Humoral antibody response and oocyst shedding after experimental infection of histocompatible newborn and weaned piglets with Cryptosporidium parvum

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Original article

Humoral antibody response and oocyst shedding after experimental infection of histocompatible newborn and weaned piglets with *Cryptosporidium parvum*

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Summary — Experimental inoculations (mono- and multi-inoculations) with *C parvum* isolated from a diarrheic child and maintained on calves, were performed on 2 histocompatible miniature (d/d haplotype) weaned 4-week-old piglets and 2 newborn piglets. In each group, 1 piglet received water at the moment of inoculation and served as a negative control. Our results showed that the piglet strain used was resistant to cryptosporidiosis. No clinical sign or oocyst shedding were observed in newborn piglets. A very weak shedding was noticed on day 6 (D6) post-inoculation in inoculated weaned piglets. Using ELISA, inoculated weaned piglets showed a peak of G, M and total antibodies on D10. Specific IgA antibody production peaked on D20. During the experiment on newborn piglets, no peak of specific IgA production was detected. Using immunoblotting, sera of both inoculated weaned piglets and one inoculated newborn piglet were shown to recognize a 14.5–16.5 kDa protein. A 23 kDa antigen was recognized by all 3 uninoculated and inoculated weaned piglets. A difference between mono and multi-inoculations was not clearly demonstrated. Age did not play any role. This pig strain does not seem to be a good model to induce acute cryptosporidiosis.

***Cryptosporidium parvum* / antibody / piglet**

Résumé — Réponse anticorps et excrétion d'oocystes chez des porcelets histocompatibles nouveau-nés et sevrés après infection expérimentale avec *Cryptosporidium parvum*. *Des*

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porcelets miniatures histocompatibles (d/d haplotype), 2 sevrés et 2 nouveau-nés, ont été inoculés per os *(mono- ou multi-inoculations) avec des oocytes d'une souche de* Cryptosporidium parvum *isolée chez un enfant diarrhéique puis multipliée régulièrement chez le veau. Un troisième porcelet dans chaque lot recevait de l'eau au moment des inoculations et était considéré comme témoin. Nos résultats montrent que cette souche de porc est résistante à la cryptosporidiose. Aucun signe clinique ni excrétion d'oocystes n'ont été observés chez les porcelets nouveau-nés inoculés et seulement une très faible excrétion à J6 chez les porcelets sevrés. Chez les porcelets sevrés inoculés un pic des anticorps totaux et d'anticorps IgG et IgM a été mis en évidence par la technique Elisa à J10. Les IgA ont augmenté plus tardivement avec un pic à J20. Chez les nouveau-nés inoculés aucun pic d'IgA spécifique n'a été décelé. Les sérums des 2 porcelets sevrés inoculés et les sérums d'un des nouveau-nés inoculés reconnaissaient une protéine de 14,5-16,5 kDa après analyse en* immunoblotting. *Un antigène de 23 kDa a également été reconnu par les 3 porcelets sevrés (inoculés et non-inoculés). Nous n'avons pas noté de différence dans la réponse immunitaire entre porcs mono- ou multi-inoculés. D'autre part l'âge n'a pas semblé jouer un rôle sur la sensibilité à l'infection. Cette souche de porc semble ne pas être un bon modèle d'étude pour reproduire une cryptosporidiose clinique.*

Cryptosporidium parvum / *anticorps* / *porcelet*

INTRODUCTION

Cryptosporidium is a protozoan parasite belonging to the *Apicomplexa* first described by Tyzzer (1907) in gastric mucosae of laboratory mice. *Cryptosporidium* parasitizes epithelial cells of the digestive and respiratory organs in a wide variety of host species, from fish (Hoover *et al,* 1981) and birds (Slavin, 1955) to mammals, including human (Meisel *et al,* 1976; Nime *et al,* 1976).

Cryptosporidium parvum (Tyzzer, 1912), specific to the mammal species, was considered as rare and not pathogenic until 1971, when it was detected in diarrheic heifer (Panciera *et al,* 1971). The interest in this parasite grew when it was found frequently in young ruminants less than 3-week-old, often associated with diarrhea (Fayer and Ungar, 1986). Prevalence and economic impact are very difficult to evaluate because other causes of disease are not always researched. Moreover, cryptosporidiosis can be asymptomatic or associated with other pathogenic agents (Fayer and Ungar, 1986).

In human medicine, *C parvum* may also cause very severe diarrhea. Immunocompromised patients, particularly those with acquired immunodeficiency syndrome (AIDS), may develop an unremitting and frequently life-threatening diarrhea (Weisburger *et al,* 1979; Weinstein *et al,* 1981; Sloper *et al,* 1982; Pitlik *et al,* 1983; Collier *et al,* 1984; Whiteside *et al,* 1984; Lewis *et al,* 1985).

