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Dose-dependent effects of specific egg-yolk antibodies on diarrhea of newborn calves

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Abstract

In a field trial on a farm in Turkey, we tested whether specific egg-yolk antibodies had a prophylactic effect on neonatal diarrhea. Owing to its antibody spectrum, this egg powder was very suitable for this farm since mainly rotavirus was identified. Regardless of the dosage (2 g, 4 g, or 8 g egg powder), the calves (n = 164) which received specific egg-yolk antibodies via the whole egg during the first 14 days of life showed significant improvements in risk and duration of diarrhea and in body weight gain compared with calves of the control group (n = 80) which had not received any egg powder. These significant differences in body weight still persisted after 3 months of life. The mortality risk in the control group amounted to 8.8%, while in all egg powder groups (n = 164) only one calf died as a result of diarrhea. Breed-dependent differences in the parameters were not observed.

Keywords: Calf; Diarrhea; Egg-yolk antibodies; Field trial; Prophylaxis

1. Introduction

Diarrhea, together with some other diseases occurring frequently in calves (e.g. respiratory diseases, navel infections), is still one of the leading causes of losses; both

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non-infectious as well as infectious forms occur. Therefore there is great interest in the prophylaxis of neonatal diarrhea.

Colostrum plays an important role, especially after protective inoculation of dams (Morgan et al., 1978; Nagy et al., 1978; Moon and Bunn, 1993). The prophylactic use of so-called probiotics, which are supposed to inhibit the propagation of diarrhea pathogens through stabilization and regeneration of the natural intestinal flora, seems to be very promising (Gedek, 1989). Another prophylactic measure is the active oral immunization of newborn calves.

The application of specific antibodies represents another possible way to control infectious diarrhea. The antibodies are extracted from either serum, colostrum, or milk (Snodgrass et al., 1977; Sadowski et al., 1983; Murakami et al., 1985; Yolken et al., 1990). Specific antibodies are also produced from chicken eggs, and are used for diagnostics as well as for treatment (Bartz et al., 1980; Jensenius et al., 1981; Lösch et al., 1986). The layer hens have to be immunized with the corresponding antigen in order to obtain specific antibodies from the egg yolk. The advantages of this method are the simple extraction of antibodies, the low costs, and the modest burden for the animals that are used for the production of antibodies. The efficacy of oral administration of egg powders containing specific antibodies which were produced from immunized layers could be demonstrated in challenge (Wiedemann et al., 1991; Ikemori et al., 1992; Yokoyama et al., 1992) and field trials (Erhard et al., 1993; Kellner et al., 1994; Lux, 1994) with calves and piglets. The field trials showed differences in body weight gain, and incidence and duration of diarrhea (Erhard et al., 1993; Lux, 1994). In those field trials 22 g, 16 g or 8 g egg powder were administered per day and per calf during the first 10 or 14 days of life.

The aim of this study was to clarify questions regarding the dosage and spectrum of pathogens to be covered by the egg powder. State-owned farms in Turkey are especially suitable for such studies, particularly since another spectrum of pathogens compared in field trials in Germany was to be expected.

2. Material and methods

This study was carried out from November 1993 until May 1994 on Karacabey Farm in Turkey. The farm owns approximately 300 dairy cows, with an average milk yield of 5100 kg per year per cow. We only used healthy calves; those which had other diseases (e.g. respiratory diseases with or without diarrhea) were excluded from the trial.

In order to test the response relationship, the newborn calves were divided into four different groups. The percentage of dams of first parity and the breeds were equally distributed among the groups. The control group did not receive any egg powder. The other three groups received 2 g, 4 g, or 8 g egg powder with specific antibodies to rotavirus types 1 and 2 (neutralisation test: minimal titer 512) and E. coli K99 pilus antigen (ELISA titer: minimal 330) each, in a drink which was divided between two meals per day during the first 14 days of life.

Calves of the Holstein Frisian breed ($n = 126$) and brown cattle ($n = 118$) were used in the trial. The first five newborn calves were put into the control group, then five
calves were put into each of the 2 g, 4 g, and 8 g groups so that the calves were allocated systematically. On five occasions, ten calves were put into the control group instead of five to make this group larger.

