Antigens of *Corynebacterium pseudotuberculosis* with promising potential for caseous lymphadenitis vaccine development: a literature review

Antígenos de *Corynebacterium pseudotuberculosis* com potencial promissor para o desenvolvimento de vacinas contra linfadenite caseosa: uma revisão de literatura

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ABSTRACT

The caseous lymphadenitis (CL) is an infectious disease of chronic evolution that affects sheep and goats, causing great economic losses in goat and sheep production. CL is caused by *Corynebacterium pseudotuberculosis*. Diagnosis is based on the isolation and identification of the agent and may be carried out serologically ELISA. Vaccination of the flock can be an important tool in preventing CL. The search for new antigens can generate vaccines that are more effective in disease control. Thus, the purpose of this article is to summarize the state of the art on the main antigens of *Corynebacterium pseudotuberculosis* with good potential for caseous lymphadenitis vaccine development. Various types of vaccines are commercially available and are based on live attenuated and/or inactivated microorganisms, microorganism extracts and/or recombinant proteins or subunits. In addition to the available forms are in the experimental stage DNA-based vaccines, and those using live recombinant microorganisms. We can realize that several studies have been conducted to find antigens for vaccine formulations that can ensure a good immune response to vaccinated animals. Some studies have reported promising antigens and others have shown that there is a need to search for new antigens for CL vaccine production more efficient. Although several studies have already been made in an attempt to develop an effective vaccine against the CL, there is a vast field to be searched and many antigens can still be discovered and studied to give a CL vaccine that is effective, inexpensive and practical.

Keywords: Prophylaxis. Goats. Sheep. Immunity.

RESUMO

A linfadenite caseosa (LC) é uma doença infecciosa de evolução crônica que afeta ovinos e caprinos, causando grandes perdas econômicas na produção de caprinos e ovinos. LC é causada por *Corynebacterium pseudotuberculosis*. O diagnóstico baseia-se no isolamento e identificação do agente e

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pode ser diagnosticada sorologicamente por ELISA. A vacinação do rebanho pode ser uma ferramenta importante na prevenção da LC. A busca por novos antígenos pode gerar vacinas que são mais eficazes no controle da doença. Assim, o objetivo deste artigo é resumir o estado da arte sobre os principais antígenos de *Corynebacterium pseudotuberculosis* com bom potencial para o desenvolvimento de vacinas contra LC. Vários tipos de vacinas estão disponíveis comercialmente e são baseadas em microrganismos vivos atenuados e / ou inativados, com extratos de microorganismos e / ou proteínas ou subunidades recombinantes. Além das formas disponíveis algumas vacinas baseadas em DNA, e que utilizam microorganismos recombinantes vivos estão em fase experimental. Podemos perceber que vários estudos têm sido realizados com o objetivo de encontrar antígenos para formulações de vacinas que possam garantir uma boa resposta imune a animais vacinados. Alguns estudos relataram a descoberta de antígenos promissores e outros têm mostrado que existe uma necessidade de pesquisa de novos antígenos para a produção de vacinas mais eficientes contra LC. Apesar de vários estudos já terem sido realizados na tentativa de desenvolver uma vacina eficaz contra a LC, existe um vasto campo a ser pesquisados e muitos antígenos podem ainda ser descobertos e estudados para se obter uma vacina contra LC que seja eficaz, de baixo custo e prática.

Palavras-chave: Profilaxia. Caprinos. Ovinos. Imunidade.

Introduction

The caseous lymphadenitis (CL) is an infectious disease of chronic evolution that affects sheep and goats, causing great economic losses in goat and sheep production mainly because of the disqualification and condemnation of carcasses and devaluation of the skin (BAIRD; FONTAINE, 2007). It is a rare occurrence of zoonosis (MOURA-COSTA *et al.*, 2008). This disease is characterized by abscess formation with purulent content of greenish yellow color and viscous. These may be presented clinically in two forms that is, internal form and external form. Internal or visceral form affects internal lymph nodes, while the external or superficial form affects palpable lymph nodes (ALVES *et al.*, 2007).