At present, no therapy is readily available. Only lasalocid (Göbel, 1987; Pongs, 1989) gave good results in calves and halofuginone lactate in infected lambs (Naciri and Yvoré, 1989) and calves (Villacorta *et al,* 1991; Naciri *et al,* 1993). Against human infections, treatments with hyperimmune bovine colostrum were assayed (Tzipori *et al,* 1986; 1987) but these attempts have not been uniformly successful (Saxon and Weinstein, 1987; Ungar *et al,* 1990).

Cryptosporidium infection in pigs is not clearly defined. Bibliographic data are contradictory. Cryptosporidiosis does not seem to represent a serious cause of diarrhea, but Tzipori *et al* (1982) reported severe symptoms: vomiting, diarrhea, and anorexia. Several papers discuss the age of infection. Experimental studies seem to show development of illness in newborn piglets (Moon

et al, 1982). Tzipori *et al* (1982) demonstrated that 1-d-old piglets are more susceptible to cryptosporidiosis than 15-d-old piglets. For unknown reasons, natural infection has been detected more in older farm-bred piglets (Kennedy *et al,* 1977; Links, 1982), especially when they are 6- to 12-week-old (Sanford, 1987).

The presence of pathogenic agents (for example, viruses and bacteria) seems to enhance the development of clinical cryptosporidiosis (Naciri, 1989), and in addition cryptosporidiosis may promote a secondary bacterial infection by injuring intestinal mucosae (Bergeland, 1977). Apparently healthy piglets, with sub-clinical infections, can show a variety of intestinal mucosae damage (Kennedy *et al,* 1977; Sanford, 1987). However, no immunological study has been performed on pigs.

Our initial aim was to immunize histocompatible miniature (d/d haplotype) pigs with *C parvum* to study *C parvum*-sensitive spleen cells *in vitro*. The absence of disease following our inoculation and the absence of bibliographic immunological data performed on pigs incited us to study the kinetics of anti-*C parvum* antibodies in the serum, and to characterize the targets of porcine humoral immune response even if only 3 newborn and 3 weaned piglets were used.

MATERIALS AND METHODS

Parasites

The strain of *C parvum* used in this study was first isolated from diarrheic child feces (Arnaud-Battandier *et al,* 1982), then was propagated on calves or lambs. Oocysts were isolated by filtration through meshes (1 000 – 250 μm), and kept at +4°C in 2.5% potassium dichromate solution to avoid bacterial proliferation. Before use, the suspension was passed through a 100 μm filter and washed 3 times for 10 min (18°C, 1 800 *g*) with tap water to eliminate potassium dichromate. The remaining lipids of the pellets were eliminated after suspension in a diethyl ether/water mixture (1:4 v/v) and vigorously shaken. The pellets containing the oocysts were submitted to 3 washing cycles with distilled water (2 000 *g*, 10 min, 18°C). The pellets were passed through 20 μm meshes and centrifuged. They were washed twice in distilled water and sterilized with commercial laundry bleach diluted to 10% in water (1.25% sodium hypochlorite) for 20 min under gentle agitation. Sterilization was stopped by 3 washes in sterile distilled water. Particles sized less than 20 μm were eliminated on a discontinuous sucrose gradient: 50–30–20 and 10% of a 100% sucrose solution, composed of 500 g sucrose in 320 ml distilled water with 6.5 g phenol. The gradient was centrifuged at 2 000 *g* for 10–15 min at 18°C, according to the technique of Heyman *et al* (1986). In this procedure, purified oocysts were harvested between layers 20 and 30% and washed 3 times with sterile distilled water. This suspension was used for piglet inoculation after numeration with Toma counting cell.

Animals and inoculations

Histocompatible miniature piglets (d/d haplotype) were originally provided by D Sachs (NIH, Bethesda, MD, USA) and have been bred at Nouzilly since July 1985.

A first group of 3 just-weaned pigs (4 weeks) was orally inoculated as follows: piglet 1 received 10^6 oocysts on the day of first inoculation (D0) and piglet 2 received 10^6 oocysts on D0, 3, 5, 7, 10 and 11. Both piglets were challenged on D28 with 10^7 oocysts. Piglet 3 received water at the moment of inoculation and served as negative control.

A second group of 3 newborn piglets was constituted. Animals were allowed to suckle their mother for 2 d to avoid newborn death. They were then artificially fed with reconstituted milk (Gloria) until weaning age (4 weeks). Newborn piglet 1 (3-d-old) received 10^6 oocysts on D0 and piglet 2 (3-d-old) received 10^6 oocysts on D0, 10^7 oocysts on D3, 5, 6, 8 and 10. Both piglets were challenged with 10^8 oocysts on D31. Piglet 3 was a non-inoculated control.

