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Vaccine development for cryptosporidiosis: Systematic review

Desenvolvimento de vacina para criptosporidiose: Revisão sistemática

Desarrollo de vacunas para criptosporidiosis: Revisión sistemática

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Abstract
Cryptosporidium spp. are opportunistic parasites with zoonotic potential transmitted by ingestion of contaminated water and food, the infection consists of severe diarrhea leading to the death of babies and immunocompromised individuals. In the veterinary, it can generate economic losses due to the death of calves, these animals being a possible source of transmission of the parasite. Nitazoxanide and halofuginone are the only drugs approved for treatment in humans and calves respectively, however there are restrictions on their use. There is still no vaccine against cryptosporidiosis in humans or animals and its development is a great challenge. Our objective with the present work was to write a systematic review of the literature addressing the progress of studies on vaccines against cryptosporidiosis. The recommended guidelines for RSL were used, with the aid of the StArt software (State of the Art through Systematic Rewiews). The research took place in the databases: Lilacs, PubMed, Scielo, Science Direct, Scopus, Embase and Medline. In 30% of the selected articles, the calves were the study animal and in 50% the mice; 40% of the studies were with vaccines derived from recombinant proteins and 30% of the research was aimed at inhibiting the parasite from entering cells and activating the host's protective immunity. So far, a vaccine with no proven efficacy or an ideal cost-benefit has been developed. Many candidate antigenic targets for a vaccine have been characterized, in addition to elucidating the immunogenicity mechanism of *Cryptosporidium* spp. in the infected individual. However, another antigenic targets for a vaccine can be searched in future studies.
Keywords: *Cryptosporidium* spp.; Coccicidiosis; Immunizatio; Zoonosis.

Resumo
Cryptosporidium spp. são parasitos oportunistas com potencial zoonótico transmitidos pela ingestão de água e alimentos contaminados, a infecção consiste em diarreia severa levando à morte de bebês e indivíduos imunocomprometidos. Na área veterinária, pode gerar prejuízos econômicos devido à morte de bezerros, sendo esses animais uma possível fonte de transmissão do parasita. A nitazoxanida e a halofuginona são os únicos medicamentos aprovados para tratamento em humanos e bezerros, respectivamente, porém há restrições ao seu uso. Ainda não existe vacina contra a criptosporidiose em humanos ou animais e seu desenvolvimento é um grande desafio. Nosso objetivo com o presente trabalho foi escrever uma revisão sistemática da literatura abordando o andamento dos estudos de vacinas contra a criptosporidiose. Foram utilizadas as diretrizes recomendadas para RSL, com auxílio do software StArt (Estado da arte por meio de revisões sistemáticas). A pesquisa ocorreu nas bases de dados: Lilacs, PubMed, Scielo, Science Direct, Scopus, Embase e Medline. Em 30% dos artigos selecionados, os bezerros foram o animal do estudo e em 50% os camundongos; 40%

dos estudos foram com vacinas derivadas de proteínas recombinantes e 30% das pesquisas tiveram como objetivo inibir a entrada do parasita nas células e ativar a imunidade protetora do hospedeiro. Até o momento, foi desenvolvida uma vacina sem eficácia comprovada ou custo-benefício ideal. Muitos candidatos a alvos antigênicos para uma vacina têm sido caracterizados, além de elucidar o mecanismo de imunogenicidade de *Cryptosporidium* spp. no indivíduo infectado. No entanto, outros alvos antigênicos para uma vacina podem ser pesquisados em estudos futuros.
Palavras-chave: *Cryptosporidium* spp.; Coccicidiose; Imunização; Zoonose.

