UNIVERSITI PUTRA MALAYSIA

MOLECULAR CHARACTERIZATION OF Corynebacterium pseudotuberculosis AND DEVELOPMENT OF RECOMBINANT VACCINE AGAINST CASEOUS LYMPHADENITIS

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By

SYAFIQAH ADILAH BINTI SHAHRIDON

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Chairperson : Professor Mohd. Zamri Saad, DVM, Ph.D
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Corynebacterium pseudotuberculosis is a causative agent for caseous lymphadenitis (CLA), a chronic disease that affects mainly small ruminants. The disease is characterized by formation of abscesses, usually in the lymph nodes and occasionally in organs of the infected animals. Caseous lymphadenitis causes great economic loss in goat and sheep industries due to low quality of milk and wool production. Vaccination has been suggested for control of CLA. Currently available commercial vaccines reduce the severity of infection but fail to control the spread of disease.

This study was conducted to characterize the various local isolates of *C. pesudotuberculosis* and to identify the candidates for development of recombinant cells against CLA. Characterization of the surface proteins of *C. pseudotuberculosis* using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) revealed 11 protein bands with two major proteins of 31 kDa and 40 kDa. The minor bands are 152, 84, 75, 69, 67, 61, 54.8, 52, 49, 44 and 25 kDa. Immunoblotting of the surface proteins revealed four immunogenic protein bands at 75, 40, 31 and 25 kDa with the 40 and 31 kDa bands showed intense reaction. Therefore, genes encoding the 31 kDa and 40 kDa surface proteins (SP) of *C. pseudotuberculosis* were amplified by polymerase chain reaction (PCR) before being cloned in pET32 Ek/LIC vector. The recombinant plasmids, pET32/LIC-SP31 and pET32/LIC-SP40 were successfully transformed into *Escherichia coli* Nova Blue strain as cloning host. Sequencing analysis showed that both genes were kept in frame with the vector sequence. Sequencing analysis of the nucleotide sequence of the SP 31 kDa showed 98% homology with putative surface anchored protein fimbrial subunit, *SpaA* gene of *C. pseudotuberculosis* strain FRC41. Meanwhile SP 40 kDa showed 99% homology with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) of *C. pseudotuberculosis* strain FRC41. Both recombinant plasmids were successfully transformed into *Escherichia coli* strain BL21 (DE3) as expression host. The subsequent SDS-PAGE and Western immunoblot analyses revealed that the expressed fusion proteins of pET32/LIC-SP31 and pET32/LIC-SP40 were approximately 67 kDa and 54 kDa, respectively.

In vivo study was carried out to determine the antibody response and protective capacity of the two recombinant cells in goats. Goats were divided into 3 groups before groups 2 and 3 were exposed intramuscularly with the pET32/LIC-SP31 and pET32/LIC-SP40 recombinant cells, respective while group 1 was the unvaccinated control. Serum samples were collected weekly to evaluate the antibody level via enzyme-linked immunosorbent assay (ELISA). Goats exposed to the recombinant cells showed significantly ($p<0.05$) higher IgG response compared with the unvaccinated group. At the time of challenge with virulent *C. pseudotuberculosis*, the IgG levels of the vaccinated goats were significantly higher than the unvaccinated goats. Following challenge, abscesses were observed in the lymph nodes of all groups and *C. pseudotuberculosis* was successfully isolated from the abscesses.

This study revealed that surface proteins of *C. pseudotuberculosis* were immunogenic. Recombinant cells carrying the surface protein were able to induce good humoral response but failed to protect the goats against challenge by live *C. pseudotuberculosis.* Thus, further studies on the method to enhance the protective capacity of the vaccine are needed considering *C. pseudotuberculosis* is an intracellular pathogen. Moreover, effective vaccination against CLA needs administration of the vaccine with adjuvant to stimulate both humoral and cell-mediated immunities.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

PENCIRIAN MOLEKULAR *Corynebacterium pseudotuberculosis* DAN PENGHASILAN REKOMBINAN VAKSIN BAGI MELAWAN PENYAKIT BISUL NODUS LIMFA

Oleh

SYAFIQAH ADILAH BINTI SHAHRIDON

Mei 2017

Pengerusi : Professor Mohd. Zamri Saad, DVM, Ph.D
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Corynebacterium pseudotuberculosis adalah penyebab bagi penyakit bisul nodus limfa (CLA), iaitu satu penyakit kronik yang memberi kesan terutamanya kepada ruminan kecil. Penyakit ini bercirikan pembentukan nanah, yang seringkali melibatkan nodus limfa dan kadang kadang organ dalaman haiwan terjangkit. Penyakit bisul nodus limfa menyebabkan kerugian ekonomi yang sangat besar dalam industri kambing dan biri-biri akibat penghasilan susu dan bulu biri-biri yang berkualiti rendah. Pemvaksinan dikatakan sebagai langkah terbaik untuk mengawal penyakit ini. Vaksin komersial yang terdapat di pasaran hanya mampu mengawal keterukan jangkitan tetapi gagal mengawal penyakit daripada merebak.

