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Evidence of pili k88 and k99 as protecting antigens : immunization against enteric swine colibacillosis by sow vaccination

Mise en évidence du rôle protecteur des pili K88 et K99 : immunisation contre l'entérite colibacillaire du port par vaccination des truies – Des truies gestantes ont été vaccinées par voie sous-cutanée avec un vaccin préparé à partir de souches entérotoxinogènes d'*Escherichia coli* (ECET) qui produisaient les pili k88 ou k99. Les porcelets nés de ces truies ont été testés par voie orale avec des souches d'ECET, k88$^+$ ou k99$^+$, différentes de celles utilisées dans le vaccin. Chez les truies, après la mise bas, le titre moyen de sérum et de colostrum en anticorps anti-k88 était de 1 : 64 mais celui des anticorps anti-k99 dans le colostrum (1 : 128) était supérieur à celui du sérum (1 : 32).
Les résultats démontrent que le vaccin est efficace pour le contrôle de la colibacillose du porc en réduisant la mortalité due aux diarrhées à colibacille entérotoxinogène et en augmentant le gain de poids moyen quotidien des porcelets. *Mots clés* : Porc – Truie – Porcelet – Colibacillose – Vaccin – Diarrhée – *Escherichia coli*.

INTRODUCTION

Among the different agents causing diarrhea in suckling pigs, *Escherichia coli* has been frequently isolated in several countries (1, 3, 4, 5). Enteropathogenic *E. coli* produces enterotoxins which cause leakage of water and electrolytes into the intestinal lumen. Another important factor in the onset of diarrhea is colonization of the small intestine (10, 11). The surface antigens denoted k88 and k99 are important in the colonization of the small intestine of suckling pigs (6, 8).

Age is a critical factor in the diarrhea induced by enterotoxigenic *E. coli*, which is observed among very young swine, in most cases during the first few days after birth, and causes dehydration, anorexia and death of untreated animals (1).

MOON (7) has reported that the prevention of colonization of enterotoxigenic *E. coli* on the surface of epithelial intestinal cells prevents enteric colibacillosis. The same author emphasizes that the presence of antibodies against surface antigens in the lumen of the intestinal tract reduces colonization.

The protective effect against diarrhea induced by enterotoxigenic *E. coli* in piglets is passively transferred by the colostrum of sows previously vaccinated against the k88 and k99 antigen (9).

The objective of the present study was to evaluate the efficiency of Colivak-88/99, a commercial bacterin carrying the surface antigens k88 and k99, in the passive protection of suckling pigs against enteric colibacillosis.

MATERIALS AND METHODS

Animals

Thirty-six selected pregnant Landrace sows from several pig farms in the state of São Paulo were divided into two groups of 21 and 15 animals each. The sows and the 295 piglets farrowed by them were used in the study.

Vaccine

The vaccine utilized in the present experiment were samples of enterotoxigenic *E. coli* that produced the pili k88 or k99, isolated from swine with enteric colibacillosis. The cultures were grown aerobically in brain heart infusion broth (BHI) for 24 hours at 37 °C. The cultures were inactivated with commercial 40 p.100 formalin diluted to 0.4 p.100 and aluminum hydroxide was used as adjuvant. Each 5 ml dose of vaccine contained 5.0 × 10^9 bacteria.

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Vaccination

Nach sow in the vaccinated group received two 5ml doses subcutaneously. The first dose was injected during the 6th week before farrowing, and the other three weeks later. The other group of 15 pregnant sows was left unvaccinated and used as control.

Serological test

Blood samples were collected from each animal 2 days before vaccination and on the day of farrowing, when colostrum samples were also obtained. The serum and colostrum samples were stored in the freezer until testing for anti-k88 or anti-k99 antibodies. Anti- k 88 or anti- k 99 antibody titres were determined by the technique of tube agglutination (2).

Challenge

The piglets were challenged soon after birth (0 to 12 hours) with a 10 ml oral dose containing 2×10^{10} viable *E. coli* bacteria per serotype [0149:k91:k88ac (H59) and 0101:k30:k99 (C19)]. Incidence of diarrhea, mortality and daily weight gain were then observed for seven days.