Assessment of infection

Fecal samples were collected daily after D3, and oocyst shedding was semi-quantified by counting 25 fields in light microscopy under 250 x magnification: 0 for no oocysts; + for < 1 oocyst on average per field; and ++ for 1–5 oocysts per field. Piglets were weighed twice a week and blood samples were taken with a dry tube from the jugular vein, 3 times a week until D40, then once a week.

ELISA technique

The presence of antibodies of G, A, M and total isotypes was titrated in the diluted serum of weaned and newborn piglets. The antigen used in each well consisted of 200 000 frozen-thawed oocysts, in 100 µl sterile distilled water. Plates were coated overnight at 37°C (until fully dry). A short blocking step was carried out over 15 min by incubating with 100 µl/well of PBS containing 5% skimmed milk (SM). Microplates were gently washed twice with deionized water and twice with a 5 min incubation step in an isotonic solution containing 0.9% NaCl and 0.05% Tween 20. Each serum sample was tested in triplicate to evaluate the reliability of the technique. Dilutions of serum were incubated for 90 min in PBS-Tween 20–5% SM. Sera of weaned piglets were respectively diluted 1/20 for isotype A detection and 1/100 for G, M and total isotypes. Sera of newborn piglets were diluted 1/10. After the washing cycle, biotinylated conjugate goat anti-pig (H + L chains, Jackson USA) was diluted 1/10 000 in PBS-0.05% Tween 20–5% SM and incubated for 90 min. The signal was amplified by biotin/streptavidine horseradish peroxidase (30 min) and revealed with 100 µl ABTS substrate (2,2'-azino-bis(3-ethylbenzylthiazoline)-6-sulfonic acid, Sigma), 40 µl H_2O_2 9% and 10 ml citrate buffer pH 4. For IgG, A and M detection, affinity purified peroxidase conjugates goat anti-pig heavy chains (Jackson, USA) were diluted 1/1 000 for µ chains, 1/500 for α chains and 1/2 000, for γ chains and incubated for 90 min. Corresponding substrate was incubated for 1 h before reading on 405 nm absorbance. The plate-reader was zeroed on 8 wells containing only antigen, serum anti-species-enzyme conjugate and substrate. The positive control was an old pig serum with a high antibody level, and the negative control was a serum from fetal piglet. Data from control piglets were subtracted from data from experimental animals.

Immunoblot procedure

Oocysts (4 x 10^7) were submitted to 3 cycles of freezing-thawing at –70°C in sample buffer (50 mM Tris HCl pH 6.8; 10% glycerol; 2% SDS; 0.1% bromophenol blue) in non-reducing conditions. The oocyst sample was heated at 100°C for 5 min, and centrifuged (7 000 *g*, 10 min). The supernatant containing *C parvum* soluble proteins was collected. They were separated by electrophoresis on 15% SDS polyacrylamide gel until the dye front reached the end of the gel at room temperature, on a Minigel-Twin G42 (Biometra) apparatus, with a constant current of 25 mA per minigel (Laemmli, 1970). Standard molecular weight markers from 14.4 to 66 kDa (Sigma) migrated together with the oocyst extract.

Using horizontal transfer half-dry Milliblot SDE system (Millipore, St Quentin, France) apparatus, the migrate was transferred onto nitrocellulose (Biometra 0.1 µm), for 1 h with a constant current of 1 mA/cm^2. Nitrocellulose was divided into individual lanes and incubated with TNT (15 mM Tris, 140 mM NaCl, 0.05% Tween 20), 5% SM for 1 h. After elimination of the buffer, serum dilutions (1/50) in TNT–SM pH 8 were incubated for 1 h. After 3 washes with TNT–SM, goat anti-pig IgA antibodies (α-chain specific, Bethyl), diluted 1/1 000, or goat anti-pig IgG (anti Fc, Bethyl), diluted 1/1 000, were incubated for 1 h in TNT–SM. Antibodies were eliminated with 3 washes in TNT–SM and alkaline phosphatase antibodies rabbit anti-goat IgG (H+L, Jackson) incubated in TNT–SM for 1 h. Alkaline phosphatase conjugate was removed with 3 washes in TNT–SM, and one in R buffer (Tris 100 mM, NaCl 100 mM, $MgCl_2$ 5 mM pH 9.5). Nitro-blue tetrazolium (NBT, Promega) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP, Promega) were added in R buffer. The reaction was stopped with 20 mM Tris, 5 mM EDTA, pH 8 in distilled water after the desired contrast was obtained. The nitrocellulose lanes were dried and prepared for photography.