CL is caused by *Corynebacterium pseudotuberculosis*. Corynebacterium genus is formed by gram-positive bacteria, in shape of small coccobacilli or filamentary, facultative anaerobic, facultative intracellular parasite. The agent is very resistant in the environment (BATEY, 1986; MOTTA *et al.*, 2010). The main agent route of elimination is the content of abscesses that when fester contaminate the environment. Transmission occurs by direct contact with secretions from the abscess or needles, shearing equipment, facilities, fomites. The gateway are superficial wounds in the skin or mucosa. It has high morbidity and low mortality. The infection may also be acquired through inhalation or ingestion of the bacterium (OREIBY, 2015).

Diagnosis is based on the isolation and identification of the agent and may be carried out serologically ELISA and hypersensitivity test known as linfadenina (DORELLA *et al.*, 2006; SEYFFERT *et al.*, 2010). Treatment of lymph nodes infarcted can be realized with a vertical incision and drainage of purulent entire content and placement of iodine solution (2%) so that there is cauterized or surgically excise the surface involved lymph nodes (NOZAKI *et al.*, 2000).

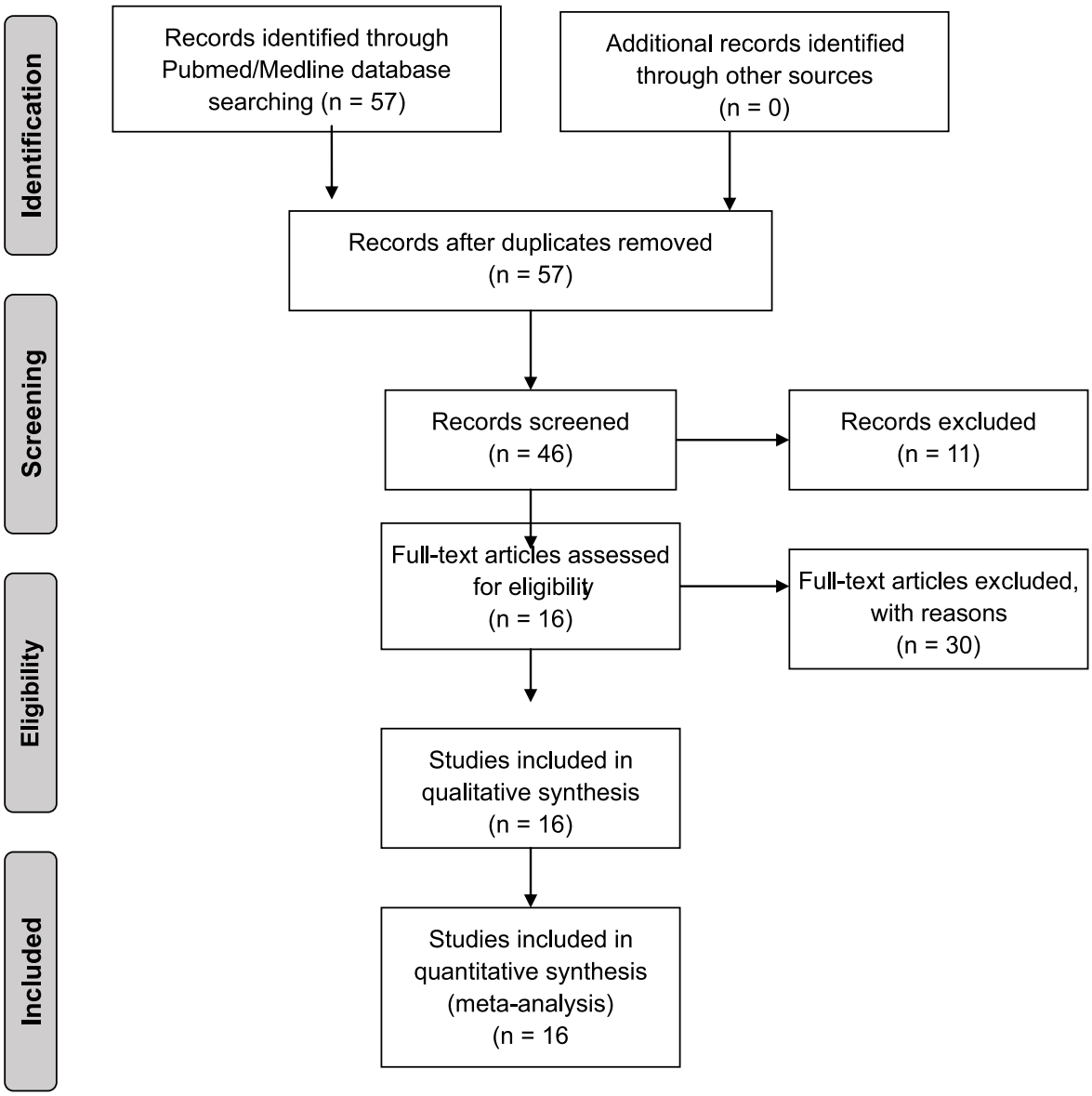
Vaccination of the flock can be an important tool in preventing CL. Immunity against a particular infectious disease can be induced by various types of vaccines which are commercially available and are based on live attenuated and / or inactivated microorganisms (first generation vaccines), microorganism extracts and / or recombinant proteins or subunits (second generation vaccines). In addition to the available forms are in the experimental stage DNA-based vaccines (third generation vaccines), and those using live recombinant microorganisms. The commercial vaccines available against CL have some disadvantages such as low protection, need for periodic booster injections, and need to be kept under refrigeration (ABBAS *et al.*, 2011).

