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EVALUATION OF THE PROPHYLACTIC POTENTIAL OF AN IMMUNOMODULATOR AGAINST RESPIRATORY DISEASE IN CALVES

By

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LARSSON, B., C. FOSSUM, M. TÖRNQUIST, P. MATSSON and S. ALENIUS: *Evaluation of the prophylactic potential of an immunomodulator against respiratory disease in calves.* Acta vet. scand. 1985, *26*, 262—272. — The prophylatic effect against respiratory disease in feedlot calves of an immunomodulator, based on an inactivated avipox virus preparation, was evaluated in a double blind, placebo controlled field trial. The effect of the immunomodulator on phagocytosis and on the reactivity of the lymphocytes was also tested.

On the day of arrival at the feedlots and 3 days later 257 calves were injected with either the immunomodulator or with placebo. All calves were then observed for respiratory disease and treatments were recorded. The immunomodulator did not reduce the frequency of disease, compared to the placebo. Thirty percent of the calves treated with the immunomodulator and 27 % of the controls were treated with antibiotics for respiratory disease.

The cell mediated immune reactivity of 7 calves treated with the immunomodulator and of 7 untreated calves was tested. Monocytes isolated from the animals were examined for their ability to ingest latex beads and lymphocytes from the animals were examined for their response to different mitogens. Sera from each of the two groups were also investigated for the effect on phagocytosis. No difference in these parameters was observed between the two groups.

phagocytosis; lymphocyte stimulation; field trial; double blind; feedlot calves.

In Sweden, as in other countries, respiratory disease is common in feedlot calves (*Hurvell & Fey* 1970). The etiology is considered to be complex, including stress, environmental factors and different kinds of microorganisms (*Hoerlein & Marsh* 1957). Different immunomodulators such as levamisole (*Babiuk*

& Misra 1982, *Saperstein et al.* 1983) and modified infectious bovine rhinotracheitis virus (*Todd et al.* 1972, *Straub & Ahl* 1976, *MacLachlan & Rosenquist* 1982) have been used in order to enhance the effectiveness of the bovine immune response to infections. Preparations of inactivated avipox virus have been reported to decrease morbidity and mortality caused by respiratory disease in feedlot calves (*Wizigmann* 1978 a, b, 1980) and to prevent disease in other species, e.g. man (*Mayr et al.* 1977), horse (*Thein et al.* 1980), swine (*Mayr & Brunner* 1980) and dog (*Bibrack* 1976). Inactivated avipox virus preparations have also been reported to enhance phagocytosis (*Meyer & Mayr* 1981, *Henschelchen* 1984), to stimulate circulating T-lymphocytes and to induce interferon production and in this way enhance non-specific resistance to infections (*Mayr* 1979).

The aim of the present study was to investigate in a double blind field trial whether an immunomodulator, based on an inactivated avipox virus preparation (Duphamun®, Duphar B. H., Amsterdam), reduced the frequency of respiratory disease of calves in feedlot herds under Swedish conditions. Further, the effect of the immunomodulator on phagocytic capacity of monocytes and its ability to enhance the stimulation of lymphocytes by different mitogens was evaluated.

MATERIAL AND METHODS

Design of Experiment 1

In this experiment 257 calves, aged 3—7 weeks, mostly of the Swedish Red and White Breed (SRB) but also of the Friesian Breed and crossbreeds were used after their arrival at 4 feedlots (A, B, C and D). The calves were kept in boxes, 4—9 in each, until the trial ended. On the day of arrival the calves were marked and injected with either 2 ml of an inactivated avipox virus preparation (Duphamun®) subcutaneously (s.c.) or with 2 ml placebo s.c. The injections were repeated 3 days later. Both preparations were coded and kindly received from Duphar B. H., Amsterdam, Holland. In herds A, B and D the calves were treated boxwise with either the immunomodulator or placebo, and in herd C every second calf was treated with the immunomodulator and the others with placebo. All calves were observed for 1 month and treatments (antibiotics for 3—5 days) for respiratory disease and enteritis were recorded daily. The frequency of disease was calculated as the number of calves treated for disease for

the first time during the first month after arrival divided by the total number of calves, by herd and treatment group.