Kajian ini dijalankan untuk mencirikan beberapa pencilan tempatan *C. pseudotuberculosis* bagi menentukan pencilan yang sesuai untuk menghasilkan sel rekombinan bagi melawan penyakit bisul nodus limfa. Pencirian protein permukaan *C. pseudotuberculosis* menggunakan elektroforesis gel poliakrilamida-sodium dodesil sulfat (SDS-PAGE) menghasilkan 11 jalur protein dengan dua jalur utama iaitu 31 kDa dan 40 kDa. Jalur sampingan adalah 152, 84, 75, 69, 67, 61, 54.8, 52, 49, 44 and 25 kDa. Pemblotan protein permukaan menunjukkan empat jalur protein yang imunogenik iaitu pada 75, 40, 31 dan 25 yang mana jalur 40 dan 31 kDa menunjukkan reaksi yang terang. Oleh itu, gen yang mengkodkan protein 31 kDa dan 40 kDa diperbanyakkan menggunakan kaedah tindakbalas rantaian polymerase (PCR) sebelum diklon ke dalam vektor pET32 Ek/LIC. Plasmid rekombinan, pET32/LIC-SP31 and pET32/LIC-SP40 berjaya dipindahkan ke dalam klon perumah *Escherichia coli* strain Nova Blue. Analisis jujukan nukleotida menunjukkan kedua-dua gen berada dalam kedudukan yang betul di dalam jujukan vektor. Analisis jujukan nukleotida SP 31 kDa menunjukkan 98% persamaan dengan protin permukaan sauh subunit fimbria, gen *SpaA*, daripada *C. pseudotuberculosis* strain FRC41. Manakala SP 40 kDa menunjukkan 99% persamaan dengan gliseraldehid-3-fosfat dehidrogenase (GAPDH) daripada *C. pseudotuberculosis* strain FRC41. Kedua-dua plasmid rekombinan

kemudian berjaya dipindahkan ke dalam perumah ekspresi *Escherichia coli* strain BL21 (DE3). Analisis menggunakan SDS-PAGE dan pemblotan kemudiannya mendedahkan bahawa protein lakuran yang diekspresikan daripada pET32/LIC-SP31 dan pET32/LIC-SP40 masing-masing adalah kira-kira 67 kDa dan 54 kDa.

Kajian *in vivo* dijalankan untuk menentukan tindak balas antibodi dan tindakan perlindungan oleh kedua dua rekombinan sel di dalam kambing. Kambing-kambing dibahagikan kepada tiga kumpulan sebelum kambing dalam kumpulan 2 dan kumpulan 3 didedahkan dengan rekombinan pET32/LIC-SP31 dan pET32/LIC-SP40 masing-masing secara suntikan intraotot manakala kambing dalam kumpulan 1 ialah kontrol yang tidak diberi vaksinisasi. Sampel serum dikumpulkan setiap minggu untuk menilai tahap antibodi melalui ujian imunoterapi terangkai enzim (ELISA). Kambing yang didedahkan kepada sel rekombinan menunjukkan gerak balas IgG yang signifikan ($p<0.05$) tinggi berbanding gerak balas IgG daripada kumpulan yang tidak diberi vaksinisasi. Pada cabaran keupayaan dengan *C. pseudotuberculosis* yang virulen, gerak balas IgG kumpulan kambing yang diberi vaksinisasi secara signifikannya ($p<0.05$) lebih tinggi berbanding kumpulan yang tidak diberi vaksin. Selepas cabaran keupayaan, bengkak bernanah telah didapati dalam nodus limfa daripada semua kumpulan kambing dan *C. pseudotuberculosis* berjaya dipencilkan daripada bengkak bernanah tersebut.