The search for new antigens can generate vaccines that are more effective in disease control. Therefore, to know and better understand the studies conducted on this subject it is necessary to chart new directions. Thus, the purpose of this article is to summarize the state of the art on the main antigens of *Corynebacterium pseudotuberculosis* with good potential for caseous lymphadenitis vaccine development.

This is a systematic review. A search was conducted in the bibliographic reference of the MEDLINE/PubMed, with the key: “caseous lymphadenitis” and vaccine. Original articles in English were considered. The classification process was performed by two independent reviewers in two stages. First, the articles were submitted to complete reading for data extraction. In the second, the resolution of discrepancies between reviewers was made by consensus, with the participation of a third independent reviewer in case of doubt. The Preferred Reporting Items for Systematic Reviews and methodology Meta-Analyses (PRISMA) (MOHER *et al.*, 2010) rules were followed, whenever possible.

This review follows an information flow as shown in Figure 1.

Figure 1 - Information flow with different phases from the literature review



Source: Prepared by the authors, 2016.

Some antigens have already been tested in vaccines against CL

First, PLD exotoxin was tested as toxoid and was able to decrease the proliferation and spread of *C. pseudotuberculosis* the site of infection to other organs, which delayed disease development (ALVES; OLANDER, 1998). Another aspect in the formulation of vaccines has been the development of live attenuated bacteria or mutants by gene recombination. This is possible through gene deletion allegedly involved in pathogen virulence. Such vaccine candidate can produce stimuli for cytokine production which is

an important factor in bacterial infection and considered key to intracellular bacteria such as *C. pseudotuberculosis* (HENSEL; HOLDEN, 1996).

A vaccine produced with a mutant strain of *C. pseudotuberculosis* to aroQ gene was able to reduce the colonization of the lymph nodes. However, this attenuated strain was not sufficient to activate an immune response capable of protecting animals from infection with the wild type strain, leading only to a decrease in clinical disease severity (SIMMONS *et al.*, 1998). Furthermore, studies have shown that mutant strains of Xa2 and ciuA of *C. pseudotuberculosis* are able to survive in the host without causing damage, and also stimulate the production of antibodies and cytokines. Thus it has been suggested that these genes are related to the virulence of *C. pseudotuberculosis* (Billington *et al.*, 2002; Ribeiro *et al.*, 2014).

A modern strategy for the production of more efficient vaccines is related to the genome of *C. pseudotuberculosis* and its molecular virulence determinants that provide new alternatives for the development of DNA and subunit vaccines (DORELLA *et al.*, 2009). When administered to the host, this DNA allows the production of the antigenic protein vaccinated host's own cells and capable of inducing specific cellular and humoral immune response with memory (GARG *et al.*, 2014). It was tested some DNA vaccines CL, and as target immunogenic proteins such as PLD membrane protein of *C. pseudotuberculosis* and protein produced by the Hsp60 gene (CHAPLIN *et al.*, 1999; COSTA *et al.*, 2011). The subunit vaccine when the host becomes an exogenous antigen. A recombinant protein that favorable results against experimental CL in mice was the CP40 (SILVA *et al.*, 2014).