Resumen

Cryptosporidium spp. son parásitos oportunistas con potencial zoonótico transmitidos por la ingestión de alimentos y agua contaminados, la infección consiste en una diarrea severa que conduce a la muerte de lactantes e individuos inmunodeprimidos. En el área veterinaria puede generar pérdidas económicas por muerte de terneros, siendo estos animales una posible fuente de transmisión del parásito. La nitazoxanida y la halofuginona son los únicos medicamentos aprobados para el tratamiento en humanos y terneros, respectivamente, sin embargo, existen restricciones sobre su uso. Todavía no existe una vacuna contra la criptosporidiosis en humanos o animales y su desarrollo es un gran desafío. Nuestro objetivo con el presente trabajo fue escribir una revisión sistemática de la literatura que aborde el avance de los estudios sobre vacunas contra la criptosporidiosis. Se utilizaron las pautas recomendadas para RSL, con la ayuda del software StArt (Estado del arte mediante revisiones sistemáticas). La investigación se realizó en las bases de datos: Lilacs, PubMed, Scielo, Science Direct, Scopus, Embase y Medline. En el 30% de los artículos seleccionados, los terneros fueron el animal de estudio y en el 50% los ratones; El 40% de los estudios se realizaron con vacunas derivadas de proteínas recombinantes y el 30% de la investigación tuvo como objetivo inhibir la entrada del parásito en las células y activar la inmunidad protectora del huésped. Hasta ahora, se ha desarrollado una vacuna sin eficacia probada ni con una relación costo-beneficio óptima. Se han caracterizado muchas dianas antigénicas candidatas para una vacuna, además de dilucidar el mecanismo de inmunogenicidad de *Cryptosporidium* spp. en el individuo infectado. Sin embargo, se pueden buscar otras dianas antigénicas para una vacuna en estudios futuros.
Palabras clave: *Cryptosporidium* spp.; Coccicidiosis; Inmunización; Zoonosis.

1. Introduction

Parasites of the genus *Cryptosporidium* spp. they are opportunistic and have zoonotic potential and can cause gastroenteric infections, via fecal-oral route or by the ingestion of contaminated water and food (Avendaño et al., 2018; Mcnair et al., 2018; Hemphill et al., 2019), with their sporulated oocysts (Lippuner et al., 2018).

Limited access to disinfectant agents, the absence of effective drugs and vaccines associated with the ability of oocysts to develop infection, makes cryptosporidiosis very important in public health (Askari et al., 2016). In developing countries, it is considered a threat, as it causes malnutrition, death of babies and immunocompromised individuals (Ryan et al., 2016; Avendaño et al., 2018; Mcnair et al., 2018).

For now, 40 species of *Cryptosporidium* have been described, among which 19 infect humans (Robinson; Elwin & Chalmers, 2019) This agent causes severe diarrhea in patients considered to be at high risk (Askari et al., 2016; Avendaño et al., 2018).

Cryptosporidium parvum infections generate significant economic losses, due to severe diarrhea, impaired development and even death of newborn calves. These can be reservoirs of this protozoan, which characterizes them as an important source of zoonotic transmission (Lippuner et al., 2018; Tomazic et al., 2018).

Currently, the only drug approved by the US Food and Drug Administration (US-FDA) to treat this disease in humans is nitazoxanide. However, this active ingredient is not effective in immunocompromised patients and cannot be used by children under one year old (Mcnair et al., 2018). In turn, in Veterinary Medicine, the therapeutic base used in calves is halofuginone, but it does not completely prevent or cure the disease (Elguero et al., 2019).

The study of the structural and functional characteristics of the candidate antigens for the synthesis of an effective immunogen is essential (Yang et al., 2016), since commercial vaccines are not available for animal or human protection or as mechanisms to prevent environmental contamination (Tomazic et al., 2018; Tosini et al., 2019).

Research has been carried out in this sense, with the use of dead or attenuated *C. parvum* oocysts and specific proteins involved in the binding and invasion of host cells, for example. Although some experiments have obtained satisfactory results in vitro, there has been no success in their application to the field (Elguero et al., 2019).

In immunocompromised patients, who may not respond to immunization and consequently develop the chronic form of the disease, it is still a challenge to develop a vaccine. However, there is an alternative to immune stimulation of B cells (Avendaño et al., 2018).

However, the study of antigens for the development of an ideal vaccine for humans and animals, can be characterized or considered as an emergency measure, given the damage that this disease can cause. Therefore, our objective in this work was to write a systematic review of the literature addressing the progress of studies on vaccines against cryptosporidiosis.

2. Methodology

For a broad approach, this review was carried out in two stages. The first was based on an informal review, where it was found that there were no reviews with the objective proposed here. In the following moment, the development of the Systematic Literature Review (RSL) was carried out.

The recommended guidelines for RSL (Kitchenham et al., 2009), contemplate the stages of Planning, Execution and Summarization. For that, the use of the StArt software (State of the Art through Systematic Rewiews) was the tool used.