TABLE I Mean final agglutinating titres in serum and colostrum of sows, vaccinated with pili k88 and k99 evaluated at different times.

Group	Nb. of sows	Nb. of piglets		Period	Titre reciprocal	
		born	with diarrhea		k88	k99
I	21	175	52	Before immunization	4	2
				Farrowing	64	32
				Colostrum	64	128
II	15	120	100	Before immunization	2	2
				Farrowing	4	4
				Colostrum	4	4

RESULTS

Table I shows that, before vaccination, the animals had low antibody titres. However, all vaccinated sows responded to immunization with an increase in serum and colostrum titres of antibodies against the k88 and k99 antigens. After farrowing the mean antibody titre against k88 in serum and colostrum was 1:64, and the mean antibody titre against k99 was higher in the colostrum (1:28) than in the serum (1 :32) of vaccinated sows. The table also shows that, after challenge, the frequency of diarrhea among group I animals was 30 p.100 and 84 p.100 among group II animals. Figure 1 clearly shows the decrease in mortality rate in the vaccinated group. Weight gain by the piglets in the vaccinated group was visibly higher than among the piglets in the control group (Fig. 2).

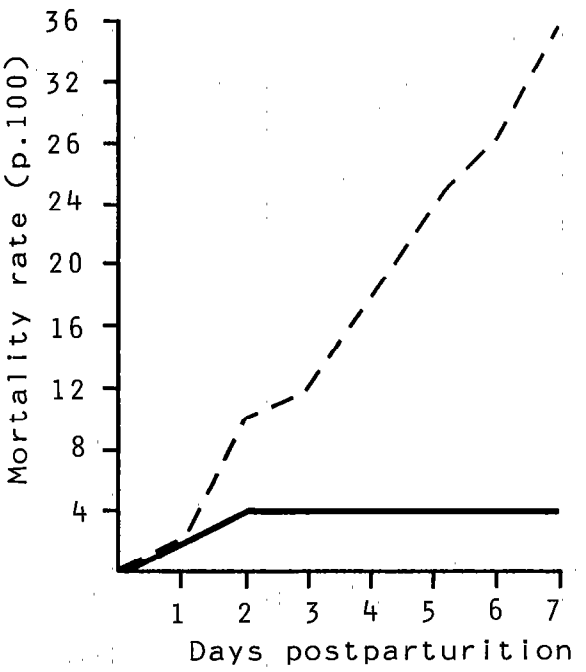

Fig. 1. Mortality rates among the piglets in the vaccinated group (– – – –) and in the control group (- - -) as a function of days after birth.

DISCUSSION

The objective of the present study was to determine the efficiency of a bacterin containing pili k88 and k99 in protecting piglets suckled by vaccinated sows. All vaccinated sows responded to parenteral immunization with an increase in serum and colostrum agglutinating titre against antigens k88 and k99. These results agree with the statement

