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THE RESPONSE OF NORMAL AND IRON ANEMIC CALVES TO NASAL INFECTION WITH AN ATTENUATED STRAIN OF PARAINFLUENZA-3 VIRUS

By

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MÖLLERBERG, LARS and J. MORENO-LÓPEZ: *The response of normal and iron anemic calves to nasal infection with an attenuated strain of parainfluenza-3 virus.* Acta vet. scand. 1975, *16*, 186—196. — Calves with experimentally induced iron deficiency anemia and normal calves, both groups deprived of colostrum, were exposed to intranasal instillation of an attenuated parainfluenza-3 virus strain.

The calves became infected, but there was no difference in the clinical picture between the 2 groups of calves. Neither was there a difference in the humoral or local immune response to parainfluenza-3 virus.

iron deficiency anemia; parainfluenza-3 virus; immune response.

It was shown in an earlier study (*Möllerberg* 1975) that 13 to 35 % of Swedish purchased calves suffered from iron deficiency anemia. In animals with iron deficiency anemia the increase of body weight is retarded and the incidence of infectious diseases is higher than in animals with normal blood values (for references, see *Baggs & Miller* 1973 and *Möllerberg et al.* 1975).

Infections with parainfluenza virus type 3 (PIV-3) resulting in disease have often been seen in Swedish beef cattle recruited from purchased calves (*Moreno & Möllerberg* unpublished).

The subject of the present study was to investigate the influence of iron deficiency anemia on susceptibility to infection and the immune response to it under experimental conditions. An attenuated PIV-3 strain was chosen as infectant.

MATERIAL AND METHODS

Calves

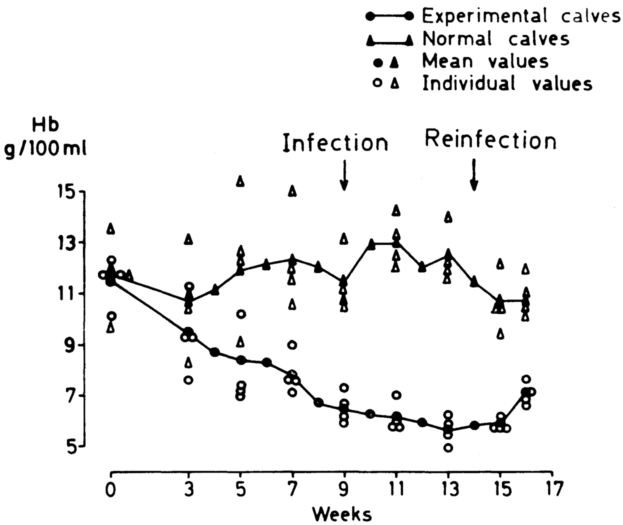
Eight colostrum deprived calves born within 10 days were purchased from stock-owners in the vicinity of Stockholm and transported to the Veterinary College as soon as possible after birth, where they were isolated from other cattle. The calves were to an average age of 8 days fed a commercial milk replacer containing 19 mg Fe/kg, and the daily ration was increased from 680 g to 2270 g of milk replacer before mixing with water (The Swedish Farmers' Purchasing Association, Stockholm). Then the calves were divided into 2 groups, experimental and normal, on the basis of their age, weight and hemoglobin values (see Table 1 and Fig. 1). The experimental calves were placed one by one in wooden boxes and were fed only milk substitute according to requirement for veal calf. From the 14th experimental week they were fed as the normal calves.

The normal calves were placed in boxes with straw beds. These calves received the equivalent of 4 l milk per day in the form of milk replacer, hay ad lib. and 0.1 to 0.5 kg concentrate during the first 8 weeks. After that the calves were fed hay and

Table 1. Status of calves as regards age, weight and Hb values at the beginning of the experiment.

Calf no.*	Age (days)	Weight (kg)	Hb (g/100 ml)
1-N	14	44	11.7
5-N	7	42	9.7
6-N	7	43	13.6
10-N	5	32	12.0
2-E	14	41	10.1
4-E	8	34	11.7
7-E	6	35	12.3
9-E	5	42	11.7
Mean for N	8.3	40.3	11.8
Mean for E	8.3	38.0	11.5

* N, kept on normal diet throughout the experiment;
E, to be exposed to iron deficiency.



F i g u r e 1. Changes in hemoglobin values in normal and experimental calves during the experimental period.

water ad lib. and afterwards even concentrate ad lib. On the basis of the iron contents of the milk substitute, hay and concentrate and the requirement for calves according to *Matrone et al.* (1957) the iron deficit was calculated. To supply the deficit the normal calves at an age of 2 and 6 weeks were treated with 800 and 500 mg Fe, respectively, given by intramuscular injection in the form of irondextrin (Cynemia®, Agricultural Division, American Cyanamid Company, Waine, N. J., USA).