Nine calves in each treatment group in herd B were also controlled daily for rectal temperature during the first 23 days. A temperature of 39.5°C or more was considered as fever.

The code (Duphamun® or placebo) was broken after the completion of the experiments and compilation of data.

Design of Experiment 2

Fourteen calves of the SRB breed were after their arrival to feedlot D divided into two groups and one group was treated with the immunomodulator as described above and the other group (control) was left untreated. The calves were bled by jugular vein puncture on the day of arrival (day 0), day 3, 6 and 10. None of these calves were subjected to antibiotic treatment. The blood was examined for total and differential white blood cell count, proportion and capacity of phagocytes among blood mononuclear cells (MNC) and the responsiveness of lymphocytes to different mitogens.

Mononuclear cells, isolated from four SRB cows were also used in a phagocytic assay in which the effect of pooled sera from each of the two groups was evaluated on day 0 and day 6.

Total and differential white blood cell count: Using EDTA blood, leucocytes were counted in a celloscope. Differential counts were performed on smears stained with May-Grünwald's and Giemsa's solutions. At least 200 cells were counted.

Phagocytic assay: The phagocytic assay was performed as described by *Matsson et al.* (1985). Mononuclear cells were isolated by gradient centrifugation of heparinized blood on Ficoll-Paque (Pharmacia Fine Chemicals AB, Uppsala, Sweden) acording to *Bøyum* (1968). The cells were washed twice in phosphate buffered saline and resuspended at a concentration of 10^6 cells/ml in RPMI-1640 growth medium supplemented with 2 mmol/l L-glutamine, 200 IU/ml penicillin, 200 μg/ml streptomycin and 10 % heat inactivated fetal calf serum or 20 % pooled heat inactivated sera from the experimental calves. Thereafter 5 μl of a stock solution of 1.72 μm fluorescent latex beads (Polysciences, Northampton, England) were added to 2 ml cell suspension (bead to cell ratio ∾ 25:1) and incubated for 20 h in minisorb test tubes (Nunc, Denmark) rotating at 0.5 rpm. After

incubation, 100 µl 1 % Triton X-100 and 25 µl propidiumiodide (1 mg/ml) were added to the sample before the analysis with a flow cytometer (FCM) (Facs-III, Becton Dickinsson). Dual fluorescence channels were used for green (beads) and red (cells) fluorescent light detection. The signals were displayed on a two parameter histogram (64×64) by a computer system (ND-6660 Multiparameter Acquisition System, Nuclear Data, Chicago). Over 10,000 cells from each sample were analyzed and the proportion of monocytes and their phagocytic capacity were calculated from the histograms. A cell with 3 or more beads was considered as a monocyte. The phagocytic capacity of the monocytes was defined as the number of cells with more than 10 beads divided by the total number of monocytes.

Lymphocyte stimulation test: Heparinized whole blood was diluted 1:10 in RPMI-1640 growth medium supplemented as described above. Then 100 µl of the sample was cultured in round bottomed microtiter plates together with 100 µl growth medium (control) or Leucoagglutinin (La) (Pharmacia Fine Chemicals AB, Uppsala), diluted to 4 µg/ml or with Concanavalin A (Con A) (Pharmacia Fine Chemicals AB, Uppsala) diluted to 10 µg/ml or with Poke Weed Mitogen (PWM) (Boehringer Mannheim, Bromma) diluted to 10 µg/ml. Each type of culture was performed in hexaplicate and incubated at 37°C for 3 days, pulsed with growth medium containing 1 µCi ^{3}H-thymidine. After another 24 h incubation the cells were harvested. The ^{3}H activity of each culture was then measured and the stimulation index (SI) calculated according to the formula:

$$SI = \frac{\text{Mean counts per minute (cpm) of stimulated cultures}}{\text{Mean cpm of the control cultures}}$$

RESULTS

Effect of treatment with an inactivated avipox virus on the frequency of disease — Experiment 1

The total frequency of treatment for respiratory disease and enteritis over the observation period varied between the herds from 15 % (A) over 22 % (B) and 46 % (D) to 77 % (C), giving an average frequency of treatment of 37 %. In the group of calves which received the avipox virus preparation 41 % were treated for respiratory disease or enteritis. The corresponding figure in the placebo group was 34 %. None of the calves died.

