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Infectivity of *Giardia* Isolates from Pig and Sheep for Dogs

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ABSTRACT

Two experimental cross-infections of beagle puppies with the *Giardia intestinalis* are described. In each experiment three littermates at the age of 18 and 24 days were included. In each group, two puppies were orally inoculated with cysts, the third animal served as a control. One of the inoculated puppies in each group was immunosuppressed by dexamethasone. In the first experiment, an isolate from lamb was used for the inoculations at a dose of 20,000 cysts pro toto, in the second experiment an isolate from pig at a dose of 30,000 cysts pro toto. Feces were examined regularly for *Giardia* cysts, blood samples were analyzed for specific anti-*Giardia* IgG and IgM antibodies by means of the IFAT technique and parameters of non-specific immunity were determined. The attempt at cross-transmission failed even in markedly immunosuppressed puppies. Neither release of cysts nor seroconversion of circulating specific antibodies were detected. We assume that cross-infection of dogs can occur only under exceptional circumstances. Dogs do not represent significant source of *Giardia intestinalis* infection for man, even during their mutual close coexistence.

INTRODUCTION

The protozoan *Giardia intestinalis,* commonly found in man and animals, is not strictly host specific. Giardiosis is therefore currently implicated as a zoonosis. Wallis and Wallis (1986) successfully infected gerbils with *Giardia* isolates from beavers, dogs and humans. The work of Gasser et al. (1987) and

Tammineli et al. (1989) brought further confirmation of the zoonotic potential of *G. intestinalis*. However, in 1988 Bemrick and Erlandsen expressed doubts as to whether giardiosis really is a zoonosis. Nevertheless, Thompson et al. (1990) suggested that the occurrence of genetically different isolates from various hosts does not exclude the zoonotic transmission of *Giardia*. Meloni et al. (1995) who studied the genetic characterization of *Giardia* isolates from animals and humans interpret their results as an evidence of possible zoonotic transmission.

The activity of immune system is essential for the course of infection. The antibody production in particular is of crucial importance for the immune response of the macroorganism (Smith 1985; Farthing and Goka 1987). Decreased total antibody levels were found in dogs with giardiosis (Toman et al. in press). Dogs living in close contact with humans are often discussed as a source of giardiosis for man (Hay et al. 1990; Collyer et al. 1992).

We studied therefore the zoonotic potential of Giardia as related to the number of species, various isolates and their specificity (McDonald et al. 1997) with regard to the coexistence of man and dog. The aim of the present study was to evaluate the susceptibility of dogs with different immune status to cross-transmission of *Giardia*.

MATERIALS AND METHODS

We performed two experimental cross-infections with isolates of *Giardia intestinalis* in the period between April and August 1996. For each of two experimental infections, one group of three beagle puppies from one litter at the age of 24 and 18 days, respectively, were used. The puppies were kept in daily disinfected kennels and fed commercial diet. One puppy from Exp. I and one puppy from Exp. II were immunosuppressed by dexamethasone (Dexona inj., Cadila Lab. Ltd., India) in four three-day cycles at a daily dose 1.1 mg/kg^{-1} i.m. The first dose was administered three days prior to infection. Before the onset of experiment, the feces of the puppies were examined three times for parasites and blood samples were collected for serological analysis and immunological profile determination.

The first experimental infection was performed on puppies 24 days old using cysts isolated from gerbils infected with *Giardia* cysts originating from a 9-week old lamb. The infected Mongolian gerbils (*Meriones unguiculatus*) were kindly provided by Dr. B. Koudela, Parasitology Institute, Academy of Science of the Czech Republic. Two puppies, the immunosuppressed and intact one, were inoculated orally with 20,000 cysts. The third puppy served as a control.

For the second experimental infection, the strain P2 MER isolated from a 6-week old piglet was used. This strain was transmitted to gerbils (infected gerbils

were also obtained from Dr. B. Koudela). Again, a healthy and an immunosuppressed puppy were inoculated orally at the age of 18 days with the infection dose of 30,000 cysts. The third puppy served as a control.

Examination of fecal samples of puppies were done every other day with the flotation method using saturated saccharose solution with specific mass of 1.3. The blood samples from puppies were collected once a week for a period of 6 weeks. Five ml of heparinized blood was used for cells counts and nonspecific activity tests. Total immunoglobulin levels and isotype specific anti-*Giardia* antibody levels were determined in serum. During the course of experiments, the health status and basic clinical parameters of puppies were examined. In the dead puppy a scraping of small intestinal mucosa was taken and the samples were stained by Giemsa. Histological preparations of the intestine were also examined.

