial transfers were made at 96-hour intervals. A calf (No. 1049) inoculated with fluid from the 20th yolk sac passage developed typical rinderpest infection from which it recovered and later proved immune to challenge.

Attempts were made to immunize rabbits with dried rinderpest vaccine prepared from the egg-propagated bovine strain of virus. Large doses of the reconstituted undiluted vaccine administered intravenously did provoke immunity in some rabbits as evidenced by their resistance when challenged 21 days later with "lapinized" virus; but the results were frequently inconsistent with potency tests in cattle. It was believed that some rabbits were absolutely refractory to the bovine vaccine, others slightly susceptible; the latter group being immune when challenged with the "lapinized" strain. This inconsistent susceptibility in rabbits precluded their use in determining the potency of the vaccine.*

When the Division of Animal Pathology assumed responsibility for the project, a further study of egg passages of the "lapinized" strain was made. It was important to determine if attenuation could be brought about which would permit using this strain as a vaccine to immunize cattle against rinderpest, at the same time retaining the "lapinized" characteristics of producing thermal reactions and immunity in rabbits. If this proved possible, rabbits could be employed to determine the immunizing properties of such a vaccine, prepared according to the technique of Hale and Walker (5), which would eliminate the expense involved when cattle have to be used to determine potency.

With the knowledge that the 20th serial yolk sac passage of the "lapinized" strain through eggs proved virulent to calves, groups of eggs were inoculated with the stock seed inoculum of the 17th, 18th and 19th yolk sac passages that had been stored in the frozen state. The yolk sac membranes and fluids were harvested from each group, bottled and stored at -20°C. on January 4, 1946, for future use as seed material.

Experimental Studies

After 86 days frozen storage, the contents of a bottle containing the 19th yolk sac passage of the "lapinized" strain were allowed to thaw at room temperature. Two series of egg passages were commenced, on 7-day incubated eggs, using as inoculum, in one, pooled yolk sac membranes and fluids and, in the other, only the fluids.

Since, as reported by Jenkins and Shope (3), the attenuation of the bovine strain of rinderpest virus apparently depended on the rapidity of transfer through eggs at 48-hour intervals, it was desirable that this procedure be followed in the contemplated egg passages of the "lapinized" virus. However, to ensure virus growth, because the stock seed virus had

*Contained in the 37th, 38th and 39th Monthly Reports submitted to the members of the Joint Canadian-United States Commission by the Director of Project, War Disease Control Station.

been in frozen storage over a period of nearly three months, two passages in each series were made first at 96-hour intervals. Thereafter, continuous 48-hour passages were made. This was found unsatisfactory in the series in which the fluids only were used for transfer, and it became necessary to recommence this series and revert to successive transfers at 96-hour intervals.

In each instance, the materials harvested for serial passage were checked for bacteriological sterility by inoculating suitable culture media and incubating under aerobic and anaerobic conditions.

Series I. — Transfers of pooled yolk sac membranes and fluids were made from the stock strain of the 19th yolk sac passage and carried through in serial order to the 29th passage. In each transfer, 0.5 cc. was injected into the yolk sac, as described by Shope et al. (6). After harvesting the 29th passage and preparing the inoculum for the following passage, 1.0 cc. of the material was injected subcutaneously into calf No. 4. In approximately 48 hours, a diarrhoea developed although the animal continued to eat and drink normally and no febrile reaction was at first observed. The diarrhoea, somewhat profuse at the beginning, gradually diminished and disappeared by the 10th day. On the 6th day, the temperature became elevated and this continued to the 10th day. No other symptoms of infection were noted. While the early appearance of the diarrhoea was somewhat confusing, the later febrile reaction was suggestive of a mild type of rinderpest infection. Otherwise, this febrile reaction appeared to be similar to that reported by Jenkins and Shope (3) and Hale and Walker (5) in calves following administration of the attenuated egg-passaged bovine strain of virus or the dried vaccine prepared from the attenuated strain.

Sixteen days after inoculation, calf No. 4 received a challenge dose of 1.0 cc. of a 10^{-2} suspension of virulent spleen from calf No. 1000, subcutaneously. No infection resulted and the animal remained normal until slaughtered 20 days later. A normal control, calf No. 9, received a similar amount of the virulent suspension and after an incubation period of between 2 and 3 days became febrile and presented typical symptoms of rinderpest infection.

