A novel anti-*Giardia* oral vaccine for domestic animals prevents establishment of infection, alleviates chronic giardiasis, and decreases parasite transmission to humans.

The flagellated protozoan *Giardia lamblia* (syn. *G. duodenalis* or *G. intestinalis*) is one of the most common causes of human intestinal disease worldwide, the most frequent cause of defined waterborne outbreaks of diarrhea, as well as a common cause of diarrhea in day-care centers, institutionalized individuals, backpackers, and travelers. In developing countries, about 200 million people have symptomatic giardiasis, with some 500,000 new cases reported each year. In regions where basic sanitation is deficient, *Giardia* infections in children are almost universal. *Giardia* has being recognized as a re-emergent infection and included in the “Neglected Diseases Initiative” by the WHO. Although *G. lamblia* is recognized worldwide as the most frequent protozoan parasite causing intestinal disease in humans, the relevance of *Giardia* infections in other mammals and its zoonotic potential is controversial. Domestic dogs and cats can be asymptomatic or can have diarrhea due to maldigestion, malabsorption and increased gut motility. In companion animals in general, *Giardia* is regarded as a potential cause of diarrhea, weight loss, and lethargy.

In veterinary, research has mostly focused on *Giardia* prevalence and on the molecular characterization of isolates from different hosts to determine their zoonotic potential. Phylogenetic studies of numerous *Giardia* isolates allowed the identification of eight *G. lamblia* assemblages (A to H). Assemblages A and B are capable to infect many mammals, which can serve as reservoirs of human infections. Importantly, humans can also be a possible *Giardia* reservoir for domestic and production animals. *G. canis* (assemblage C), *G. cati* (assemblage F) and *G. bovis* (assemblage E) seem to be restricted to particular species or type of hosts, whereas other *Giardia* species have a broad host range, including the human assemblages A and B. In addition to the livestock assemblage E, the zoonotic assemblage A and, occasionally, the human assemblage B have been found in production and experimental animals.

Unfortunately, efficient vaccines against *Giardia* are not currently available. A few anti-parasitic drugs are used to treat giardiasis, but re-infections are common and drug resistant strains have already been reported. *Giardia* undergoes antigenic variation, a mechanism that allows parasites to evade the host’s immune response, producing chronic and/or recurrent infections. Antigenic variation is characterized by the continuous switching in expression of a family of homologous genes encoding surface antigens. Recently, we found that the mechanism controlling variant-specific surface proteins (VSPs) switching in *Giardia* involves components of the RNA interference (RNAi) pathway. Only one VSP, of a repertoire of ~200 VSP genes, is expressed on the surface of individual trophozoites, but disruption of the RNAi machinery generates trophozoites expressing the entire repertoire of VSPs. We then hypothesized that trophozoites expressing all potential VSPs on their surface may be able to confer protection against subsequent infections. Therefore, we performed experiments using altered parasites of the *Giardia* WB isolate (assemblage A) in the gerbil model of giardiasis. Our results showed that initial infection with cells expressing all VSPs encoded in their genome highly protected the animals against subsequent infection by *Giardia* clones expressing a unique VSP on their surface or by cysts obtained from infected individuals. Additionally, immunization with the entire repertoire of VSPs purified from these transgenic cells, using a monoclonal antibody (mAb) directed to the 5-amino acid conserved cytoplasmic tail, also conferred protection against secondary infections. Our results showed, for the first time in any parasite, the direct experimental evidence that antigenic surface variation is an essential mechanism responsible for evasion of the host immune response. We also showed that secretory IgA anti-variable surface antigens are crucial for avoiding parasite colonization of the host’s intestine. Serum and intestinal content obtained from infected animals were able to agglutinate trophozoites belonging to assemblages A and B, indicating the presence of antibodies against VSPs in those fluids after immunization and an important cross-reaction among VSPs of one assemblage to those belonging to another. We had previously demonstrated that the murine parasite *G. muris* also undergoes antigenic variation and that many mAbs generated to VSPs of this species cross-react with VSPs of *G. lamblia* assemblages A and B, suggesting that, despite their heterogeneity, VSPs would share common epitopes.

In this new work, we used wild type and transgenic *Giardia* parasites to determine the course of experimental infections in young and adult domestic animals. In addition, we generated a simple and sensitive diagnostic test to monitor the release of cysts by infected animals to be used in large-scale field studies. Subsequently, we used an oral vaccine formulation comprising VSPs purified from *Giardia* WB isolate expressing the full repertoire of their VSPs and tested its efficacy in experimental infections in puppies and kittens and in dogs and cats inhabiting a *Giardia* endemic shanty town.

We found that disruption of antigenic variation in *G. lamblia* can generate viable parasites that complete their life cycle *in vivo* and *in vitro*. These cells are capable of infecting domestic animals and conferring protection against subsequent infections. Similarly, oral immunization with the entire repertoire of variable surface antigens prevented the establishment of infection by trophozoites or cysts both in experimental and domestic animals; alleviated the symptoms of the disease in already infected animals and, importantly, abolished the release of cysts into the environment, decreasing the transmission of the infection among susceptible hosts. Remarkably, due to the intrinsic characteristics of the VSPs (resistance to proteolytic digestion, acidic pH, etc.), this oral vaccine is the only one composed solely by native proteins and capable to generate a strong mucosal and systemic immune response. For this reason, VSP may constitute an excellent carrier to shuttle other candidate vaccines by the oral route, avoiding all the problems associated with vaccines given by injection.