The detection of specific anti-*Giardia* antibodies was performed by indirect fluorescent antibody test (IFAT) using conjugates Dog IgG-FITC and Dog IgM-FITC (Immunotech and Coulter Company). *Giardia* cysts isolated from feces of inoculated gerbils were used as an antigen. Cysts were purified by sucrose gradient flotation (Roberts-Thompson et al. 1976) and the suspension containing *Giardia* cysts was applied to slides and antigen was fixed with acetone and stored at -20°C. Blood sera were tested simultaneously to IgG and IgM in twofold dilutions starting at titer 10 up to the positive end titer. Reliability of the serological reaction was checked by inclusion of the following controls: negative - without serum and with repeatedly negative sera; positive - with repeatedly positive sera.

Total leukocyte counts were determined using cell counter Dicell 500. Differential leukocyte counts were done from blood smears by routine hematologic procedures. Flow cytometry (Coulter EPICS II) was used for determination of lymphocyte subsets. A panel of five monoclonal antibodies against surface antigens of leukocytes (kindly provided by Dr. E. Kremer, Munich and Dr. P. F. Moore, Davis) were used in the first step and swine anti-murine FITC conjugate (ÚSOL Prague) in the second step. The phagocytic activity was determined by counting ingested metacrylate (HEMA, ARTIM Prague) particles in neutrophils and by calculating phagocytizing cells. The metabolic activity of the phagocytizing cells was determined by chemiluminiscence method in the luminometer BioOrbit 1251 using luminol as luminophore. Respiratory burst of cells was determined after stimulation with 1% rice starch. The activity of lymphocytes was assessed in lymphocyte transformation test. Mononuclear cells were isolated on gradient and cultivated in media RPMI 1640 with mitogens (phytohemagglutinin and concanavalin A) and the incorporation of ^{3}H labeled thymidin into the newly formed DNA was measured. The immunoglobulin concentration was determined spectrophotometrically measuring the turbidity

resulting from addition of zinc sulfate. Detailed description of the method was published elsewhere (Toman et al. 1997).

RESULTS

The first experimental infection --- the isolate from lamb

Prior to infection

Parasitological examination of feces of puppies aimed at the shedding of *Giardia* was negative. Only specific IgG antibodies against *Giardia* at low titers 40-80 were present. In the dam, IgG was identified at the titer 160. IgM was found neither in the puppies nor in their dam. The leukocyte counts and parameters of non-specific activity of the immune system of experimental puppies prior to infection corresponded to the stage of ontogenesis. Higher percentage of B lymphocytes and lower proportion of T lymphocytes was characteristic.

After infection

We could not find release of *Giardia* in any puppy by parasitological examination. Low level of specific IgG was found in puppies by means of IFAT. The maximal titer was 80 and it decreased gradually. Anti-*Giardia* IgM was not detected. After infection, only minimal changes of non-specific parameters of the immune system were determined. The minimal Ig level in experimental animals was achieved at the age of 5 weeks, while in the control dog at the age of 5-6 weeks. In the infected and not immunosuppressed puppy, transient decrease in phagocytic activity was detected 3 weeks after experimental inoculation. In the immunosuppressed puppy, increase in neutrophils and decrease of lymphocyte counts and activity were observed.

Neither the control nor the infected puppy showed clinical symptoms of disease and their development during the course of the experiment corresponded to their age. On the contrary, in the immunosuppressed puppy increased water intake (*polydipsia*) and excessive urination (*polyuria*) were observed. The growth of the puppy was markedly retarded, it was lethargic and its abdomen was enlarged. Numerous pustules (*impetigo*) appeared on the skin of lower abdomen and inguinal area.

The second experimental infection --- the isolate from pig

Prior to infection

The parasitological examination of feces did not reveal shedding of *Giardia* cysts. No specific immunoglobulins G and M to *Giardia* were detected by serological analysis in the puppies and their dam. The parameters of non-specific activity of the immune system were similar to those found in puppies in the Exp.

I.

After infection

During the course of the experiment, no puppy was shedding *Giardia* cysts. Specific anti-*Giardia* immunoglobulins G and M were not detected by serological analysis. Similarly, only minimal changes in non-specific parameters of the immune system were found. The minimal levels of immunoglobulins were found in the infected puppies in the 5th week, while in the control puppy in the 5th and 6th week.

The immunosuppressed puppy died at 11 DPI without major alteration of the activity of the immune system. At necropsy, enlarged fragile liver, enlarged kidneys and effusion in abdominal cavity were found. Neither *Giardia* trophozoites nor cysts were observed during the examination of the small intestinal mucosal scraping and histological preparations.