The percentage of calves treated for respiratory disease was 30 % in the avipox group and 27 % in the placebo group (Table 1). Five calves in each group had been treated for enteritis prior to developing respiratory disease. Three calves in the avipox group and 5 in the placebo group were treated twice for respiratory disease. The onset of respiratory disease occurred 12.5 ± 7.0 days (mean ± s) after arrival in the apivox group and after 12.0 ± 6.8 days in the placebo group.

Table 1. Frequency of calves treated for respiratory disease during the first month after arrival at 4 feedlots. The calves were injected with either inactivated avipox or placebo on the day of arrival and 3 days later.

Feetlot	Inactivated avipox virus		Placebo	
	number	treated (%)	number	treated (%)
A	34	9	32	10
B	38	18	43	2
C	28	57	28	68
D	27	44	27	44
Total	127	30	130	27

All of the 18 calves (herd B) daily controlled for rectal temperature during the first 23 days had fever (≧ 39.5 °C) for at least 1 day. The average number of days with fever was 6.7 ± 3.6 and 5.0 ± 3.3 (mean ± s) for the avipox group and the placebo group, respectively. Only 1 of these calves, belonging to the avipox group, was subjected to antibiotic treatment.

The effect of treatment with inactivated avipox virus on total and differential white cell count, phagocytosis and lymphocyte stimulation — Experiment 2

The mean values (7 calves in each group) for total white cell count on day 0, 3 and 6 were in the range of 11.3—12.0 × 10^9 cells/l in the avipox group and 11.4—12.4 × 10^9 in the untreated group. The mean values decreased on day 10 to 8.4 × 10^9/l and 9.1 × 10^9, respectively. The decrease in total white cell count was mainly due to a drop in the number of circulating neutrophilic granulocytes, while the number of lymphocytes and monocytes remained rather stable.

The FCM measurement showed that there was no significant difference in phagocytic capacity between monocytes isolated from avipox treated and untreated calves. A rather constant mean capacity of 0.3 was obtained for both groups (Table 2). Individual capacities ranged from 0.05 to 0.50 and the mean capacity for each calf for the 4 sampling occasions ranged from 0.20 ± 0.08 to 0.41 ± 0.03 (mean ± s). The proportion of phagocytes (monocytes) among blood MNC did not differ between the two groups.

Table 2. Phagocytic capacity of monocytes isolated from calves treated with inactivated avipox virus and from control calves, after their arrival at the feedlot (mean ± s, n=7).

Days after arrival	Phagocytic capacity: Inactivated avipox virus	Phagocytic capacity: Control
0	0.37 ± 0.14	0.33 ± 0.09
3	0.33 ± 0.14	0.30 ± 0.13
6	0.27 ± 0.14	0.30 ± 0.06
10	0.36 ± 0.05	0.36 ± 0.08

As shown in Table 3, there was no difference between sera from the avipox treated calves and sera from the untreated calves to influence the phagocytosis when monocytes isolated from adult cattle were used in the assay. However, more monocytes were able to phagocytose when pooled sera collected on day 0 were used in the test compared to pooled sera from day 6.

Table 3. Proportion of phagocytes and their phagocytic capacity among blood mononuclear cells[1] incubated with pooled sera from the experimental calves before (day 0) and after (day 6) treatment with inactivated avipox virus and from control calves (mean ± s).