From this result it is evident that the yolk sac membrane and fluid suspension of the 29th yolk sac passage contained virus which was apparently attenuated, as evidenced by the resistance of the calf to a subsequent inoculation of virulent virus.

Further yolk sac passages were continued to the 36th passage, at which time the harvested yolk sac membranes and fluids were used to prepare a vaccine, using the method of lyophilization with dry ice and alcohol described by Hale and Walker (5). The vaccine was reconstituted two days later with an appropriate amount of sterile physiological saline solution, and a dose of 0.5 cc. administered subcutaneously into Calf No. 14. At the same time, Rabbits No. 5, No. 6 and No. 7 received 1.0 cc. of the vaccine intravenously. These animals were non-immune when later challenged, as shown in Table I.

Series II.

The stock strain of the 19th yolk sac passage contained pooled yolk sac membranes and fluids and was used for the original passage in this series. For all subsequent transfers, which were carried out at 48-hour intervals, the allantoic and amniotic fluids were harvested for inoculation

purposes. After the 30th yolk sac passage, the fluids were harvested and 1.0 cc. inoculated subcutaneously into Calf No. 3. No evidence of infection was observed during a 16 day post-inoculation period. A challenge dose of 1.0 cc. of a 10^{-2} suspension of virulent spleen from Calf No. 1000 was injected subcutaneously, and typical rinderpest infection resulted with death on the 8th day after challenge. This indicated the absence of virus in the 30th egg passage of fluids, which probably resulted from rapidity

Table 1

IMMUNE RESPONSE TO EGG-PASSAGED LAPINIZED VIRUS

Animal	Inoculum	Clinical Effects Following	Days Later	Source of Virulence Virus	Result
Calf No. 4	Fluids and y. s. membs. 29th y. s. passage (48-hour intervals)	Mild Infection	16	1.0 cc. of a 10^{-2} suspension of spleen from calf No. 1000	Not Infected
Calf No.3	Fluids from the 30th y.s. passage (48-hr. intervals)	None	16	1.0 cc. of a 10^{-2} suspension of spleen from calf No. 1000	Infection
Calf No. 14	Vaccine from Fluids and y. s. membs. 36th y.s. passage (48-hour intervals)	None	14	1.0. cc. of 10^{-2} suspensions of spleen from Calf No. 9	Infection
Rabbit No. 5	Vaccine from Fluids and y. s. membs. 36th y. s. passage (48-hour intervals)	Normal	21	1.0 cc. of a 10^{-2} suspension of spleen from calf No. 826	Infection
Rabbit No. 6	Vaccine from Fluids and y. s. membs. 36th y.s. passage (48-hour intervals)	Normal	21	1.0 cc. of a 10^{-2} suspension of spleen from calf No. 826	Infection
Rabbit No. 7	Vaccine from Fluids and y. s. membs. 36th y. s. passage (48-hour intervals)	Normal	21	1.0 cc. of a 10^{-2} suspension of spleen from calf No. 826	Infection
Calf No. 15	Vaccine from fluids 29th y. s. passage (96-hour intervals)	Normal	14	1.0 cc. of a 10^{-2} suspension of spleen from calf No. 9	Not Infected
Rabbit No. 8	Vaccine from fluids 29th y. s. passage (96-hour intervals)	Normal	21	1.0 cc. of a 10^{-2} suspension of spleen from calf No. 826	Not Infected
Rabbit No. 9	Vaccine from fluids 29th y. s. passage (96-hour intervals)	Normal	21	1.0 cc. of a 10^{-2} suspension of spleen from calf No. 826	Not Infected
Rabbit No. 10	Vaccine from fluids 29th y. s. passage (96-hour intervals)	Normal	21	1.0 cc. of a 10^{-2} suspension of spleen from calf No. 826	Not Infected