Kajian ini mendedahkan bahawa protein permukaan daripada *C. pseudotuberculosis* adalah imunogenik. Sel rekombinan yang membawa protein permukaan tersebut mampu untuk meningkatkan tindak balas humoral tetapi gagal memberikan perlindungan daripada cabaran keupayaan *C. pseudotuberculosis* yang hidup kepada kambing. Oleh itu, kajian lanjut mengenai kaedah untuk meningkatkan keupayaan perlindungan vaksin tersebut diperlukan memandangkan *C. pseudotuberculosis* adalah patogen intrasel. Tambahan pula, vaksinisasi yang berkesan untuk melawan CLA memerlukan pemberian vaksin bersama-sama dengan adjuvan untuk meransang keimunan humoral dan sel-perantara.

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May ALLAH bless all of you. Thank you.

I certify that a Thesis Examination Committee has met on 26th May 2017 to conduct the final examination of Syafiqah Adilah binti Shahridon on her thesis entitled "Molecular Characterization of *Corynebacterium pseudotuberculosis* and Development of Recombinant Vaccine Against Caseous lymphadenitis" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Degree of Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

%	Percentage
α	Alpha
β	Beta
γ	Gamma
δ	Delta
°C	Degree celcius
µg	Microgram
µl	Microliter
µm	Micrometer
µM	Micromolar
Amp^R	Ampicilin resistance
APC	Antigen Presenting Cells
APS	Ammonium persulfate
BLAST	Basic local alignment search tool
bp	Base pair
BSA	Bovine serum albumin
Cfu	Colony forming unit
CMI	Cell mediated immunity
DTT	Dithiothreitol
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
Dntp	Deoxynucleotide triphosphate
EDTA	Ethylene-diamine-tetraacetic acid (disodium salt)
ELISA	Enzyme linked immunosorbent assay
ERIC-PCR	Enterobacterial repetitive intergenic consensus
g	Gram
H_2O	Water
H_2S	Hydrogen sulfide
Hsps	Heat shock proteins
IFN	Interferon
IgG	Immunoglobulin G
IL	Interleukin
In vitro (*in vitro* systems.	In an experimental situation outside the organism. Biological or chemical work done in the test tube is Latin for “in glass”) rather than in living
In vivo	in a living cell or an organism
IPTG	Isopropyl-β-D-thiogalacosidase
kb	kilobase pair
LB	Luria-Bertani
L	Liter
M	Molar
mA	Miliampere
mAB	monoclonal antibody
$MgCl_2$	Magnesium chloride
mRNA	Messenger ribonucleic acid
MW	Molecular weight
NaH_2PO_4	di-sodium hydrogen phosphate

NaCl	Natrium chloride
NaH_2PO_4	Sodium di-hydrogen peroxide
NaOH	Sodium hydrogen peroxide
$(NH_4)_2SO_4$	Ammonium sulfate
NK	Natural killer
OD	Optical density
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
pET32/LIC-SP31	Recombinant plasmid (pET32/LIC+SP 31 kDa gene of *C. pseudotuberculosis*)
pET32/LIC-SP40	Recombinant plasmid (pET32/LIC+SP 40 kDa gene of *C. pseudotuberculosis*)
PFGE	Pulse field gel electrophoresis
pH	Puissance hydrogen (hydrogen ion concentration)
PVDF	Polyvinyl diflouride
RAPD	Random amplified polymorphic DNA
Rpm	Rotation per minute
RT	Room temperature
s	Seconds
SDS	Sodium dodecyl-sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SP	Surface protein
Taq	*Thermus aquaticus* YT-1
TBE	Tris-boric EDTA
TBS	Tris-buffer saline
TE	Tris-EDTA buffer
TEMED	N,N,N',N'-tetramethylethylene diamine
Tris-HCl	Tris (hydroxymethyl) aminomethane hydrochloride
U	Unit
UV	Ultra-violet
V	Voltan/volt
v/v	Volume per volume
w/v	Weight per volume

Amino acid	Single/Three letter	Amino Acid Code
Alanine	A	Ala
Arginine	R	Arg
Asparagine	N	Asn
Aspartic Acid	D	Asp
Glutamine	Q	Gln
Glutamic Acid	E	Glu
Glycine	G	Gly
Isoleucine	I	Ile
Leucine	L	Leu
Lycine	K	Lys
Methionine	M	Met
Phenylalanine	F	Phe
Proline	P	Pro
Serine	S	Ser
Threonine	T	Thr
Tryptophan	W	Trp
Valine	V	Val

CHAPTER 1

INTRODUCTION

Caseous lymphadenitis (CLA) is a chronic disease of sheep and goats caused by *C. pseudotuberculosis*. It occurs in many countries from all continents worldwide but is of most concern in large sheep-producing areas such as Australia, New Zealand, South Africa and the American continent (Schreuder *et al*., 1986; Moore *et al*., 2010). The disease is characterised by formation of caseous abscessation in the lymph nodes and internal organs (Stefanska *et al*., 2008). Apart from enlargement of lymph nodes, pneumonia, arthritis and mastitis have also been reported (Mittal *et al*., 2010).

