Interferon, Antibody Responses and Protection Induced by an Intranasal Infectious Bovine Rhinotracheitis Vaccine

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SUMMARY

The interferon and antibody response induced by an intranasal infectious bovine rhinotracheitis vaccine was followed in 22 calves over a nine month period and the ability of these vaccinated calves to withstand challenge with virulent infectious bovine rhinotracheitis virus was assessed.

Interferon was detected two to three days postvaccination and disappeared by the tenth day. Nasal and serum antibodies appeared by day 7 and persisted for nine months.

The calves challenged three days postvaccination came down with disease typical of infectious bovine rhinotracheitis, whereas calves challenged three weeks, three months or nine months postvaccination resisted infection.

RÉSUMÉ

Interféron, réponse immunitaire et protection consécutifs à la vaccination intra-nasale contre la rhino-trachéite infectieuse bovine

Au cours d'une période de neuf mois, les auteurs suivirent le profil de l'interféron et des anticorps, chez 22 veaux auxquels ils avient administré un vaccin intra-nasal contre la rhino-trachéite infectieuse bovine. Ils vérifièrent aussi la réaction de ces veaux à une infection de défi avec une souche virulente du virus de la rhino-trachéite infectieuse bovine.

Ils démontrèrent la présence d'interféron, de deux à trois jours après la vaccination; huit jours plus tard, il avait cependant disparu. Les anticorps sériques et nasaux apparurent sept jours après la vaccination et persistèrent durant neuf mois.

Les veaux soumis à une infection de défi, trois jours après leur vaccination, développèrent une rhino-trachéite infectieuse typique, contrairement à ceux qui subirent cette infection au bout de trois semaines et trois ou neuf mois après leur vaccination.

INTRODUCTION

Efforts to improve vaccination procedures for the control of infectious bovine rhinotracheitis, have led to the development and use by intranasal administration, of modified live virus vaccines (1, 2, 5, 6). Todd and co-workers (4, 5) were among the first to demonstrate the presence of interferon as well as secretory antibody in the nasal secretions of calves exposed to an intranasal vaccine and they associated the early resistance of the calves challenged with virulent infectious bovine rhinotracheitis virus (IBRV) to the presence of interferon in the nasal secretions. The studies reported here were undertaken in an attempt to evaluate this premise and to provide additional data on the duration of immunity produced by intranasal vaccination of calves.

MATERIALS AND METHODS

Over an eight month period, four groups of four to six week old calves were purchased at a local salesbarn and shipped to the isolation unit at the Ontario Veterinary College. Following a three day acclimatization period, a commercially available intranasal IBRV vaccine[1] was administered to each of 22 calves according to the manufacturer's instructions. Calves were held in isolation rooms for a further three week observation period and then transferred to a one-acre fenced plot. Prior to challenge the calves were again returned to the isolation rooms and remained there until the termination of the experiment. Groups of calves were challenged with virulent Cooper strain of IBRV[2] at three days, three weeks, three, five or nine months postvaccination (see Table I), and an additional four calves were used as unvaccinated controls. A stock of Cooper strain of IBRV with a titer of $10^{6.7}$ cell culture infectious dose 50 ($CCID_{50}$) per 0.1 ml was prepared in secondary embryo bovine kidney (EBK) cell cultures and aliquots stored at -70°C until used. Six ml of the undiluted stock virus was introduced into the nasal passages of each calf at the time of challenge in the same manner as the vaccine virus. Nasal swabs, nasal secretions, and blood samples were collected at predetermined intervals and assayed for the presence of virus, antibody and interferon. Virus assays were done in duplicate EBK cell culture tubes. Nasal secretions were collected as described by Rouse and Angulo (3) and serum and nasal antibody levels were determined by a standard virus neutralization technique in cell culture tubes using 100 $CCID_{50}$ of IBRV (Cooper). The nasal

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[1]Jensen-Salsbery Laboratories, Kansas City, Missouri 64141.

[2]Obtained through the courtesy of Dr. Boylan, Connaught Medical Research Laboratories.