The status of the infected and control puppy was again identical, i.e. corresponding to the growth stage and without clinical signs of disease. In the immunosuppressed puppy, the clinical changes observed in the first experiment were not noted until the death of the puppy.

DISCUSSION

The giardiosis occurs in dogs often in relation to the age and the system of keeping. *Giardia* cysts were found in 5.5% of dogs in the Czech Republic. The release of cysts was more common in young animals and in large kennels (Svobodová et al. 1987). The prevalence of specific antibodies (36.5%) suggests the contact of dogs with this often latent or opportunistic infection during their life (Svobodová et al. 1995).

Isolates from lamb and pig were used for experimental infection of puppies, since the release of *Giardia* cysts is frequently encountered in young farm animals which are also considered as a potential source of infection for humans (Kirkpatrick et al. 1989). Due to higher concentrations in which animals are reared we can consider the relatively easy transmission to dogs living in their vicinity. We inoculated the puppies with relatively high doses of cysts (20,000 and 30,000 pro toto) which exceed several times the amount of cysts sufficient for natural infection (Rendtorff 1954; Kirkpatrick 1987; Eckert 1989). We used exactly defined *Giardia* isolates in order to confirm the successful infection in case of cysts release and to exclude other possible sources, e.g. autochthonous infection. However, no cysts shedding occurred during the six-week study.

The detected changes in cell numbers and non-specific activity of the immune system were associated with the maturation of the immune system or

immunosuppression in selected puppies. The differences in the decrease of the total immunoglobulin levels in individual puppies can be attributed to individual variation as well as to different levels of colostral immunity, not to the influence of infection with *Giardia*. Similarly, the decrease in phagocytic activity in one puppy three weeks after experimental infection cannot be attributed to this inoculation. In our previous studies (Toman et al. in press) we found low levels of immunoglobulins, neutropenia and in some cases decreased phagocytic activity in dogs with giardiosis. We did not note these changes in experimental infection. The puppies did not even respond to the inoculation by the production of specific circulating IgG and IgM antibodies, that is, an observation compatible with the fact that actually no transmission and multiplication of the inoculated strains occurred. Low IgG titers found in puppies in the first experiment were probably derived from the dam's colostrum since their levels were very similar prior to and following the inoculation. We assume the elimination of the introduced *Giardia* by means of intestinal immune mechanisms, particularly by intestinal IgA (Heyworth et al. 1988). The *Giardia* had been thus destroyed before they were able to initiate the synthesis of specific circulating antibodies. Data in the available literature suggest that the decreased activity of immune system represents a predisposition to giardiosis rather than being its consequence (Smith 1985; Toman et al. in press). However, in our experiments we even could not facilitate the cross-transmission in cases with immunologically and clinically markedly expressed immunosuppression. Isolates used in puppies confirm the low specificity of *Giardia*, because the transmission from pig and lamb to gerbils and kids was successful (Koudela and Vítovec in press). However, gerbils are very susceptible to infection with *Giardia* and they were infected with various isolates including those from dogs (Wallis and Wallis 1986; Gasser 1987). Considering our own observation, the transmission of *Giardia* isolated from dogs to gerbils is often unsuccessful, in contrast to isolates e.g. from ruminants. If cross-infection occurs in dogs, it represents probably an exceptional situation, as described by Woo and Paterson (1986), who unsuccessfully attempted to infect puppies and kittens with cysts isolated from children's feces.

We can assume that the transmission of *Giardia* from the dog to man is also an exception which is confirmed indirectly by the results of analysis of feces from owners and staff taking care of infected dogs. In no case, including families with small children, concurrent infection was found (Svobodová et al. 1987; Castor and Linguist 1990; Pospísilová and Svobodová 1992; Hrejs and Koudela 1994). Of course, these results do not definitely exclude the possible transmission from dog to man with regard to many genetically different isolates of *Giardia* originating from animals and man in various geographical regions (Gasser 1990; Thompson

et al. 1990). In contrast to Warburton et al. (1994) and Collyer et al. (1992), in our opinion dogs are often infected with *Giardia* during their life, however, they do not represent major source of infection for man and are not responsible for significant number of cases of human giardiosis. Glaser et al. (1994) do not even exclude the coexistence of pets with HIV-positive patients suffering from general immunodeficiency, since the positive effect of such relationship outweighs the relatively small risk of transmission. Of course, all dogs passing out *Giardia* cysts should be treated in order to minimize the danger of infection for man and animals.

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