For weaned piglets, A and G isotype detections were done on D0, 12, 25, 40, 52, 65, 77, 84, 98, 112 (piglets 2 and 3) and 105 instead of 112 (piglet 1) and for newborn piglets on D0, 13, 22, 38, 45, 59 and 73.

RESULTS

Symptomatology of cryptosporidiosis

The 2 groups of pigs (weaned and newborn) showed no symptom of illness. Each day from D3 to D30 no oocyst shedding was observed, except on D6, noted (+) in feces of inoculated weaned piglets. For newborn piglets, no oocyst shedding was detected. The weight–growth curves of inoculated animals within both groups were found to be comparable to weight–growth curves of corresponding controls and so these data are not shown.

Immune response

Optical densities obtained by ELISA were very low, even when serum sample dilution was 1/10.

Weaned piglets

Piglets had no specific antibodies to *C parvum* antigens before the first inoculation. Kinetics of M, G and total isotypes showed a peak on D10 after the first inoculation (fig 1A, B, D). Multi-inoculated piglet 2 showed a weaker antibody response than mono-inoculated piglet 1 for G and total isotypes

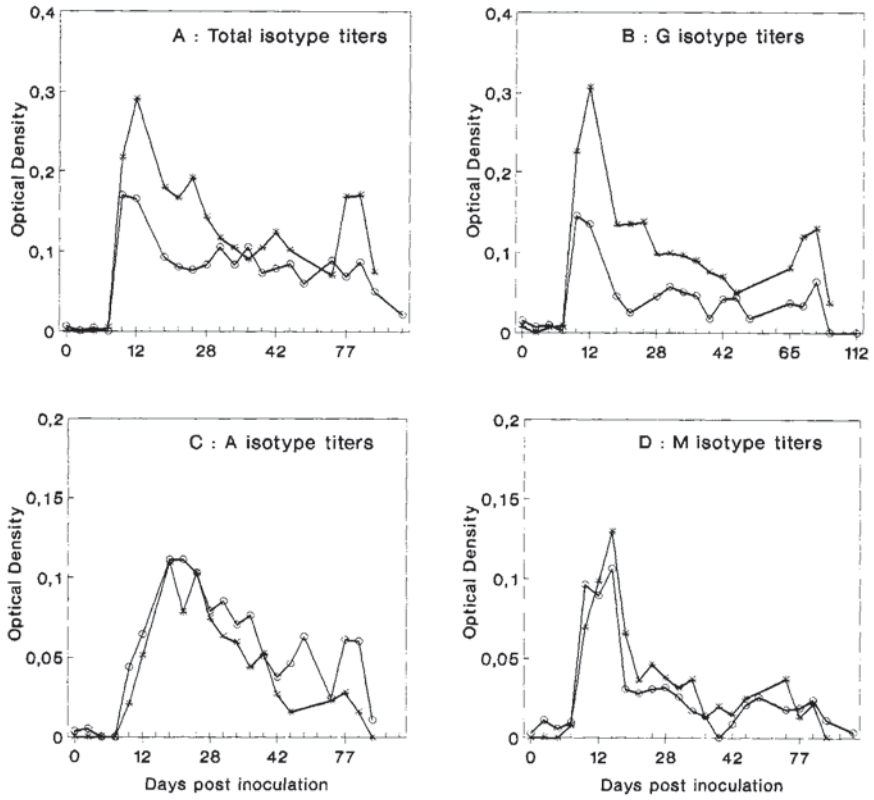

Fig 1. Serum titers of specific antibodies directed against *C parvum* in weaned piglets. Piglet 1 (—※—) was orally inoculated on D0 (10^6 oocysts) and 28 (10^7 oocysts). Piglet 2 (—o—) was orally inoculated on D0, 3, 5, 7, 10, 11 (10^6 oocysts) and 28 (10^7 oocysts). Data of the control piglet (non-inoculated) were subtracted from the data of piglets 1 and 2.

(fig 1A and B). Both inoculated piglets showed a similar response for IgM and A (fig 1C and D). A peak of IgA was observed on D20 (fig 1C).

Using immunoblotting, IgG detection performed on D0, 12, 25, 40, 52, 65, 77, 84, 98, 112 (piglets 2 and 3) and D105 (piglet 1) showed 2 main antigens of 23 kDa and 14.5–16.5 kDa strongly recognized by serum of the piglets 1 and 2 from D12. Serum from control piglet 3 weakly recognized a 23 kDa antigen from D25 to the end of the experiment (fig 2).

Sera from inoculated piglets recognized a 14.5–16.5 kDa antigen after D12, which decreased with time. Recognition by piglet 2 seemed to persist longer than recognition by piglet 1.

Newborn piglets

In ELISA, optical densities obtained were very low though sera were very little diluted (1/10). All 3 newborn piglets presented an optical density of 0.1 (IgM and IgA) and 0.4 (IgG and total isotypes) before inoculation. These maternal rates decreased to 0 on D13 and are not shown on our curves.