After the first administration of colostrum by nipple-drinking, the calves were taken away from the dams and kept individually on straw. The calves received 0.5–1.0 l colostrum three times on the first day of life, and then 1.5–2.0 l cow’s milk three times per day on Days 2–5. After the first 5 days of life, the calves received 2.0–2.5 l cow’s milk twice a day, increasing slowly to 3.0–4.0 l cow’s milk per meal until the 4th week of life. From the 10th day, fresh water and hay were also available to the animals. At the age of 3 weeks, commercial compound feed and hay were offered.

As in the study by Erhard et al. (1993), the infectious agent, the cumulative incidence of diarrhea in the groups, the duration of diarrhea (from the first sign of diarrhea to the recovery), body weight gain, and mortality were examined during this trial. Clinical diagnosis was done by a veterinarian who had no knowledge of the experimental groups. Diarrhea was diagnosed according to the parameters faecal consistency score and the condition of the calves. Treatment of diarrhea was similar across groups, depending on the clinical state of the calves. If necessary, calves got electrolytes and/or antibiotics (tetracycline). Faecal samples were taken on Day 7 and, in cases where diarrhea was diagnosed, immediately prior to treatment. Identification of the infectious agent was carried out in all faecal samples by means of the Lactovac* ELISA (Hoechst Veterinär GmbH, Unterschleißheim, Germany). This test can identify bovine rota- and coronaviruses, and E. coli K99 pilus antigen, as well as cryptosporidia. Probes (1:2 diluted in PBS, pH 7.2) were interpreted as positive if the extinction at 450 nm was above 0.1 and the negative control was below 0.1. The minimal difference in extinction was set at 0.1.

Statistical analysis was made by the Mann–Whitney test (unpaired) or χ² test. All tests were two-tailed, and alpha was 0.05. The number of calves was higher in the control group (n = 80) because all egg powder groups were only compared with the control group.

3. Results

The cumulative incidence of diarrhea was significantly (p < 0.005) reduced in all three egg-powder groups (14.3–15.1% of the calves) compared with the control group (38.8%). The average duration of diarrhea in the control group (3.5 days, sd 1.3) was significantly higher than in the egg-powder groups (2 g egg powder (n = 56), 2.0 days (sd 0.0, p < 0.001); 4 g egg powder (n = 53), 2.8 days (sd 1.8, p < 0.05); 8 g egg powder (n = 55), 2.3 days (sd 0.5, p < 0.05)). Furthermore, 26.3% of the calves in the control group had to receive an antibiotic and liquid-electrolyte treatment. Nevertheless, 8.8% of the calves in the control group died as a result of diarrhea, whereas only 12.5%, 9.1% (p < 0.05), and 12.5% (p < 0.05) of the calves in the 2 g, 4 g, and 8 g egg-powder groups, respectively, required additional treatment measures and only one calf died. Differences (by inspection) between the different egg-powder groups were not found (Table 1).
Table 1
Incidence risk and duration of diarrhea in the calves, in relation to the different test groups, in the period from birth to 14 days of life, together with the percental proportion of calves that were also treated with antibiotics and the mortality. The diagnoses were blind and the clinical treatments used for diarrhea were the same for all groups.

| Groups                        | Incidence of diarrhea (%) | Duration of diarrhea (days) | Antibiotic treatment (%) | Mortality (%) |
|-------------------------------|---------------------------|-----------------------------|--------------------------|---------------|
| Control (without egg powder, n = 80) | 38.8                      | 3.5 1.3                     | 26.3                     | 8.8           |
| Egg powder (2 g per day, n = 56)  | 14.3*                     | 2.0 0.0*                    | 12.5                     | 0.0           |
| Egg powder (4 g per day, n = 53)  | 15.1*                     | 2.8 1.8*                    | 9.4*                     | 1.9           |
| Egg powder (8 g per day, n = 55)  | 14.5*                     | 2.3 0.5*                    | 9.1*                     | 0.0           |

* p < 0.05 (vs. control); ** p < 0.005 (vs. control); *** p < 0.001 (vs. control).

