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THE EFFECT OF VACCINATION ON TITRES OF ANTIBODY TO ROTAVIRUS IN COLOSTRUM AND MILK

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Résumé.

EFFET DE LA VACCINATION SUR LE TITRE EN ANTICORPS ANTIROTAVIRUS DANS LE COLOSTRUM ET LE LAIT. — La présence continue d'anticorps antirotavirus dans l'intestin du ruminant nouveau-né est importante pour la protection contre les maladies cliniques associées à l'infection par le rotavirus. Cet effet protecteur dépend de la quantité d'anticorps contenue dans la ration. Les titres en anticorps antirotavirus peuvent être relativement élevés dans les sécrétions mammaires du ruminant juste après la parturition, mais diminuent rapidement au cours de la première journée. On a étudié l'influence de la vaccination sur le titre en anticorps, la concentration en immunoglobulines et leur rythme respectif de décroissance dans la sécrétion mammaire après la parturition. Les brebis vaccinées deux à trois semaines avant l'accouplement avec une préparation inactivée de rotavirus d'agneau produisaient du colostrum et du lait qui contenaient significativement plus d'anticorps spécifique que la sécrétion mammaire des non-vaccinées. L'activité anticorps semble associée aux IgG, et les auteurs suggèrent que la vaccination peut être un moyen intéressant pour atténuer les diarrhées néonatales associées au rotavirus chez les bovins et les ovins.

The continued ingestion of colostrum antibody has been shown to be important in the protection of newborn gnotobiotic lambs against diarrhoea associated with lamb rotavirus infection (Snodgrass and Wells, 1976). In further experiments, we have attempted to investigate the possible existence of a dose-response relationship between the quantity of antibody ingested and prevention of disease and virus excretion (Snodgrass and Wells, 1978).

These studies examined the influence of feeding standard doses of serum from three different serum pools with reciprocal neutralising antibody titres to lamb rotavirus of 3,200, 10 and <2.5 respectively. Lambs re-

ceived 30 ml of serum given three times daily from the second to the fourth day of life and through delaying initiation of feeding serum until absorption of immunoglobulin from the gut had ceased, all lambs remained agammaglobulinaemic under this regime of treatment.

Lambs receiving serum with a reciprocal neutralising titre of 3,200 obtained from sheep hyperimmunised with lamb rotavirus were completely protected against the effects of infection with lamb rotavirus on the second day of life. However, lambs fed serum from normal adult sheep with a reciprocal titre of 10 were incompletely protected, virus being excreted from both lambs treated in this

way and diarrhoea being observed in one. Serum obtained from gnotobiotic lambs which contained immunoglobulin but no detectable antibody to lamb rotavirus, was not protective. Lambs treated with this negative serum exhibited a similar degree of virus excretion and clinical diarrhoea to that resulting from infection of untreated control lambs. Specific antibody is therefore implicated as important in the protective effect and there is evidence of a gradation of protective efficacy as the quantity of antibody administered is altered.

To further clarify this dose-response relationship lambs were treated with lower doses of the hyperimmune serum than the standard dose of 30 ml given orally three times daily on the second, third and fourth days of life. It was found that two doses of 20 ml given orally twice daily over the same period was equally as protective as the higher dose. However, some virus excretion occurred in lambs infected with rotavirus on the second day of life and fed hyperimmune serum at a dosage rate of 10 ml given twice daily on the second to the fourth days of life. Apparently this low dose approached the end-point of effective protection. It is suggested that such an end-point may be reached and exceeded as the antibody content of the mammary secretion declines in the transition from colostrum to milk. While relatively high titres of neutralising antibody to rotavirus are present in bovine colostrum collected within four hours of calving, there is a rapid decline in the content of antibody in colostrum during the first two days after calving (Woode *et al.*, 1975). It was considered possible that vaccination of the dam might result in the production of antibody to rotavirus in colostrum and milk to higher titres and over a longer period after parturition. Indeed some support for this contention comes from the findings that there was a reduced incidence of diarrhoea in calves suckled by cows treated with an inactivated vaccine of rotavirus (Mebus *et al.*, 1973). To study this possibility, antibody titres to rotavirus in the mammary secretions of vaccinated and unvaccinated ewes were monitored during a 10 day period after parturition (Wells *et al.*, 1978). This is period when the antibody content of the diet would appear to be significant to the suckled neonate.

Ewes were injected intramuscularly prior to mating with a vaccine consisting of for-

malin-treated lamb rotavirus or a placebo vaccine emulsified in incomplete Freund's adjuvant. The serum antibody response to vaccination was measured during pregnancy and while there was no evidence of an immune response in the control ewes, a marked serological response was observed in the group treated with the rotavirus vaccine. Colostrum samples were collected within six hours of lambing and thereafter daily milk samples were collected for a period of 10 days. Neutralising antibody titres to lamb rotavirus were determined in the whey prepared from colostrum and milk samples. Relatively high titres of antibody were present in colostrum collected within four hours of lambing from the ewes treated with placebo vaccine but no antibody was detectable in the mammary secretion within a further three days. It is of interest to note the similarity between titres of lamb rotavirus neutralising antibody observed in the mammary secretions of the control ewes and those of neutralising antibody to calf rotavirus in mammary secretions of normal adult cows in the first 4 days after calving reported by Woode *et al.* (1975). By comparison, vaccinated ewes had very high neutralising antibody titres to lamb rotavirus in their colostrum on the first day after lambing and titres in their mammary secretions remained significantly higher ($p < 0.01$) than in the control group throughout the 10 days of sampling. In contrast there was no significant difference between immunoglobulin concentrations in colostrum and milk from either group. This indicates that the increased titres of antibody in mammary secretions of vaccinated ewes was not due to an increased content of total immunoglobulin but to an increase in the content of immunoglobulin with a specificity towards the lamb rotavirus. Gel filtration fractionation of pooled whey samples collected on the first, fifth and tenth days after lambing showed that the antibody activity was associated largely with IgG during the 10 day sampling period.

By vaccinating ewes with this inactivated preparation, colostrum and milk antibody titres to lamb rotavirus have been markedly increased to the extent that the geometric mean neutralising antibody titres in milk on the tenth day after lambing was 380. Previous experiments have shown that lambs receiving orally 20 ml of serum with a neutralising antibody titre of 3200 are protected

against experimental rotavirus infection (Snodgrass and Wells, 1978). Such a daily oral intake of antibody would therefore appear feasible in lambs suckled by vaccinated ewes even at 10 days after lambing. Further experiments will show whether vaccination can elevate mammary secretion anti-

body titre for longer than the first 10 days after parturition and whether a similar response can be stimulated in cattle. Clearly the potential value of a vaccine which can prevent neonatal diarrhoea associated with rotavirus infection will be greater in the bovine species.

Summary

The continued presence of antibody to rotavirus in the gut of the neonatal ruminant has been shown to be important in protecting against clinical disease associated with rotavirus infections. This effect is dependant upon the amount of antibody included in the diet. Titres of antibody to rotavirus may be relatively high in ruminant mammary secretions immediately after parturition but decline rapidly over the course of the first day. The influence of vaccination on the titres of antibody, the concentrations of immunoglobulin and their respective of decline in the mammary secretion following parturition has been studied. Ewes vaccinated two to three weeks prior to mating with an inactivated preparation of lamb rotavirus produced colostrum and milk after the subsequent pregnancy which contained significantly higher titres of antibody to the virus than did the mammary secretions from non-vaccinates. The antibody activity appears to be associated with IgG and it is suggested that vaccination may be of value in the alleviation of neonatal diarrhoea associated with rotavirus infection in cattle and sheep.

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