The Table 1 shows the selected studies in this review of the literature obtained from the MEDLINE/PubMed database. It is noteworthy that 57 articles were retrieved by the search keys used. After reading the summaries by two independent expert review, 11 were excluded because they do not address directly the development of vaccines against CL or are review articles. Still, 30 were excluded for not obtaining the full text by MEDLINE/PubMed. They then selected 16 articles for full reading, also by two independent expert review.

Table1 - Summary of studies in the systematic review of “caseous lymphadenitis” and vaccines

Author(s)	Some target antigens	Authors ‘conclusions
Santana-Jorge *et al.*, 2016	SpaC, PknG, NanH	“*In silico* analyses show that the putative virulence factors SpaC, PknG and NanH present good potential for CLA vaccine development.”
Radusky *et al.*, 2015	3-isopropylmalate dehydratase small subunit, 50S ribosomal protein L30, Chromosomal replication initiator protein DnaA	“Overall we provide valuable information of possible targets against *C. pseudotuberculosis* where some of these targets have already been reported in other microorganisms for drug discovery projects, also discarding targets that might be physiologically relevant but are not amenable for drug binding. We propose that the constructed *in silico* dataset might serve as a guidance for the scientific community to have a better understanding while selecting putative therapeutic protein candidates as drug able ones as effective measures against *C. pseudotuberculosis*.”
Hassan *et al.*, 2014	tcsR, mtrA, nrdI, ispH, adk, gapA, glyA, fumC, gnd, and aspA	“We propose that some of these proteins can be selectively targeted using structure-based drug design approaches. Our results facilitate the selection of *C. pseudotuberculosis* putative proteins for developing broad-spectrum novel drugs and vaccines. A few of the targets identified here have been validated in other microorganisms, suggesting that our model one strategy is effective and can also be applicable to other pathogens.”
Silva *et al.*, 2014	rCP40, CP09	“rCP40 is a promising target in the development of vaccines against caseous lymphadenitis.”
Ribeiro *et al.*, 2014	CZ171053	“Because iron acquisition in intracellular bacteria is considered one of their most important virulence factors during infection, these results demonstrate the immunogenic potential of this mutant against CLA.”

Continua

Pinto *et al.*, 2014	sigma factors varied	"Despite the veterinary importance of *C. pseudotuberculosis*, the bacterium is poorly characterised; therefore, effective treatments for caseous lymphadenitis have been difficult to establish. Through the use of RNAseq, these results provide a better biological understanding of this bacterium, shed light on the most likely survival mechanisms used by this microorganism in adverse environments and identify candidates that may help reduce or even eradicate the problems caused by this disease."
Bastos *et al.*, 2013	*C. pseudotuberculosis*-secreted antigens adjuvanted with Quillaja saponaria saponins	"Resistance to *C. pseudotuberculosis* infection is supported by the early acute phase response, in which up-regulation of haptoglobin and IgM were predictive of a lower risk of CLA lesion development. Because the immunogen used in this study induced a high production of both Hp and IgM, Quillaja saponaria saponin should be considered a promising candidate in vaccine formulations against sheep CLA."
Santos *et al.*, 2012	150 genes, out of 377 from the whole ISPPE, representing 750 *locus_tags,* 227 genes account for 1135 *locus_tags*	"The *in silico* prediction of exported proteins has allowed to define a list of putative vaccine candidate genes present in all five complete *C. pseudotuberculosis* genomes. Moreover, it has also been possible to define the *in silico* predicted dispensable and unique *C. pseudotuberculosis* exported proteins. These results provide *in silico* evidence to further guide experiments in the areas of vaccines, diagnosis and drugs. The work here presented is the first whole *C. pseudotuberculosis in silico* predicted pan-exoproteome completed till today."
Costa *et al.*, 2011	hsp60 gene	"This vaccination induced significant anti-hsp60 IgG, IgG1 and IgG2a isotype production. However, immunization with this DNA vaccine did not confer protective immunity."