In the first stage, Planning, the stages to be followed to obtain the articles of interest were defined. For RSL to have a starting point, the objective would need to be clear and direct, given the question to be answered. Thus, the objective was "To investigate the experimental studies published in the last five years for the development of a vaccine for animal cryptosporidiosis". Thus, the main question was: "Is there a vaccine that develops an adequate immune response against cryptosporidiosis?"

The databases searched were: Lilacs, PubMed, Scielo, Science Direct, Scopus, Embase and Medline. These databases were chosen because they have wide access to health publications. To develop the search expressions, the so-called “string”, the “PICO” method was established as follows: P of population, animal was established; I intervention as a vaccine; C is the control, that is, vaccinated population and O of result designated as the development of an adequate immune response against the protozoan *Cryptosporidium* spp.

In all databases, the same search expressions were inserted, which refine the results, so the application of filters makes the methodology more judicious, they are: *Cryptosporidium* spp. AND vaccine; *Cryptosporidium* spp. AND DNA vaccine; *Cryptosporidium* spp. AND vector vaccine; *Cryptosporidium* spp. AND proteine vaccine; *Cryptosporidium* spp. AND animal vaccine; Vaccine effetiveness AND criptosporidiosis.

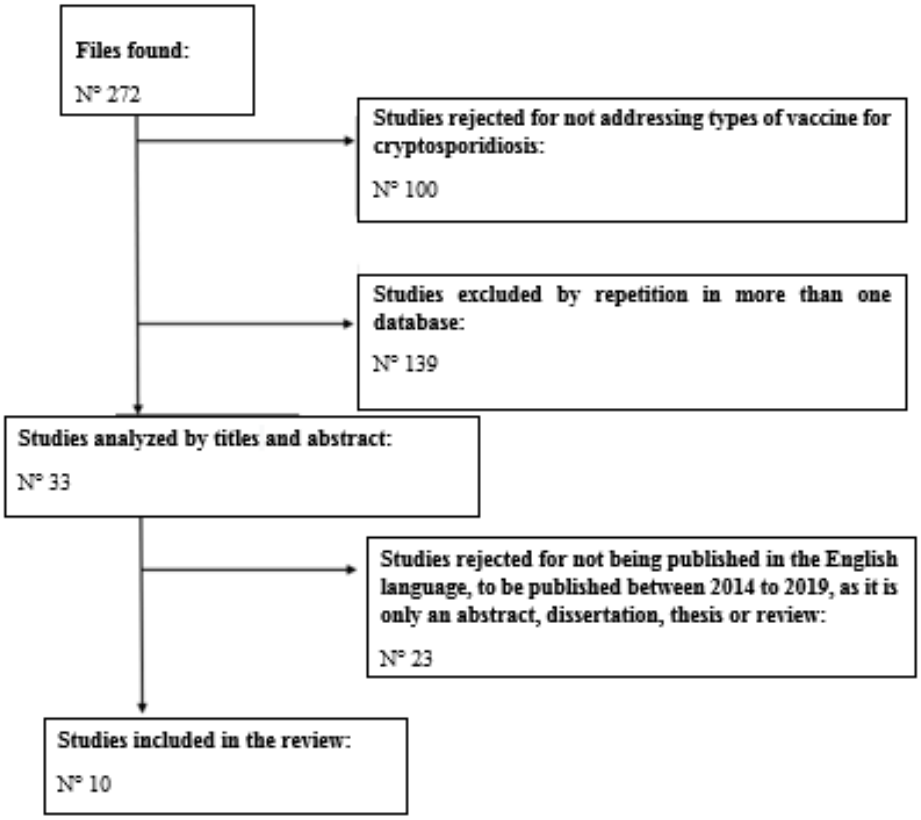
The citations were exported from the databases, in the format supported by the software, for subsequent import. Therefore, the titles and abstracts were read, selecting the articles according to the inclusion and exclusion criteria, the latter should not have any. The inclusion criteria were: (i) Have experimental evaluation of vaccines against cryptosporidiosis in animals; (ii) Be published between 2014 to 2019; (iii) English language; (iv) Animal population; (v) Addresses types of vaccine against cryptosporidiosis. And the exclusion criteria were: (i) The publication is only an abstract, dissertation or thesis; (ii) Literature review.