In figure 3A, piglet 1 did not show any specific response. Piglet 2 showed a maximal total isotype response (optical density 0.3) on D40. This rate then decreased (OD

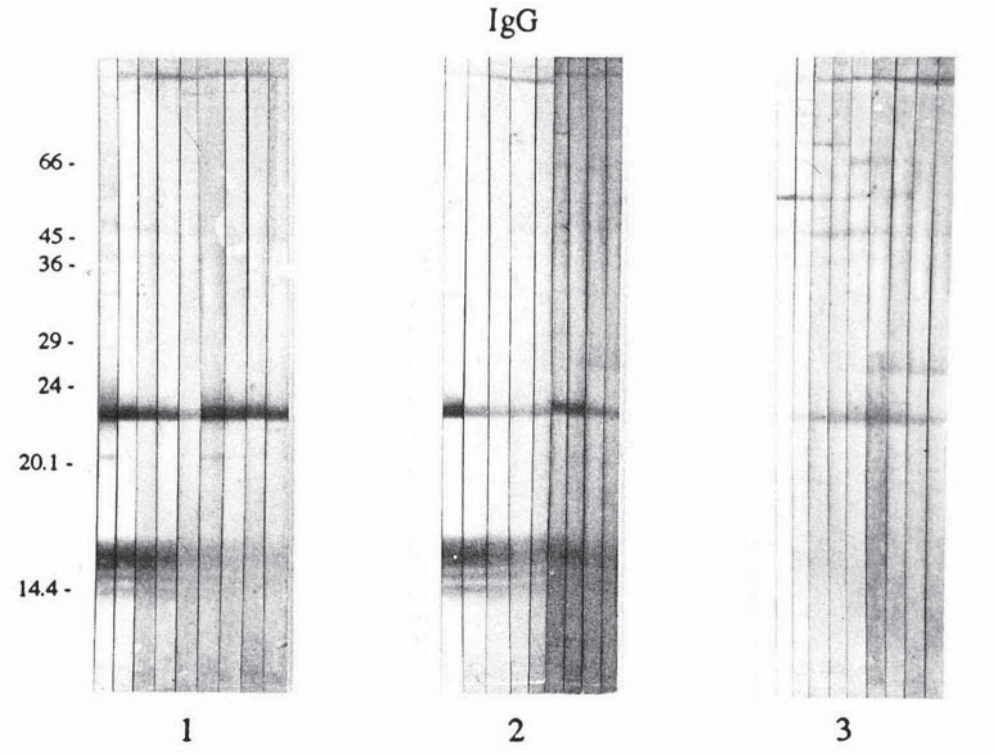

Fig 2. Immunoblots performed with sera of weaned piglets infected with *C parvum* revealing IgG on total parasite extract. Immunoblot 1: serum of piglet inoculated on D0 (10^6 oocysts) and 28 (10^7 oocysts). Immunoblot 2: serum of a piglet inoculated on D0, 3, 5, 7, 10, 11 (10^6 oocysts) and 28 (10^7 oocysts). Immunoblot 3: serum of control piglet, non-inoculated. Immunoblots were performed on D0, 12, 25, 40, 52, 65, 77, 84, 98, 112 (piglets 2 and 3), and 105 instead of 112 (piglet 1).

0.15). Isotype G response began after D13 and peaked on D43 and D73 (fig 3B). Isotype A and M responses were very weak (fig 3C and D).

As revealed by immunoblotting, serum IgA and IgG from piglet 2 (multi-inoculated) recognized a 14.5–16.5 kDa protein from *Cryptosporidium*, from D22 to D45 for IgA (fig 4), and from D22 to D73 (while decreasing) for IgG (figs 4 and 5). This recognition was not observed for the 2 other newborn piglets (non-inoculated and inoculated on D0 and D28) (fig 5). IgG in the serum of multi-inoculated piglet 2 recognized a 22 kDa antigen from DO to the end of the experiment (figs 4 and 5). This antigen was also recognized by the control piglet and piglet 1 between D45 and D73 (fig 5). Piglet 1 serum binding was weaker. The serum from the mother showed reactivity to several antigens of *C parvum*. Maternal IgG and IgA antibodies bound the 22 kDa antigen recognized by the serum of the piglets but did not recognize the 14.5–16.5 kDa antigen (fig 6).