TABLE I

RESPONSE OF CALVES AFTER VACCINATION WITH INTRANASAL IBR VACCINE

No. of calves in group	Period between vaccination and challenge	At time of challenge: Virus neutralization activity	At time of challenge: Interferon activity	Response to challenge
4	Unvaccinated	—	—	IBR
3	3 days	—	+	IBR
4	3 weeks	+	—	No clinical response
5	3 months	+	—	No clinical response
5	5 months	+	—	No clinical response
5	9 months	+	—	No clinical response

secretions used for interferon determinations were first dialysed against 0.1 M HCl-KCl buffer pH2 at 4°C for 24 hours, and then dialysed against phosphate buffered saline pH 7.2 for another 24 hours. Dilutions ranging from undiluted to 1:160 were made in phosphate buffered saline (PBS) and 0.5 ml inoculated in EBK culture tubes. After overnight incubation at 37°C, the culture tubes were washed with PBS, and 0.1 ml of vesicular stomatitis virus (VSV) containing 1000 $CCID_{50}$ was added to each tube before refeeding with fresh medium. A complete set of controls were included in all interferon assays.

To confirm the hypothesis that the inhibition of VSV was due to the presence of interferon, selected samples of nasal secretions were tested and shown to satisfy the following criteria: 1) activity stable at pH 2, 4°C for 24 hours, 2) activity stable at 56°C for 30 minutes, 3) absence of toxicity in cell cultures, 4) retention of antiviral activity after thorough washing of cell cultures prior to inoculation with VSV, 5) absence of inactivating effect on the virus (VSV) alone and 6) an inhibitory effect against heterologous virus, i.e. adeno and BVD viruses.

RESULTS

The main findings are summarized in Table I and Figure 1.