Economic loss caused by CLA is an important issue in small ruminant industries such as sheep and goats due to the reduced in weight gain, reproductive efficiency as well as condemnation of carcasses and devaluation of hides (Sood *et al*., 2012). Australia has reported a decreased in clean wool production resulting in annual cost of approximately $15 million. Caseous lymphadenitis has also been associated with $12 to 15 million losses annually at abattoir due to carcase losses and the costs of meat inspection and trimming of CLA affected carcases (Paton *et al*., 2003). In five regions of the western United States, 42.4% of 4,089 culled sheep were CLA positive and in western Australian abbatoir, 53.7% of 4,574 slaughtered adult ewes exhibited the disease (Ilhan *et al*., 2013). In Malaysia, actual economic importance of this disease have been underestimated due to the lack of serological studies to determine the prevalence of CLA and reliable figures for specific financial losses (AbdiNasir *et al.*, 2012).

Effective program in controlling the disease should include clinical inspection, periodic serology of animals in flock and culling of the affected animals. However, it is difficult to be accomplished due to the rapid dissemination of the bacterium within flock and also difficulties in identifying animals that show subclinical form of the disease (Guimaraes *et al*., 2011b). Control using antibiotics is generally ineffective and is not recommended (Barh *et al*., 2011). Thus, immunization or vaccination has been the main strategy for control of CLA in countries where the disease is endemic (Colom-Cadena *et al*., 2014).

Several vaccination programs have been developed in order to reduce the prevalence of the disease with variable outcomes. Commercial vaccines based on inactivated cell culture supernatant and phospholipase D (PLD) combined with antigen from other pathogen are available in several countries (Dorella *et al*., 2009). Although the available vaccines such as Glanvac ™ and Caseous D-T ™ help in decreasing the prevalence of the disease, adjustment in the vaccination program should be considered before use such as doses to be administered according to age and weight of the animals and also revaccination issue (Dorella *et al*., 2009). Therefore, development of single-dose vaccines is desperately needed to improve the performance of the vaccine (Hodgson *et al*., 1994).

Identification of antigen with high immunogenicity and protective capacity is important in developing efficient vaccines. Apart from PLD exotoxin that has been recognized as the main virulence factor of *C. pseudotuberculosis*, the cell surface proteins has also been among the suitable candidates for vaccine preparation against CLA. Thus, the potential of recombinant-based vaccine that encodes the surface protein of local isolates of *C. pseudotuberculosis* is evaluated in this study. Therefore, the objectives of this study were:

1. to characterize the deoxyribonucleic acid (DNA) and surface proteins of five isolates of *C. pseudotuberculosis* isolated from cases of goat CLA in Malaysia.

2. to identify the suitable vaccine candidate from the five different isolates of *C. pseudotuberculosis* for the development of recombinant vaccine

3. to prepare and evaluate a crude recombinant vaccine against CLA in goats.

Hypothesis:

1. There are suitable candidates from local isolates of *C. pseudotuberculosis* for preparation of recombinant cells
2. The newly developed recombinant vaccine encoding the surface protein of local *C. pseudotuberculosis* isolate is able to induce the humoral response and protects host animals against challenge by live virulent *C. pseudotuberculosis*.

REFERENCES

Abdinasir, Y. O., Jesse, F. F. A. and Aziz, A. S. (2012) Sero-prevalence of caseous lymphadenitis evaluated by agar gel precipitation test among small ruminant flock in East Coast Economic Regions in Peninsular Malaysia. Journal of Animal and Veterinary Advances. 11(19): 3474-3480

Abed, K. and Hassan, H. Z. (2008) Genetic fingerprinting and relationships of some *Corynebacterium* isolates based on DNA polymorphism. Arab Journal of Biotechnology. 12(1): 121-132

Adza Rina, M. N., Zamri-Saad, M., Jesse, F. F. A. Saharee, A. A., Haron, A. W. and Shahirudin, S. (2013) Clinical and pathological changes in goats inoculated *Corynebacterium pseudotuberculosis* by intradermal, intranasal and oral routes. Online Journal of Veterinary Research. 17(2): 73-81