Continua

Chaplin *et al.*, 1999	targeting DeltaPLD as a CTLA-4 fusion protein	"We propose that targeting antigens to antigen-presenting cells offers a generic strategy for enhancing the efficacy of DNA vaccines."
Simmons *et al.*, 1998	aroQ and pld mutants	"The results suggest that aroQ mutants of *C. pseudotuberculosis* may be overly attenuated for use as a CLA vaccines or as vaccine vectors."
Stanford *et al.*, 1998	Bacterial cells (0.86 mL/ dose) and MDP-GDP (0.4 mL/dose)	"Sheep vaccinated with WC+ MDP-GDP also had a reduced incidence of putative CLA abscesses, although confirmation of the presence of *C. pseudotuberculosis* was only successful in a small number of instances."
Simmons *et al.*, 1997	aroB and aroQ genes	"These studies support an important role for IFN-gamma in control of primary *C. pseudotuberculosis* infections and indicate that aroQ mutants remain attenuated even in immunocompromised animals. This is the first report of an aroQ mutant of a bacterial pathogen, and the results may have implications for the construction of aromatic mutants of *Mycobacterium tuberculosis* for use as vaccines."
Hodgson *et al.*, 1994	phospholipase D (PLD)	"These results confirm the importance of PLD as a protective antigen and demonstrate both the potential for developing an oral caseous lymphadenitis vaccine and *C. pseudotuberculosis* Toxminus as a live vaccine vector."
Walker *et al.*, 1994	40-kDa antigen	"These results strongly suggest that the 40-kDa antigen plays a major role in immunity to caseous lymphadenitis."
Menzies *et al.*, 1991	Each dose consisted of sterile saline, mineral oil with 3 % Arlacel A and either 5.0 mg dried whole C. pseudotuberculosis cells	"The vaccine appeared to be efficacious in reducing the proportion of sheep that developed CLA when challenged naturally in a field situation."

Source: Prepared by the authors, 2016.

We can realize that several studies have been conducted to find antigens for vaccine formulations that can ensure a good immune response to vaccinated animals. Some studies have reported promising antigens and others have shown that there is a need to search for new antigens for CL vaccine production more efficient. The *in silico* analyzes are important to make a possible screening antigens which have a good potential to be used in vaccines. The results from *in silico* analyzes should be tested experimentally to validate the results. The experimental analysis must start with *in vitro* tests and evolve for the tests using laboratory animals and finally verify the effectiveness of the vaccine in the target species.

Studies of vacines against CL of sheep and goat go in this direction in an effort to produce effective vaccines that can be inserted into the programs of control and eradication of CL.

Final considerations

The CL is a disease that affects sheep and goats and occasionally man. The ineffectiveness of drug therapy and failure in its early clinical diagnosis makes it difficult to control the disease. Prophylaxis and control of CL can be conducted by draining lymph nodes of the affected surface or surgical removal thereof. Another method is vaccination the herd.

The course focused on research related to CL and its causative agent is still wide, aiming to reach their full understanding, and consequently obtain effective control of the disease. Prospects for research in thier CL based on some key points. Among them are included the study of the kinetics of the immune response in goats and sheep in different age groups; evaluating the type of vaccine and the vehicle; the study of efficacy of existing vaccines under natural conditions of exposure to disease and in a controlled environment; biochemical and genetic characterization of strains of *C. pseudotuberculosis* in the region; the characterization and identification of major antigens of *C. pseudotuberculosis* recognized by antibodies present in the sera of infected goats and sheep or naturally immunized against CL; the study of the relationship between the antigen recognition pattern of *C. pseudotuberculosis* in different phases of the disease; the production of DNA vaccine against CL and testing in mice, goats and sheep; and, finally, implementation and evaluation of control programs based on vaccination, associated with good production practices in regions and properties, in the real environment.

Many studies have been conducted to obtain vaccines that induce high level of protection of animals against CL. These vaccines may be comprised of dead bacterial cells (bacterins), live bacterial cells, the inactivated toxin (toxoid) of *C. pseudotuberculosis* or the combination of these components, with or without some type of adjuvant. Besides these, studies have focused on developing vaccines from changes in the genetic material of the organism, in order to obtain advances on the immune protection, for they offered. To perform a proper evaluation of vaccine efficacy, potency, safety, feasibility, among others, should be considered for proper analysis.

Given that there are prospects for further development in the segment of creation of goats and sheep, because Brazil still imports most of the meat consumed, it is important to combat diseases that affect and cause losses to the creation of these animals. Although several studies have already been made in an attempt to develop an effective vaccine against the CL, there is a vast field to be searched and many antigens can still be discovered and studied to give a CL vaccine that is effective, inexpensive and practical. The ultimate goal is to seek vaccine production strategies increasingly better.

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