The 22 calves that were vaccinated with the

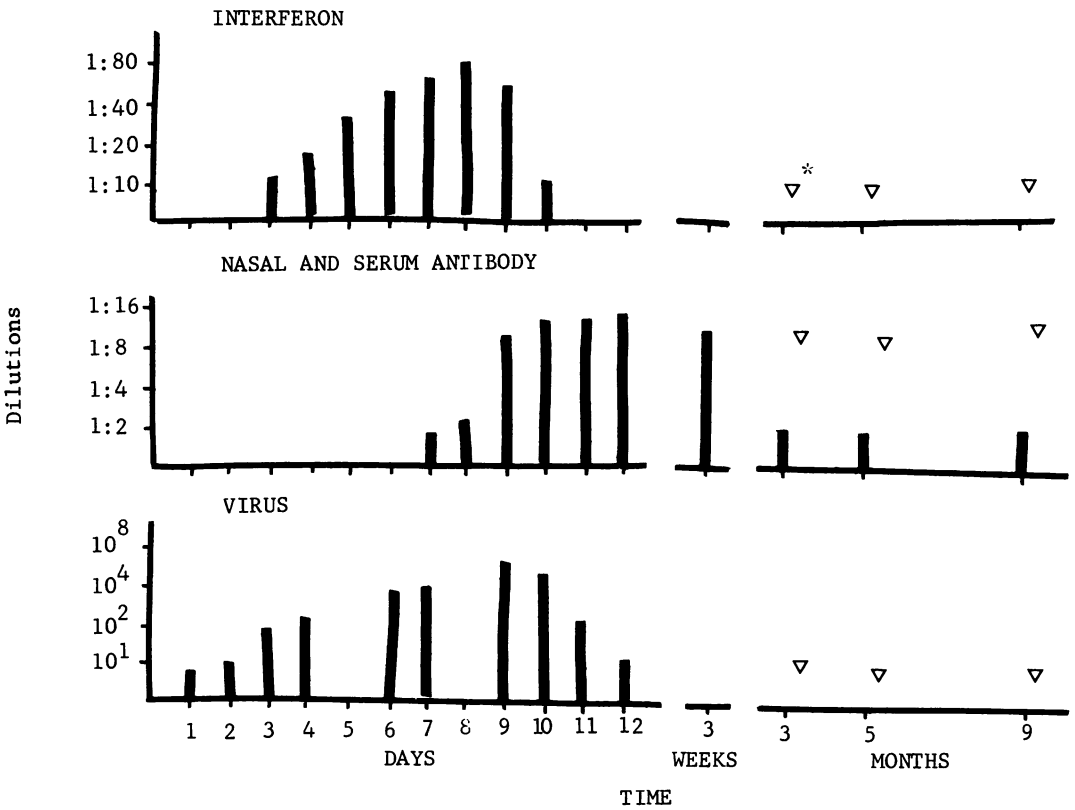

FIGURE 1. Average levels of interferon, antibody and virus in calves vaccinated with an intranasal IBR vaccine.
*Average levels three to five days after challenge with virulent IBR virus.

commercial intranasal IBR vaccine did not manifest any overt clinical signs of IBR disease following vaccination, even though they had been subjected to the stress associated with shipping and handling. The calves did appear to be dull and lethargic on the third day postvaccination and had temperatures of 41°C or greater, but the rise in temperature did not persist for more than two days.

Following vaccination, the calves shed virus for an average period of two weeks. On day 1 postvaccination, we recovered 10^0 to $10^{2.5}$ $CCID_{50}$ per 0.1 ml of nasal secretions. The virus titer in nasal secretions started to increase on day 2 and by day 9 titres varying between 10^4 and 10^6 $CCID_{50}$ were detected. After day 9, the virus titre in nasal secretions decreased until at the 14th day postvaccination, the virus was detected only in undiluted nasal secretions (Figure 1).

All of the vaccinated calves developed both serum and nasal antibody titres to levels between 1:2 and 1:16. In ten calves, serum and nasal antibodies appeared by the seventh day and by the 22nd day all calves had significant antibody titres. The antibody levels then began to decrease but low levels (1:2-1:4) persisted in the five calves held for nine months prior to challenge (Figure 1).

The nasal secretions of all 22 vaccinated calves contained detectable levels of interferon three days after vaccination, and by the fifth day to the eighth day postvaccination, the interferon titres had risen to 1:80. The levels then declined rapidly so that by the ninth day or tenth day, interferon activity was detected only in undiluted nasal secretions (Figure 1).

The groups of vaccinated animals were challenged with IBRV (Cooper strain) at three days, three weeks or three, five or nine months postvaccination. The three calves challenged at three days postvaccination had interferon titres of 1:32-1:64 and were shedding vaccine virus in their nasal secretions immediately prior to challenge. Following challenge, these three calves developed clinical signs typical of IBR with temperatures of 41°C persisting for four to five days, inflammation of the nasal mucosa, a serous to mucopurulent nasal discharge, coughing, dyspnea and inappetance. Virus titres in the nasal secretions ranged from 10^3 to 10^5 on the third and fourth day postchallenge, but by day 6 and 7, virus being shed had fallen to levels just detectable in undiluted samples. None of the remaining 19 vaccinates that were challenged manifested any clinical signs of disease other than mounting a mild temperature response (38°C) and appearing dull for two to three days following challenge. However, IBR virus (presumably the challenge virus) was isolated from the nasal secretions for six or seven days postchallenge and a secondary immune response was observed among all of the calves. That is, starting at five days postchallenge, antibody levels increased and by the 14th day, at least a fourfold rise was recorded in all animals. This response occurred in both serum and nasal antibody levels, although the serum response generally appeared earlier. For example, in 14 calves there was no increase in nasal antibody levels by the seventh day postchallenge. Interferon activity was detected at low levels in the challenged calves on days 3 and 4, and was not detected after day 5.

Nasal secretions containing interferon from vaccinated calves were tested in cell cultures for antiviral activity against a variety of viruses often associated with respiratory disease in cattle. Infection by the following viruses was inhibited or prevented: PI-3, BVD and adenovirus 1 and 3. This was evidenced by the absence of CPE or hemadsorption in EBK cell cultures pretreated with the nasal secretions in dilutions of up to 1:20, whereas partial to complete CPE was observed in the cell cultures pretreated with dilutions of 1:32 and in control cultures. However, pretreatment of the cell cultures that were exposed to the Cooper strain of IBR or three local IBR isolates or a bovine rhinovirus, failed to cause any inhibition of CPE, i.e. manifested viral infection.