Clinical examinations

The calves were observed and their body temperature measured daily from 2 days before the first infection to the end of the experiment.

Hematological examinations

Blood samples for the hematological examinations were taken the day before the calves were divided into 2 groups and then at intervals as indicated in Figs. 1 through 3. The methods for hemoglobin (Hb), serum iron (SI) and unsaturated iron binding capacity (UIBC) were the same as described previously (*Möllerberg* 1975).

Mode of infection, serological test and skin test

The Norden strain of a commercial attenuated vaccine Resbovac (Norden Laboratories, Lincoln, Nebraska, USA) with 10^7 TCID50/ml was used for infection; we obtained this strain from B. Morein. To each of 8 calves, 10 to 11 weeks old, 2 ml of this strain was instilled intranasally. This was carried out gently through a rubber tube along the ventral meatuses of the nose (*Morein* 1972). A second dose was given 37 days later.

Collection and processing of nasal secretion, collection of serum samples, virus recovery, hemagglutination (HA) and hemagglutination inhibition (HI) tests were carried out as described by *Morein* (1970). Samples of nasal secretion and serum were taken daily during the first 2 weeks after infection and after reinfection, and then every second day until day 56. Samples of nasal secretion and serum were heated for 30 min. at 56°C before testing for HI antibodies.

The skin test to PIV-3 was carried out according to *Morein & Moreno-López* (1973). This test was performed 14 days after the first infection.

RESULTS

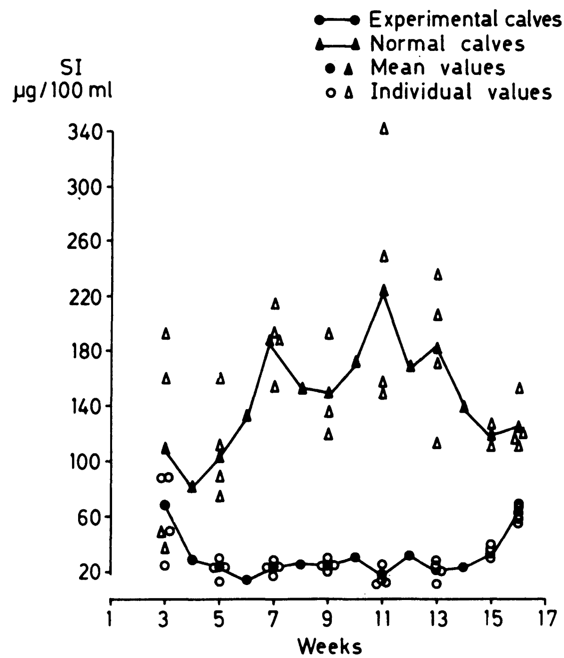
Experimental iron deficiency anemia

Hematology

The hemoglobin levels are shown in Fig. 1. There was no difference in mean Hb value between the 2 groups at the start (week 0) of the experiment. The mean Hb values were 11.5 g/100 ml in the normal group and 6.5 g/100 ml in the experimental group 9 weeks after the start of the experiment. The calves were then inoculated with PIV-3. The individual values in the 2 groups come near to the mean values at the end of the experimental period.

The SI values followed the same trend as Hb (Fig. 2). Nine weeks after the start of the experiment the mean value of the experimental calves was very low, 24 µg/100 ml. In the experimental group these increased after the 14th experimental week.

The changes in UIBC are shown in Fig. 3. At the first inoculation with PIV-3 (9th experimental week) the mean values were 736 µg/100 ml and 272 µg/100 ml in the experimental and normal groups, respectively, a great difference depending on a



F i g u r e 2. Changes in serum iron values in normal and experimental calves during the last 13 weeks of the experimental period.

sharp initial increase in the experimental group. The mean values in the experimental group were high till the 14th experimental week when the values started decreasing.

Clinical signs

The only noted clinical illness was non-purulent nasal discharge from the calves the days after the infection. The nasal discharge was most pronounced 5 to 7 days after infection and was absent on day 10.

The mean values of the body temperatures are shown in Fig. 4. There was a small increase days 4 and 5 after the infection for the normal and experimental calves, respectively.