DISCUSSION

Histocompatible weaned and newborn piglets mono- or multi-inoculated with *C parvum* did not show any symptom of illness. This strain of *C parvum* isolated in a diarrheic child and regularly maintained on calves is known in our laboratory for its virulence used in the same conditions (NaOCl

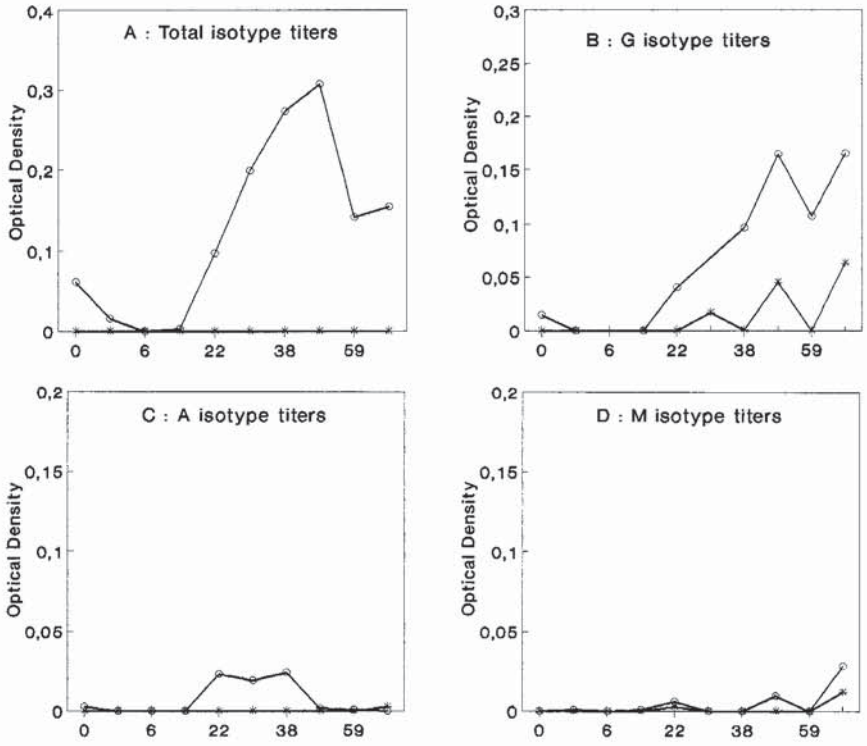

Fig 3. Serum titers of specific antibodies directed against *C parvum* in newborn piglets. Piglet 1 (—※—) was orally inoculated on D0 (10^6 oocysts) and 31 (10^8 oocysts). Piglet 2 (—o—) was orally inoculated on D0 (10^6 oocysts), 3, 5, 6, 8, 10 (10^7 oocysts) and 31 (10^8 oocysts). Data of the control piglet (non-inoculated) were subtracted from data of piglets 1 and 2.

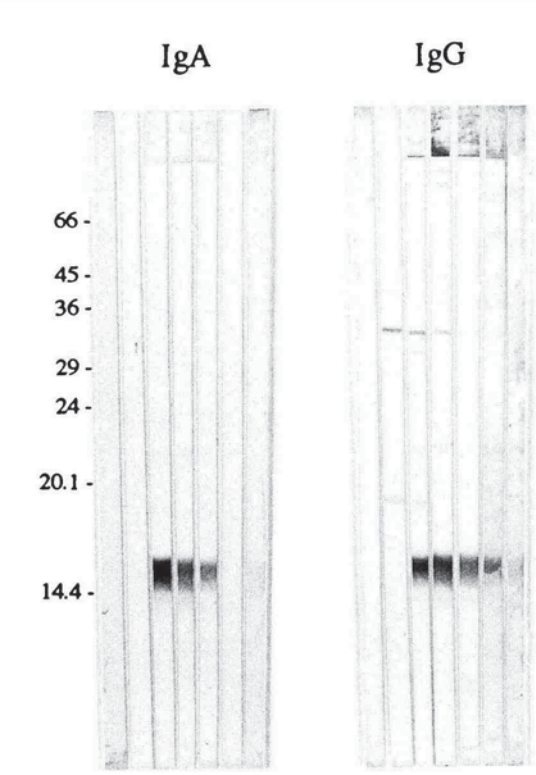

Fig 4. Immunoblot performed with serum of multi-inoculated newborn piglet infected with *C parvum*. IgA (left) and IgG (right) were revealed on total parasite extract on D0, 13, 22, 38, 45, 59 and 73.

treatment) in neonatal ruminants (calves, kids, lambs). The few oocysts found in feces of the mono- or multi-inoculated weaned piglets on D6 show that the development of the parasite in the intestine was limited. These results seem to confirm the data from conventionally farm-raised pigs, which generally develop subclinical infections (74% of infected pigs, according to Sanford, 1987).