	Inactivated avipox virus		Control	
	Day 0	Day 6	Day 0	Day 6
Phagocytic cells (%)	4.6 ± 1.3	3.3 ± 0.4	5.9 ± 1.5	3.8 ± 0.8
Phagocytic capacity	0.52 ± 0.07	0.46 ± 0.03	0.52 ± 0.04	0.49 ± 0.08

[1] isolated from 4 adult cattle.

The cpm values in cultures with unstimulated cells were close to 1000 cpm in both groups except on day 6, when especially the avipox group had an increased average, mainly due to a single high value. The lymphocytes obtained from the calves of the

Table 4. Mitogen stimulation of lymphocytes collected from calves treated with inactivated avipox virus and from control calves after their arrival at the feedlot. Counts per min in unstimulated cell cultures and stimulation index (SI) of La, Con A and PWM (mean ± s, n=7).

Days after arrival	Unstimulated cells		SI, La		SI, Con A		SI, PWM	
	Inactivated avipox virus	Control	Inactivated avipox virus	Control	Inactivated avipox virus	Control	Inactivated avipox virus	Control
0	904±401	1106±365	2.4±1.2	4.6±4.7	27.5±16.9	27.1± 9.3	8.6±5.2	9.5±5.0
3	943±310	1134±390	8.9±8.7	11.2±9.6	19.9± 7.0	20.3±11.6	12.3±4.1	12.3±7.0
6	1593±1879	1153±561	5.7±5.5	6.7±6.3	9.3± 6.4	10.7± 5.0	6.5±5.7	9.1±6.0
10	1048±891	1131±665	7.9±5.5	8.8±6.2	9.9± 4.7	15.5±11.0	6.7±3.0	9.0±6.3

two groups had the same responsiveness to the mitogens used. The SI of PWM was rather stable whereas that of La had a greater day to day variation. The SI of Con A decreased almost three times from day 0 to day 6, where it remained until day 10 (Table 4).

DISCUSSION

The total frequency of antibiotic treatment varied considerably between the 4 farms included in the study. This difference could either be due to a true difference in frequency of disease or to differences in the judgement when to treat an animal or not. In any case no significant difference in frequency of disease between the avipox and placebo group was found at any farm or in the 4 farms combined (Table 1). This result is in contrast to *Wizigmann* (1978) who reported a reduced morbidity from 35 % to 9 % (2,478 animals in 83 herds), based on observations in problem herds before and after treatment with inactivated avipox virus.

Blood samples collected from the calves at arrival at the farms revealed that the total leucocyte counts were elevated, mainly due to an increased number of neutrophils. The increase in white blood cell count remained for 6 days after arrival and thereafter returned to normal values. The elevated white blood cell count could be due to the stress after transportation, with increased cortisol levels in serum (*Hartmann et al.* 1973, *Simensen et al* 1980).

Elevated serum cortisol levels have been shown to depress the reactivity of bovine lymphocytes to the mitogens PHA and Con A (*Muscoplat et al.* 1975, *Roth et al.* 1982). In the present study we found a low response to La but a high response to Con A at day 0 compared to days 3, 6 and 10. However, as shown in Table 4, both avipox treated and untreated calves reacted similarly to the mitogens.

The method used to measure the proportion of monocytes and their phagocytic capacity is sensitive to changes in the phagocytic capacity, as revealed by differences between individual calves. However, no difference neither in the proportion of phagocytic mononuclear cells nor in their phagocytic capacity was found between the avipox treated and the untreated calves. This is in contrast to *Henschelchen* (1984) who found an increased phagocytic capacity of monocytes as well as of neutrophils in

cows treated with an inactivated avipox virus daily for 4 days post partum. *Henschelchen's* results were based on counting the number of latex beads ingested by 20 monocytes and 50 neutrophils in each sample. The incubation with latex beads was performed in whole blood cultures, for which reason serum factors might have been responsible for the observed increase in phagocytic capacity, an effect which has been reported from studies in germ free rats (*Meyer & Mayr* 1981). In the present study, however, sera from avipox treated calves did not increase the phagocytic capacity of MNC isolated from adult cattle (Table 3).