The daily increase in body weight during the first 14 days of life (Fig. 1)—a parameter which can be measured impartially—additionally reflects the conditions. The calves of the control group gained 2.4 kg (sd 1.2) on average during the first 2 weeks of life, whereas body weight gains of 3.5–3.9 kg were recorded for the calves of the egg-powder groups (p < 0.0001).

![Body weight gain of Turkish dairy calves during the first 14 days of life](image)

Fig. 1. Body weight gain of Turkish dairy calves during the first 14 days of life. Egg powder containing antibody to rotavirus at different dosages was administered to some calves. The controls did not receive any egg powder. Bars indicate sd.
In some of the calves the birth weight could be compared with the body weight after 3 months of life. The birth weight of all groups was between 38.2 kg and 38.8 kg (sd between 1.6 and 3.2) on average. After 3 months the calves in the control group weighed only 85.2 kg (sd 3.9) compared with the significantly higher weights in the egg-powder groups; these were 88.5 kg (sd 2.3, \( p < 0.001 \), 2 g egg powder), 89.5 kg (sd 3.6, \( p < 0.0001 \), 4 g egg powder), and 89.8 kg (sd 2.3, \( p < 0.0001 \), 8 g egg powder; Table 2).

With regard to the pathogens routinely identified on Day 7 on this farm, intestinal rotavirus infections (38.1% of the calves) predominated. In the diarrheic calves, 78.2% of the calves showed rotavirus and 34.5% cryptosporidia in faecal samples before

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Table 2

| Groups                              | Birth weight (kg) | Body weight gain (kg after 14 days) | Body weight (kg after 3 months) |
|-------------------------------------|-------------------|-------------------------------------|---------------------------------|
|                                     | \( \bar{x} \) | sd   | \( \bar{x} \) | sd   | \( \bar{x} \) | sd   |
| Control (without egg powder, \( n = 27 \)) | 38.8  | 2.9  | 2.8  | 1.0  | 85.2  | 3.9  |
| Egg powder (2 g per calf, \( n = 23 \)) | 38.7  | 3.2  | 3.4  | 0.5* | 88.5  | 2.3* |
| Egg powder (4 g per calf, \( n = 32 \)) | 38.6  | 1.6  | 3.8  | 0.8* | 89.5  | 3.6* |
| Egg powder (8 g per calf, \( n = 30 \)) | 38.2  | 2.5  | 3.8  | 0.8* | 89.8  | 2.3* |

* \( p < 0.05 \) (vs. control); ** \( p < 0.001 \) (vs. control); *** \( p < 0.0001 \) (vs. control).

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Fig. 2. Identification of pathogens by means of the Lactovac® test in the fecal samples of Turkish dairy calves by routine tests on Day 7 (routine subclinical sample), or of diarrheic calves.
additional treatment. Polyinfections were recorded in 15.3% of the calves from the routine faecal samples on Day 7, and in 47.3% of the faecal samples of the diarrheic calves (Fig. 2).

Differences attributable to the breeds, which were similarly distributed among the groups, did not occur with any parameter (data not shown).

4. Discussion

In this study dosages of 8 g, 4 g, and 2 g egg powder per calf per day were administered. In spite of the clear reduction in the egg powder amount compared with previous studies in Germany (Erhard et al., 1993; Lux, 1994), significant improvements were seen in risk and duration of diarrhea, mortality, and body weight gain in the egg powder groups. The significant differences in body weight persisted after 3 months compared with the control group without egg powder.

Since infections occurring on the farm were mainly from rotavirus, the egg powder containing specific antibodies against rotavirus and E. coli K99 pilus antigen covered a large proportion of the pathogens. Based on our results, 2 g of the egg powder would be sufficient for the prevention of neonatal diarrhea in calves on this farm. Since the yield of egg powder per egg amounts to approximately 14 g, two eggs would be required per calf for the 2-week prophylaxis period. In cases where there is a different distribution of pathogens, the spectrum of specific antibodies in the egg powder would have to be altered. Additional nonspecific effects of the egg cannot be excluded.

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