For the weaned piglets, repeated inoculations did not lead to a higher immunological response, as suggested by multi-inoculation (McDonald *et al,* 1991). We could conclude that multi-inoculation was useless. However, we must bear in mind that the difference may be due to individual variation, as this study was not performed on groups of animals. Nevertheless, we always found the best response on D10 for IgG and M (Fig 1A,B,D) and D20 for IgA (fig 1C). These results seem to be different from those obtained with 5-d-old infected lambs, which developed an IgA peak on D11, a constant IgM level after D7, and an increasing rate of IgG (Hill *et al,* 1990). Hill (1989), Peeters *et al* (1992) and Répérant *et al* (1992) observed that oocyst shedding stopped when the IgA peak began. Oocyst detection in pig feces has met with disappointing results (Kennedy *et al,* 1977; Tzipori *et al,* 1981). The inoculated weaned piglets may have excreted oocysts at a low rate until D20 (IgA peak, fig 1C) but this shedding was under the detection limit of the technique.

The specific response in ELISA (peak on D10, D20, fig 1) was confirmed on immunoblot by showing recognition of a 14.5–16.5 kDa protein from D12, which disappeared after D52 for the piglet 1 and lasted longer for the piglet 2 (fig 2). This recognition lasted longer for the multi-inoculated piglet than for the mono-inoculated piglet and thus contradicts ELISA results, which showed better results for the mono-inoculated piglet (fig 1). Serum from the control piglet did not recognize the 14.5–16.5 kDa antigen. The 15–17 kDa band is a good marker of infection. This band was always intensively recognized by the serum of our ruminants (calves, lambs and kids) experimentally infected with *C parvum* (Répérant *et al,* 1994). A 23 kDa protein also bound strongly with serum of weaned piglets 1 and 2 and weakly with that from the control piglet (fig 2). Even if the piglets were not kept in isolators but in separate cages in the same room, it is difficult to explain the contamination of the control piglet by inoculated piglets considering the low oocyst shedding

of the latter and the maintenance conditions. Hill (1989), Hill *et al* (1990), Tilley *et al* (1990, 1991) and Fayer *et al* (1991) found a 15 kDa and a 23 kDa protein which could be the same antigens as the one we describe here, but our 23 kDa antigen seems to be less specific. The 23 kDa antigen was also found by Ungar and Nash (1986) in sera of AIDS and non-AIDS patients. Mead *et al* (1988) found a 20 kDa protein in convalescent sera from experimentally infected calves and naturally infected horses and humans. This protein could correspond to the 23 kDa.

Weaning did not constitute a stress factor favorable to the development of illness in pigs (Sanford, 1987; Tacal *et al*, 1987; Naciri, 1989).

Newborn piglets did not seem to react in the same way as newborn calves or lambs (Harp *et al*, 1990; Hill *et al*, 1990), and did not show any symptom of illness. These results contradict experimental data on newborn piglets (Tzipori *et al*, 1981, 1982), but corroborate data from farm-raised piglets (Sanford, 1987). Piglets were allowed to suckle their mother during the first 2 d to prevent death, and it is possible that factors including maternally derived lactogenic immunity might have operated to reduce infection (Sanford, 1987). However, in mice and ruminants the maternal antibodies would not be protective. In mice, Moon *et al* (1988) showed that immune dams did not protect their suckling pups. According to Naciri *et al* (1994), lambs suckling their