To extract the data that answer the question of this RSL, fields were created that systematized the information that should be observed, such as establishing the study population; principle, mechanism of action of the tested vaccine, as well as its effectiveness and cost benefit.

3. Results

Among the articles included in the review (Table 1, Figure 1), in 30% (3/10) of them calves were used as the study population, in 50% (5/10) mice were involved, the research was carried out in vitro in 1% (1/10) and in 1% (1/10) the work was developed in chickens.

Figure 1. Graphical representation of the process of choosing and applying filters from the StArt software.



Source: Authors (2020).

Table1. Articles selected from the protocol established in the StArt too.

Title	Authors	Journal
The *Cryptosporidium parvum* gp60 glycoprotein expressed in the ciliate Tetrahymena thermophila is immunoreactive with sera of calves infected with *Cryptosporidium* oocysts	(ELGUERO et al., 2019)	Veterinary Parasitology
Profiling the diversity of *Cryptosporidium* species and genotypes in wastewater treatment plants in Australia using next generation sequencing	(ZAHEDI et al., 2018)	Science of The Total Environment
Identification of novel vaccine candidates against cryptosporidiosis of neonatal bovines by reverse vaccinology	(TOMAZIC et al., 2018)	Veterinary Parasitology
Cryptosporidium spp. CP15 and CSL protein-derived synthetic peptides' immunogenicity and in vitro seroneutralisation capability	(AVENDAÑO et al., 2018)	Vaccine
A canine model of experimental infection with *Cryptosporidium canis*	(CUI et al., 2018)	Experimental Parasitology
RNA-Seq analysis during the life cycle of *Cryptosporidium parvum* reveals significant differential gene expression between proliferating stages in the intestine and infectious sporozoites	(LIPPUNER et al., 2018)	International Journal for Parasitology
Efficacy of chitosan, a natural polysaccharide, against *Cryptosporidium parvum* in vitro and in vivo in neonatal mice	(MAMMERI et al., 2018)	Experimental Parasitology
W A A V P guideline for evaluating the efficacy of anticoccidials in mammals (pigs, dogs, cattle, sheep)	(JOACHIM et al., 2018)	Veterinary Parasitology
Inflammasome components caspase-1 and adaptor protein apoptosis-associated speck-like proteins are important in resistance to *Cryptosporidium parvum*	(MCNAIR et al., 2018)	Microbes and Infection
Modulation of cyptosporidiosis by CD4 levels in chronic diarrhoea HIV/AIDS individuals visiting Tarkwa Municipal hospital, Ghana	(KWAKYE-NUAKO et al., 2016)	Asian Pacific Journal of Tropical Disease

Source: Authors (2020).

Of the selected articles, in 40% (4/10), 20% (2/10) and 10% (1/10), respectively, work was carried out with recombinant protein vaccines, surface antigens and nucleic acid vaccine carrying the rhomboid gene.

In 30% (3/10) of these studies, possible mechanisms for the development and rational production of an efficient and effective vaccine were discussed, with investigation of the humoral immune response.

Regarding the mode of action of the tested vaccine, variation was observed according to the principle used, with 30% (3/10) inhibiting parasite entry into cells, 10% (1/10) interrupting the biological cycle of the parasite, in 30% (3/10) the activation of protective immunity in the host and in 10% (1/10) the performance of the immunogen in the process related to parasitic adhesion and invasion to host cells was verified. In the other articles, the mode of action of the tested vaccines was not mentioned. It is important to note that in the selected studies there were no issues related to the costs of vaccines in tests.

4. Discussion

Until now, a vaccine with no proven effectiveness or an ideal cost-benefit ratio for its production and commercialization has been developed. Many candidate antigenic targets for a vaccine have already been characterized, in addition to elucidating the immunogenicity mechanism of *Cryptosporidium* spp. in the infected individual.

The development of a vaccine synthesized from synthetic peptides has advantages when compared to conventional vaccines in terms of safety, manufacture and storage conditions (Avendaño et al., 2018).

In studies, proteins and glycoproteins anchored to the parasite's surface have been identified via glycosylphosphatidylinositol (GPI), responsible for the motility, fixation and invasion of host cells, which are considered targets for generating an appropriate antibody response (Askari et al., 2016; Tomazic et al., 2018).