Antibody response to infection

Preinfectional antibody activity

Samples of serum and nasal secretion were collected a few days after birth, 1 week prior to the infection and on the day of infection. No HI antibody activity to PIV-3 was found in the sera. The samples of nasal secretion had HI titers varying between 1:4 and 1:16.

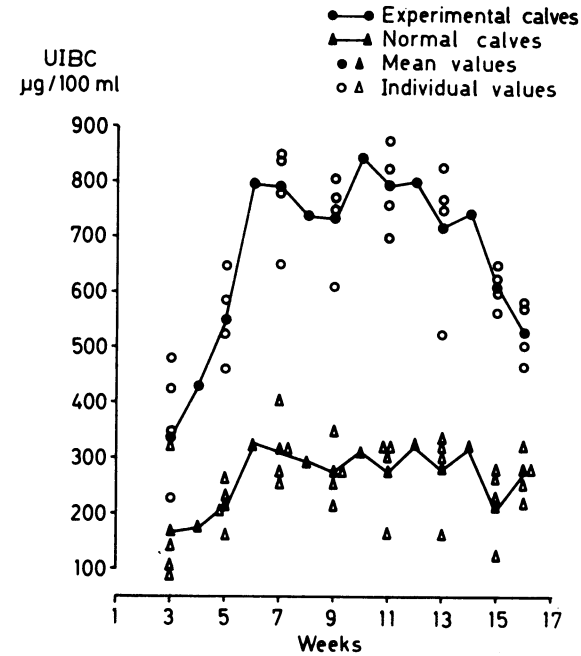


Figure 3. Changes in unsaturated iron binding capacity in normal and experimental calves during the last 13 weeks of the experimental period.

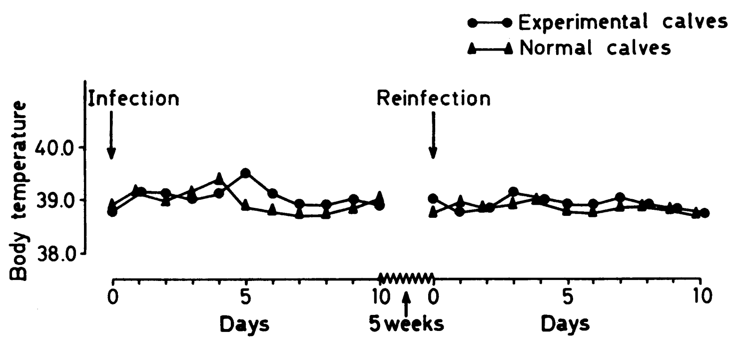


Figure 4. Mean values of body temperature of normal and experimental calves 10 days after infection and reinfection.

Postinfectional antibody activity

The time lapses between the infection and the increase of HI titers of normal and experimental calves are shown in Table 2. No differences were noted between HI titers of normal and experimental calves. An increase in HI titers in the serum started on day 7 after infection and in the nasal secretion on days 5 through 7. The highest serum titers were found in the time period between 10th and 24th day, and the highest nasal secretion titers on the 6th to 9th day. All the calves showed a decrease of HI titers in serum and nasal secretion from day 22 to 33 after infection. The HI titer increased again on days 2 through 6 after reinfection. The highest serum titers were noted on days 4 through 13 and the highest nasal secretion titers on days 5 through 10.

Table 2. Similar antibody response to parainfluenza-3 virus in calves with experimental iron deficiency anemia and normal calves as measured by hemagglutination inhibition (HI).

Calf group	No.	Hi titers in serum*			HI titers in nasal secretion*		
		prior to infection	after infection	after reinfection	prior to infection	after infection	after reinfection
	1	< 8	64(10)	64(42)	16	32(7)	64(47)
normal	5	< 8	64(24)	128(44)	8	32(7)	64(43)
	6	< 8	64(22)	64(42)	8	64(7)	64(43)
	10	< 8	32(15)	64(41)	8	32(8)	64(47)
with iron	2	< 8	64(13)	128(50)	16	64(9)	64(40)
deficiency	4	< 8	64(22)	128(45)	16	32(6)	32(43)
anemia	7	< 8	32(14)	64(41)	16	32(8)	64(43)
	9	< 8	64(18)	64(43)	4	16(7)	64(47)

* Reciprocal of serum dilution respectively of nasal secretion.
HI: highest HI titer noted; Figures in brackets indicate the day when the highest HI titer was observed.

PIV-3 was recovered from nasal secretion on days 2 through 7 after infection; no virus was recovered after reinfection.

Skin hypersensitivity

The skin test with a PIV-3 concentrate was used to estimate the degree of a cell-mediated immune response to infection. A significant skin reaction to PIV-3 was found in both groups of the calves and 48 hrs. after inoculation, but there was no difference in the degree of reaction between these groups (Table 3).