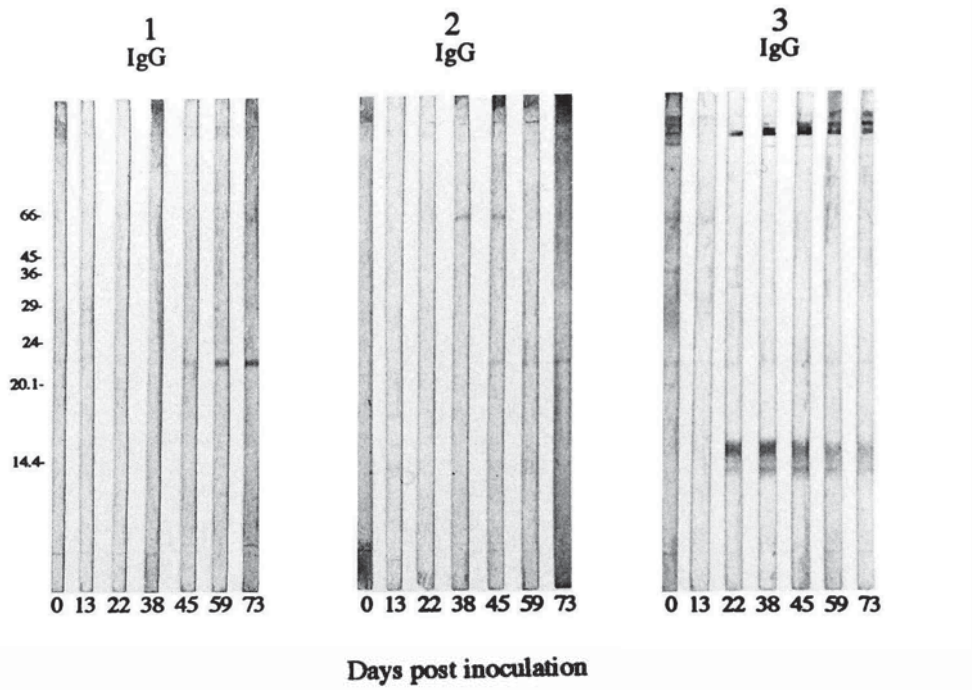

Fig 5. Immunoblots performed with sera of newborn piglets infected with *C parvum* revealing IgG on total parasite extract. Immunoblot 1: serum of control piglet, non-inoculated. Immunoblot 2: serum of piglet 1 inoculated on D0 and D28. Immunoblot 3: serum of piglet 2 inoculated on D0, 3, 5, 6, 8, 10 and 31. Immunoblots were performed on D0, 13, 22, 38, 45, 59 and 73.

hyperimmunized dams were not protected against *C parvum* infection in spite of their high titers of serum specific anti-*C parvum* immunoglobulins. The efficiency of hyperimmune colostrum is local, in the lumen of the gut. The colostral antibody levels should be high and needs long-term administration to be effective. Perhaps, these piglets react as other mammals, such as rabbits, cats and dogs, which do not develop usually clinical cryptosporidiosis. Treatments with immunosuppressive drugs (cyclophosphamide and corticosteroids) or an immunosuppressive retrovirus infection (feline leukemia virus or feline immunodeficiency virus in cats) cancel this innate resistance. Only an experiment with piglets separated from their dams at birth and reared exclusively with artificial milk in isolators would allow us to study the resistance of this strain of piglets to *C parvum* infection.

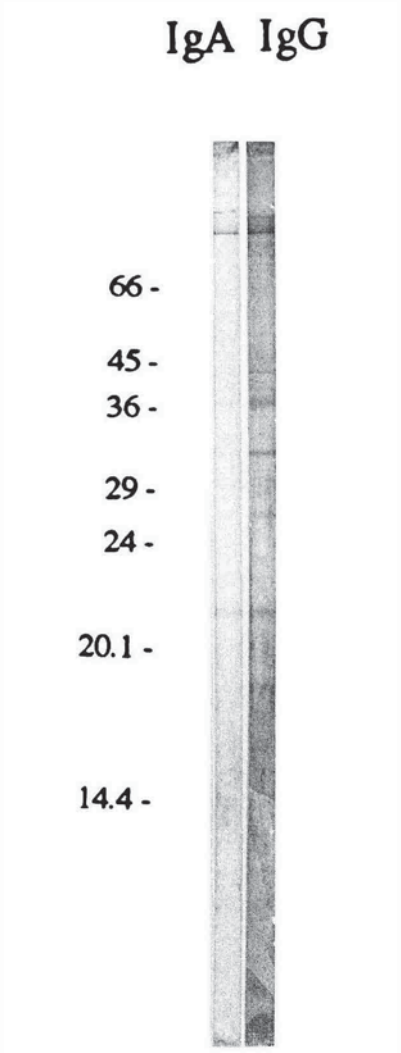

Fig 6. Immunoblot performed with serum of the mother of the newborn. IgA and IgG are detected on total parasite extract.

With the ELISA technique, the 2 inoculated newborn piglets reacted very differently. Piglet 1 (inoculated at D0 and D28) did not react to the antigenic stimulations (figs 3 and 5). Oocyst suspension seemed to be swallowed normally, but we did not verify the oocysts shedding a few hours after inoculation and did not find any oocyst later on. Piglet 2 (multi-inoculated) showed a very weak G isotype response (fig 3B). An IgA response seemed to appear, but would have needed to be amplified for verification. The protective role of immunoglobulin secretion is still to be demonstrated: newborn 1 never developed symptoms of illness with binding on immunoblot or ELISA detection. Thus, specific serum antibodies do not seem to be necessary to protect from illness.

This is the first immunological study performed on piglets. We have chosen the 2 main ages which could be susceptible to cryptosporidiosis, but inoculations were inefficient. This strain of piglets did not develop clinical illness. Weaned piglets developed a peak of G and M isotypes on D10 (fig 1A, B, D), and A isotype on D20 (fig 1C). Immunoblots showed a recognition of a 14.5–16.5 kDa protein, as well as a 23 kDa protein (fig 2). This pig strain had been chosen for its physiological and immunological analogies with man. It could

have been a good model to study human infection, to consider an immunological therapy with porcine monoclonal antibodies.

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