CONTROLS Calf No. 9 (for Calves No. 3 and No. 4	- -	1.0 cc. of a 10^{-2} suspension of spleen from calf No. 1000	Infection
Rabbit No. 1 (for lapinized strain)	—	1. 0 cc. of a 10^{-4} suspension of spleen from calf No. 826	Not Infected
Rabbit No. 2 (for lapinized strain)	—	1.0 cc. of a 10^{-3} suspension of spleen from calf No. 826	Infection
Rabbit No. 3 (for lapinized strain)	—	1.0 cc. of a 10^{-2} suspension of spleen from calf No. 826.	Infection
Calf No. 19 (for lapinized strain)	—	1.0 cc. of a 10^{-2} suspension of spleen from calf No. 826	Infection
Calf No. 16 (for bovine strain)	—	1.0 cc. of a 10 suspension of spleen from calf No. 9	Infection

of the transfers. The yolk sac passage of fluids from this series was discontinued and a new series commenced with the serial transfers being made at 96-hour intervals.

The fluids from the 29th yolk sac passage were harvested, bottled, shell-frozen and dried similar to that mentioned in connection with the 36th passage yolk sac membranes and fluids of Series I. The vaccine prepared from the fluids was reconstituted two days later and 0.5 cc. inoculated subcutaneously into calf no. 15. Simultaneously, rabbits No. 8, No. 9 and No. 10 received 1.0 cc. of the vaccine intravenously. These were challenged later and proved to be immune. (Table I.)

It was found that an immune response in calves began as early as the 8th day following vaccination and, except in rare instances, was always present between the 10th and 12th days. Consequently, challenge inoculations were carried out in all potency tests on or about the 14th day. In rabbits, however, the immune response was slower, thus, usually, 21 days were allowed to elapse before the challenge dose of virulent material was administered.

The challenge inoculum for calves No. 14 and No. 15 was a suspension of virulent spleen from calf No. 9. This calf, previously used as a normal control, had been inoculated with virulent spleen from calf No. 1000, which was a serial passage of the original bovine virus supplied for the rinderpest studies.

The challenge inoculum from calf No. 826 was the "lapinized" strain for the rabbits and control calf No. 19. Calf No. 826 was originally infected with the spleen from a rabbit of the 5th serial transfer after five alternate calf-rabbit passages, and the spleen harvested served as material for the experimental egg passages of the lapinized strain of virus.

Discussion

From the results presented in Table I, it will be noted that, in the series in which the yolk sac membrane and fluids were used, the 29th yolk sac passage apparently produced a mild type of infection in calf

No. 4, but a vacccine prepared from the same tissues harvested from the 36th serial passage failed to either infect or cause an immune response in calf No. 14. There is the possibility of rapid attenuation occurring between these passages. However, since the 29th serial passage produced a mild infection, it is more likely that the virus was accidently inactivated during the egg passages. Lyophilization could not be responsible for the loss of viability, since the vaccine produced from the fluids of the other series that stimulated an immune response in both rabbits and a calf was dried during the same "run."

The results obtained from a vaccine prepared from the 29th serial yolk sac passage of fluids harvested at 96-hour intervals definitely indicate an attenuation of the "lapinized" strain of virus. Calf No. 15 and rabbits No.8, No. 9 and No. 10 remained normal throughout the post-vaccination period and were subsequently immune to challenge with the bovine and "lapinized" strains of virus respectively. The egg-attenuated "lapinized" virus produced immunity in a calf against a challenge dose of the bovine virus and at the same time the "lapinized" characteristics persisted sufficiently to completely immunize rabbits against the challenge inoculation of the "lapinized" virus. Similarly, the "lapinized" virus suspension used for challenge produced infection in the control calf No. 19 as well as in the control rabbits.

Summary

The "lapinized" strain of rinderpest virus became attenuated after 36 successive passages through developing chick embroyos, the first 7 passages being on the chodio-allantoic membrane and the remaining 29 passages in the yolk sac. A lyophilized vaccine prepared from fluids of the 29th yolk sac passage induced immunity in a calf and rabbits.

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Sommaire

LA VIRULENCE d'une souche du virus de la peste bovine adaptée au lapin est atténuée après 36 passages consécutifs par l'embryon du poulet; dans les sept premiers passages le virus est culitvé sur la membrane chorio-allantoïde, dans les vingt-neuf autres, le virus est inoculé dans le sac vitellin. Un vacicn lyophilitique préparé à partir de liquide vitellin, obtenu au vingt-neuvième passage, confère l'immunité au veau et au lapin.

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