However, the absence of a means of propagating this protozoan in vitro makes it difficult to identify these surface antigens. One solution found was the use of reverse vaccinology and through bioinformatics tools it was possible to identify the candidate antigens for the development of a vaccine (Tomazic et al., 2018).

C. parvum GP60, CpH1 and CpSUB2 surface antigens have been identified and characterized as vaccine candidates, generating a humoral immune response in calves (Tomazic et al., 2018). The synthetic peptides CP15, sporozoite surface antigen, and CSL, circumsporozoite type antigen, from *C. parvum* can induce the production of antibodies in mice. Especially CP15-1 and CP15-3, in the presence of adjuvant, could stimulate the production of antibodies, neutralizing the entry of this protozoan in cultured cells in vitro (Avendaño et al., 2018).

The GP60 expressed in *Tetrahymena thermophila*, a ciliated protozoan, proved to be immunoreactive, since it was specifically recognized by the IgG antibodies generated by calves after infection by *C. parvum*, being a candidate for the development of a recombinant vaccine. *T. thermophila* has the advantage of being easily grown in an inexpensive culture medium at high cell densities and of expressing surface proteins in large quantities (Elguero et al., 2019).

In turn, intranasal immunization with the enteric vector Salmonella typhi, expresses two recombinant antigens (ClyA and Cp15) but, however, has limited efficacy (Bartelt et al., 2016).

Other proteins that make up *Cryptosporidium* spp. they can also induce an immune response in infected individuals. The recombinant protein CpSushi has an immunoprotective effect, with a reduction of 68.91% elimination of oocysts in the feces of mice challenged with *Cryptosporidium tyzzeri* (Huang et al., 2017). Recombinant peptides SA35 and SA40, portions of two *C. parvum* proteins, improve humoral and cell-mediated immune responses in adult mice (Tosini et al., 2019).

In children, it has been possible to observe resistance after primary infection, which has also been seen in mice infected with *C. tyzzeri*, which may have significantly less infection after a secondary challenge. Likewise, a live-attenuated vaccine by means of gamma irradiation, with single or multiple exposures, can confer immunity similar to individuals exposed to the live agent (Sateriale et al., 2019).

In neonates, passive immunization may be an alternative to a conventional vaccine. In the case of newborn calves, ingestion of colostrum plays a significant role in the control and prevention of the disease (Askari et al., 2016; Tosini et al., 2019).

Calves that receive colostrum from cows immunized with the recombinant P23 protein, surface glycoprotein, and that are subsequently exposed to *C. parvum*, show no signs of cryptosporidiosis. Also, a significant reduction in the excretion of oocysts eliminated in the feces of these animals was noted (Askari et al., 2016). In mice, this immunization has also been observed, resulting in a marked reduction in the intestinal parasitic burden of puppies infected with *C. parvum*, with mothers previously inoculated with SA35 and SA40 antigens, intranasally (Tosini et al., 2019).

The nucleic acid vaccine also has advantages when compared to traditional vaccines, with emphasis on simple preparation, lower cost, convenient storage and transport, in addition to inducing a more comprehensive immune response. And finally, in chickens, vaccinated with recombinant DNA from the CbROM gene in high doses (100 μg) of *C. baileyi*, intramuscularly, and later infected, protective immunity has been conferred, minimizing the rate of shedding of oocysts by up to 71, 3% (Yang et al., 2016).

Molecular biology is another area of research that has promoted advancement or progress in the search for vaccine candidates, through the cloning, identification and expression of these genes. Thus, there are about 30 possible vaccine candidate molecules already reported (Yang et al., 2016). RNA sequencing can also be performed, with the aim of identifying important genes for the completion of the parasite's life cycle, which are possible targets for drugs or vaccines (Lippuner et al., 2018).

However, additional studies are needed to achieve an ideal vaccine, ensuring adequate protection in order to reduce the damage caused by the disease.

5. Conclusion

We have shown that, to date, a vaccine with no proven efficacy or an ideal cost-benefit has been developed for its production and commercialization. Many candidate antigenic targets for a vaccine have been characterized, in addition to elucidating the immunogenicity mechanism of *Cryptosporidium* spp. in the infected individual. However, another antigenic targets for a vaccine can be searched in future studies.

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