DISCUSSION

The studies reported here confirmed the findings of Todd *et al* (4, 5) that interferon is induced in the nasal passages of cattle following intranasal vaccination with an avirulent strain of IBRV. Detectable levels of interferon first appeared three days after vaccination and persisted for nine or ten days. The same pattern was established in the group of control calves that received only the challenge virus, whereas, in the vaccinates that were subsequently exposed to challenge virus interferon appeared again three days later, but persisted for only two to three days. These differences in persistence of interferon following a second exposure to the virus may be explained by the fact that extensive virus replication may be necessary for the production of high interferon titres. Moreover, we found that the interferon produced in calves and tested in cell cultures, prevented infection of cell cultures by PI-3, BVD and bovine adenoviruses, but not by IBR or bovine rhinoviruses. The suggestion (4, 5) that interferon induced in the nasal secretions by the vaccine virus is responsible for subsequent resistance to virulent virus infection was not supported in our studies, since the three calves challenged three days after vaccination developed clinical disease, despite the presence of interferon at the time of challenge. These experimental findings support field observations (1) in which the same vaccine failed to induce early protection against IBR.

The presence of even low antibody levels in serum and nasal secretion was indicative of resistance to IBR infection with virulent virus although evidence of a secondary response appeared in the serum five to seven days

postchallenge. Challenge of the calves at intervals after vaccination indicated a duration of immunity of at least nine months.

Finally, the shedding of virus from vaccinates may be advantageous since other nonvaccinated animals brought in contact with the vaccinates could become "inoculated" with the vaccine virus via contact and thereby, also become protected.

ACKNOWLEDGMENTS

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BOOK REVIEW

Diphyllobothriasis in Man. Bertel von Bonsdorff. Published by Academic Press, London. 1977. 189 pages. Price $18.50.

Dr. von Bonsdorff has produced an exhaustive text on human diphyllobothriasis with particular emphasis on the Scandinavian disease due to *Diphyllobothrium latum* and its sometimes associated picture of megaloblastic anaemia.

The book in effect takes over the story where it was left off by Birkeland in 1932 and carries it through to the current decade.

From a North American point of view it might seem that 182 pages is rather much to devote to a topic that is given little coverage in most medical texts but it must be realised that this was written, originally in Swedish, in a part of the world where broad tapeworm infection and the associated problem of anaemia is of far greater frequency today than on this continent, and in the past was a much greater problem still.

The first half of the book, comprising three chapters on the biology, the epidemiology and the control of *Diphyllobothrium* infections is both interesting and readable. The second half which comprises two chapters dealing with the clinical manifestations and the pathogenesis of "Tapeworm pernicious anaemia" was for me rather heavy going in that I found it quite tediously repetitive. One had the feeling of having been pulled through the case history of every patient with megaloblastic anaemia associated with *Diphyllobothrium latum* infection in the last half century. Dr. von Bonsdorff is nothing if not thorough and leads us along every trail. The constant use of abbreviations was also visually disturbing.

The entire text bears the stamp of authority and quite evidently represents the distillation of a lifelong interest. It is probably worth shelving for the references alone which follow each chapter and number between 700 and 800 in all, though there is some duplication.

The book is commendably error free and shows evidence of careful editing, though a few inevitable mistakes have crept in. These are most apparent where they involve figures but are readily picked out and therefore unlikely to confuse the reader.

If there is one place where I would really take issue with the contents it is with the suggestion that fish wastes should be sterilised by boiling before being discarded in the belief that this would reduce the carriage of *D. latum* by mammals and *D. dentriticum* by gulls. If one could rely on the gulls to bring in the fish they catch themselves for cooking maybe, but in the present order of things I doubt that such a recommendation would make the slightest difference.

The fact that this book is a translation is not obtrusive. Only a few dropped definite and indefinite articles and the use of Scotch for Scottish in relation to coregonids make one realise that it was the work of one for whom English is not the mother tongue.

In summary this is a valuable acquisition to all biologists and clinicians with an interest in Diphyllobothriasis. *R.D.P. Eaton.*