Table 3. Similar degree of skin hypersensitivity to parainfluenza-3 virus (PIV-3) in normal (N) and iron deficiency anemic calves (E).

Calves			HI titer*	Skin reaction**		Skin fold thickness (mean increase in mm)		
group	age	number		1 hr.	48 hrs.	1 hr.	48 hrs.	range
N	12—13 weeks	4	32 or 64	—	+	0.0	2.0	1—3
E	12—13 weeks	4	32 or 64	—	+	0.0	2.0	1—3

* Reciprocal of serum dilution.
** Only the results with the PIV-3 antigen are tabulated. The results with the control antigen were negative.

DISCUSSION

The hematological findings show a significant difference in Hb, SI and UIBC values between experimental and normal calves indicating that an iron deficiency anemia developed in the experimental calves.

In our experiment the milk replacer contained increasing doses of Fe, i.e. from 10 to 40 mg daily. The dietary requirement of iron in calves varies between 30 and 100 mg daily (*Blaxter et al.* 1957, *Matrone et al.* 1957). The experimental calves were fed milk replacer according to the requirement for veal calves which means that these calves had an optimal growth rate and thus a great iron requirement.

In the experimental calves the mean Hb and SI values decreased during the milking period. Similar results have been reported by *Scheidegger* (1973) and *Möllerberg et al.* (1975). Only *Scheidegger* reported on SI and TIBC values in veal calves and these are in accordance with those in the present study. However, high UIBC values and decreased SI values are characteristic in persons with iron deficiency anemia and in late pregnancy (*Bothwell & Finch* 1962).

At the end of the experiment, when the experimental calves received the same food as the normal calves, their mean SI values increased from 24 to 62 μg/100 ml and Hb from 5.8 to 7.1 g/

100 ml. Obviously these anemic calves resorbed iron very fast. With iron deficiency anemia in man there is regularly an increased iron absorption (*Höglund* 1968), and probably there is a similar situation regarding calves. Four or 5 days after the first infection there was a slight increase of mean body temperature in both the normal and experimental calves. At the same time a nasal discharge appeared in all calves, and PIV-3 was recovered from samples of nasal secretion. Since the nasal discharge was non-purulent, we considered it as a sign of viral rhinitis. No signs of discharge were observed at the time of reinfection. The virus strain of the present study was completely avirulent when used for vaccination by *Morein* (1972).

As a criterion of cell-mediated immune response we used a skin test according to *Morein & Moreno-López* (1973). A positive skin hypersensitivity to intradermal PIV-3 injection both in normal and experimental calves was found. However, when some other microbial agents or their products were tested on human patients with iron deficiency anemia, e.g., Candida albicans or tuberculin (PPD), a negative reaction was noted.

There was no difference between the groups of calves in their immune response to nasal infection with an attenuated PIV-3 strain. However, since only PIV-3 was tested, it is unknown how the calves should have responsed if exposed to other microbial agents. In man there was a depressed cell-mediated immune response in patients with iron deficiency anemia (*Joynson et al.* 1972). The reason for that is unknown. However, there are some data suggesting that iron may play a crucial role in the mitotic process since addition of iron-chelating agents to living cells caused a selective inhibition of DNA synthesis (*Robbins & Pederson* 1970). Furthermore, an impairement of DNA synthesis has been found in bone-marrow cells from patients with iron deficiency anemia (*Hershko et al.* 1970).

Scheidegger et al. (1974) have found a significantly higher number of leukocytes and lymphocytes in normal calves than in calves with iron deficiency anemia. Other authors (*Greatorex* 1957 and *Holman* 1956) did not observe any difference. Leukocyte counts were carried out in the present study, and the results confirm *Scheidegger et al.*'s results. However, since the number of calves was small, the significance of difference in the leukocyte count could not be estimated. The difference in counts may be within the normal range (*Holman* l.c.).

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SAMMANFATTNING

Immunitetsvaret hos normala kalvar och kalvar med järnbristanemi efter en nasal infektion med en attenuerad parainfluensa-3 virus stam.

Kalvar med experimentellt framkallad järnbristanemi och normala kalvar infekterades intranasalt med en attenuerad parainfluensa-3 virus stam. Kalvarna hade ej tillåtits dricka råmjölk.

Samtliga kalvar visade lindriga symtom på en infektion. Det var ej någon skillnad varken i det humorala eller i det lokala immunitetssvaret mellan de två grupperna.

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