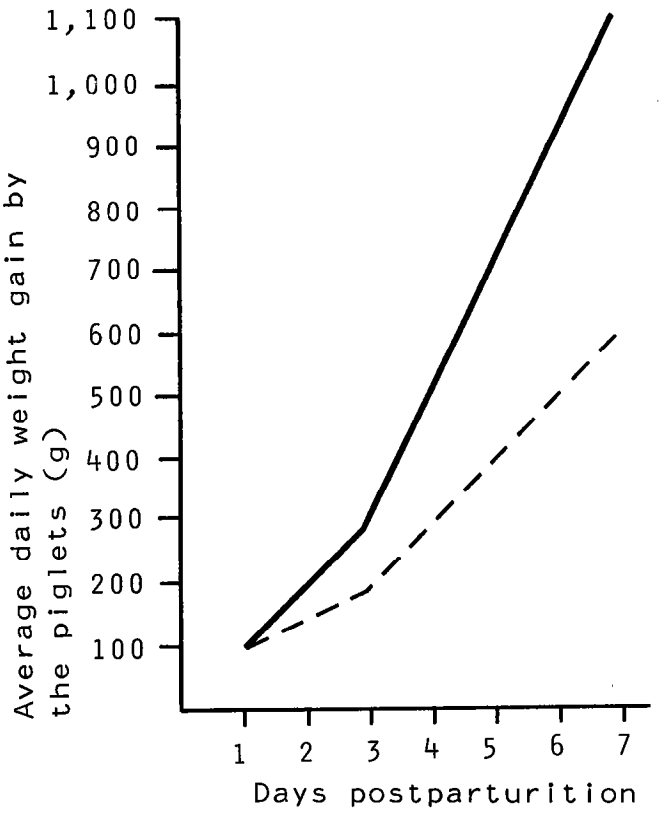

Fig. 2. Average daily weight gain by piglets farrowed by vaccinated (– – – – –) and by control (- - -) sows.

made by MORRIS *et a.* (9) that the protective effect against diarrhea induced by enterotoxigenic *E. coli* is passively transferred to the piglets through the colostrum of sows vaccinated against the two antigens. The bacteria used in the present study were prepared under conditions that favored the expression of pili k88 and k99, and the control of these antigens was done with specific hyperimmune serum. All of the sows used, both in the vaccinated and in the control group, showed agglutinating titres before immunization. The presence of antibodies in the colostrum of control sows was not sufficient to avoid the high percentage of piglets with diarrhea (84 p.100) and the large number of deaths (36 p.100). Antibody titres of 1:64 or more in the colostrum against antigens k88 and k99 were sufficient to protect the piglets against severe diarrhea which may have caused death after challenge with the H59 and C19 strains. The cases of diarrhea that occurred in the piglets produced by the vaccinated group were of low severity and of short duration, and perhaps were due to the high challenging dose. Comparison of daily weight gain by the piglets produced by the vaccinated group with the weight gain by the piglets produced by the control group showed a large difference, with the piglets of the vaccinated group gaining on average 100 g more per day than the control piglets (Fig. 2).

The results of the present study show that the vaccine used protected the piglets against severe diarrhea and death, and favored greater daily weight gain. ■

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Pregnant sows were vaccinated by the subcutaneous route with a vaccine prepared with samples of enterotoxigenic *Escherichia coli* (ETEC) which produced pilus k88 and k99. Piglets farrowed by these sows were challenged by the oral route with k88+ or k99+ ETEC differing from those of the vaccine samples. After farrowing, the mean antibody titre against k88 in serum and colostrum was 1:64, with the mean antibody titre against k99 in colostrum (1:128) being higher than in serum (1:32) of vaccinated sows. The results show that the vaccine behaved efficiently in controlling swine colibacillosis by decreasing mortality in cases of ETEC induced diarrhea and increasing daily weight gain by the piglets. *Key words* : Swine – Sow – Piglet – Colibacillosis – Vaccine – Swine diarrhea – *Escherichia coli.*

AVILA (F. A.), AVILA (S. H. P.), SCHOCKEN-ITURRINO (R. P.), MARQUES (M. A.). Puesta en evidencia del papel protector de los "pili" k88 y k99 : inmunización contra la enteritis colibacilar del cerdo por vacunación de las marranas. *Rev. Elev. Méd.vét. Pays trop.*, 1986, **39** (3-4) : 293-296.

Se vacunaron marranas preñadas por vía subcutánea con vacuna preparada de *Escherichia coli* a partir de cepas enterotoxigénicas (ETEC) las cuales producian "pilus" k88 o k99. Lechones procedentes de estas puercas fueron sometidos a una prueba por vía oral con cepas de ETEC, $k88^+$ o $k99^+$ diferentes de aquellas utilizadas para la preparación de la vacuna. En las marranas, después del parto, la media del título de anticuerpos contra k88 observada en el suero y en el calostro fue de 1:64, y la media de anticuerpos contra k99 en el calostro (1:128) fue mayor que la del suero (1:32). Los resultados muestran que la vacuna es eficaz para luchar contra la colibacilosis porcina disminuyendo la mortalidad en los casos de diarrea por ETEC y aumentando la ganancia de peso medio diario de los lechones. *Palabras claves* : Marrana – Lechón – Cerdo – Colibacilosis – Vacuna – Diarrea – *Escherichia coli.*

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