Following the treatment recommended by the manufacturers we were not able to demonstrate any effect of treatments with inactivated avipox virus (Duphamun®) on the frequency of disease in feedlot calves. Further, no effect was found on the phagocytosis by monocytes or on the responsiveness of lymphocytes to mitogens.

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REFERENCES

Babiuk, L. A. & V. Misra: Effect of levamisole in immune responses to bovine herpesvirus — 1. Amer. J. vet. Res. 1982, *43*, 1349—1354.

Bibrack, B.: Bekämpfung des Welpensterbens durch aktive Interferonisierung. (The use of an active Interferon inducer against mortality in puppies). Fortschr. Vet. Med. 1976, *25*, 222—226.

Bøyum, A.: Isolation of leucocytes from human blood. A two phase system for removal of red cells with methyl cellulose as erythrocyte aggregating agent. Scand. J. Clin. lab. Invest. 1968, *21*, suppl. 9, 4—29.

Hartmann, H., H. Meyer, G. Steinbach & B. Finger: Zur Reaktion des Kälberorganismus auf Transportbelastungen. (Response of calf organism to transport stress). Mh. Vet.-Med. 1973, *28*, 647—651.

Henschelchen, O.: Stimulierung der Körpereigenen Abwer in der Geburthilfe. (Stimulation of defense mechanisms at delivery). Tierärztl. Umschau 1984, *39*, 229—232.

Hoerlein, A. B. & C. L. Marsh: Studies on the epizootiology of shipping fever in calves. J. Amer. vet. med. Ass. 1957, *131*, 123—127.

Hurvell, B. & H. Fey: Comparative studies on the gammaglobulin level in sera of market calves in relation to their health. Acta vet. scand. 1970, *11*, 341—360.

MacLachlan, J. & B. Rosenquist: Duration of protection of calves against rhinovirus challenge exposure by infectious bovine rhinotracheitis virus-induced interferon in nasal secretions. Amer. J. vet. Res. 1982, *43*, 289—293.

Matsson, P., C. Fossum & B. Larsson: Evaluation of flow cytometry and fluorescence microscopy for the estimation of bovine mononuclear phagocytes. J. immunol. Methods 1985, *78*, 13—24.

Mayr, A.: Erfahrungen mit dem Paramunitätsinducer PIND-AVI in der Tiermedizin. (Experience in veterinary medicine with PIND-AVI paramunity inducer). Collegium veterinarium 1979, *60*, 35—40.

Mayr, A. & R. Brunner: Untersuchungen über die Wirksamkeit einer Paramunisierung zur Bekämpfung von Ferkelaufzuchtverlusten und der enzootischen Pneumonie der Schweine (Ferkelgrippe). (Studies on the effectiveness of paramunization to control piglet breeding losses and piglet enzootic pneumonia). Zbl. Vet. Med. B 1980, *27*, 589—598.

Mayr, A., H. Stickl, M. Westhues, W. Gillesberger, D. Schwarz & B. Bibrack: Therapie von Herpes Zoster durch aktive Prämunisierung. (Therapy of Herpes zoster by a paramunizing inducer). Fortschr. Med. 1977, *95*, 119—122, 152—153.

Meyer, H. & A. Mayr: Untersuchungen über die clearance und Phagozytose gegenüber Pseudomonas aeruginosa an isoliert perfundierten Lebern von keimfreien Ratten nach Vorbehandlung mit dem Paramunitätsinducer „PIND-AVI". (Studies on clearance and phagocytosis of Pseudomonas aeruginosa in isolated perfused livers of germ-free rats after pre-treatment with paramunity inducer "PIND-AVI"). Zbl. Vet. Med. B 1981, *28*, 46—58.

Muscoplat, C. C., R. E. Shope, A. W. Chen & D. W. Johnsson: Effects of corticosteroids on responses of bovine peripheral blood lymphocytes cultured with phytohemagglutinin. Amer. J. vet. Res. 1975, *36*, 1243—1244.

Roth, J. A., M. L. Kaeberle & W. H. Hsu: Effects of ACTH administration on bovine polymorphonuclear leucocyte function and lymphocyte blastogenesis. Amer. J. vet. Res. 1982, *43*, 412—416.

Saperstein, G., S. B. Mohanty, D. D. Rockemann & E. Russek: Effect of levamisole on induced bovine viral diarrhea. J. Amer. vet. med. Ass. 1983, *183*, 425—427.

Simensen, E., B. Laksesvela, A. K. Blom & O. V. Sjaastad: Effects of transportations, a high lactose diet and ACTH injections on the white blood cell count, serum cortisol and immunoglobulin G in young calves. Acta vet. scand. 1980, *21*, 278—290.

Straub, O. C. & R. Ahl: Lokale Interferonbildung beim Rind nach intranasaler Infektion mit avirulentem IBR/IPV-Virus und deren Wirkung auf eine anschliessende Infektion mit Maul- und Klauenseuche-Virus. (Local production of interferon in cattle after intranasal infection with avirulent IBR/IPV virus and its effect on a subsequent challenge infection with foot- and mouth disease virus). Zbl. Vet. Med. B 1976, *23*, 470—482.

Thein, P., W. Leistner & H. Hechler: Erfahrungen mit dem Einsatz des Paramunitätsinducers PIND-AVI in der Pferdepraxis. (Experiences with using a paramunizing inducer PIND-AVI in equine practice). Zbl. Vet. Med. B 1980, *27,* 499—512.

Todd, J. D., F. J. Volenec & I. M. Paton: Interferon in nasal secretions and sera of calves after intranasal administration of avirulent infectious bovine rhinotracheitis virus: Association of interferon in nasal secretions with early resistance to challenge with virulent virus. Infect. Immun. 1972, *5,* 699—706.

Wizigmann, G.: Erfahrungen mit einer aktiven Paramunisierung zur Prophylaxe der Crowding diesase bei der Kälber- und Bullenmast. (Experiences with active paramunization as a prophylactic treatment of crowding disease in calf and bull fatstock). Fortschr. Vet. Med. 1978a, *28,* 51—53.

Wizigmann, G.: Weitere Erfahrungen mit einer aktiven Paramunisierung in der Rinderpraxis. (More experience of an active paramunizing inducer in bovine practice). Prakt. Tierärzt. 1978b, *59,* 765—766.

Wizigmann, G.: Prophylaxis of crowding disease in calves by means of paramunization. Proc. XI. Int. Congr. Diseases of Cattle, Tel Aviv 1980, p. 441.

SAMMANFATTNING

Utvärdering av en immunomodulator som profylax mot respirationssjukdomar hos kalv.

En immunomodulator, baserad på ett inaktiverat avipoxvirus, användes i ett placebokontrollerat fältförsök, där den profylaktiska effekten mot luftvägsinfektioner hos förmedlingskalvar utvärderades. Immunomodulatorns effekt på fagocytos och på lymfocyternas stimulerbarhet undersöktes också.

På ankomstdagen till köttdjursbesättningarna och tre dagar senare behandlades 257 kalvar med antigen immunomodulatorn eller med placebo. Under en månads tid observerades kalvarna med avseende på sjukdomssymtom och behandlingar med antibiotika mot luftvägsinfektioner noterades. Immunomodulatorn minskade ej sjukdomsfrekvensen. Trettio procent av de kalvar som behandlats med immunomodulatorn och 27 % av kalvarna i placebogruppen behandlades med antibiotika mot luftvägsinfektioner.

Det cellmedierade immunsvaret mättes hos 7 kalvar behandlade med immunomodulatorn och hos 7 obehandlade kontroller. Monocyter, som isolerats från djuren, undersöktes på sin förmåga att fagocytera latexpartiklar, och lymfocyter från djuren testades på sin förmåga att svara på olika mitogen. Sera från kalvar i båda grupperna undersöktes i en fagocytostest. Ingen skillnad i dessa parametrar observerades mellan de två grupperna.

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