Al-Gaabary, M. H., Osman, S. A., Ahmed, M. S. and Oreiby, A. F. (2010) Abbatoir survey on caseous lymphadenitis in sheep and goats in Tanta, Egypt. Small Ruminant Research. 94: 117-124

Aslanidis, C., Jong, P. J. and Schmitz, G. (1994) Minimal length requirement of the single-stranded tails for ligation-independent cloning (LIC) of PCR products. PCR Methods Application. 4: 172-177

Augustine, J. L. and Renshaw, H. W. (1986) Survival of *Corynebacterium pseudotuberculosis* in axenic purulent exudate on common barnyard fomites. American Journal of Veterinary Research. 47(4): 713-715

Babalola, O. O. (2003) Molecular techniques: An overview of methods for the detection of bacteria. African Journal of Biotechnology. 2 (12): 710-713

Baird, G. J. and Fontaine, M. C.(2007) *Corynebacterium pseudotuberculosis* and its role in ovine Caseous lymphadenitis. Journal of Comparative Pathology. 137: 179-210

Barh, D., Jain, N., Tiwari, S., Parida, B. P., D'Afonseca, V., Li, L., Ali, A., Santos, A. R., Guimaraes, L. C., Soares, S. C., Miyoshi, A., Bhattacharjee, A., Misra, A. N., Silva, A., Kumar, A. and Azevedo, A. (2011) A novel comparative genomics analysis for common drug and vaccine targets in *Corynebacterium pseudotuberculosis* and other CMN group of human pathogen. Chemical Biology and Drug Design. 78: 73-84

Bastos, B. L., Portela, R. W. D., Dorella, F. A., Ribeiro, D., Seyffert, N., Castro, T. L. P., Miyoshi, A., Oliveira, S. C., Meyer, R. and Azevedo, V. (2012) *Corynebacterium pseudotuberculosis*: Immunological responses in animal models and zoonotic potential. Journal of Clinical and Cellular Immunology. S4: 005. doi: 10.4172/2155-9899.S4-005

Billington, S. J., Esmay, P. A., Songer, J. G. and Jost, B. H. (2002). Identification and role in virulence of putative iron acquisition gene from

Corynebacterium pseudotuberculosis. FEMS Microbiology Letters. 208: 41-45

Braga, W. U. (2007) Protection in alpacas against *Corynebacterium pseudotuberculosis* using different bacterial components. Veterinary Microbiology. 119: 297-303

Britz, E., Spier, S. J., Kass, P. H., Edman, J. M. and Foley, J. E. (2014) The relationship between *Corynebacterium pseudotuberculosis* biovar equi phenotype with location and extent of lesions in horses. The Veterinary Journal. 200: 282-286

Brown, J. S. and Holden, D. W. (2002) Iron acquisition by Gram-positive bacterial pathogens. Microbes and Infection. 4: 1149-1156

Brown, T. A. (2002) Genomes: Chapter 7, Understanding a genome sequence (2nd Edition).Oxford: Wiley-Liss

Burkovski, A. (2013) Cell envelope of Corynebacteria: Structure and influence on pathogenicity. International Scholarly Research Notices: Microbiology. 935736

Cameron C. M. and Maria M. (1971) Mechanism of immunity to *Corynebacterium pseudotuberculosis* in mice using inactivated vaccine. Journal of Veterinary Research. 38 (2):73-82

Carne, H. R. (1940) The toxin of *Corynebacterium ovis*. Journal of Pathology and Bacteriology. 51:199-212

Carne, H. R., Wickham, N. and Kater, C., (1956) A toxic lipid from the surface of *Corynebacterium ovis.* Nature (London). 178: 701-702

Casadevall, A. (2003) Antibody-mediated immunity against intracellular pathogens: two dimensional thinking comes full circle. Infection and Immunity. 71(8): 4225-4228

Centikaya, B., Karahan, M., Atil, E., Kalin, R., De Baere, T. and Vaneechoutte, M. (2002) Identification of *Corynebacterium pseudotuberculosis* isolates from sheep and goats by PCR. Veterinary Microbiology. 88: 75-83

Chaplin, P. J., De Rose, R., Boyle, J. S., McWaters, P., Kelly, J., Tennent, J. N., Lew, A. M. and Scheerlinck, J. Y. (1999) Targetting improves the efficacy of a DNA vaccine against *Corynebacterium pseudotuberculosis* in sheep. Infection and Immunity. 67(12): 6434-6438

Chirini-Zarraga, C., Scaramelli, A. and Rey-Valeiron, C. (2006) Bacteriological characterization of *Corynebacterium pseudotuberculosis* in Venezuelan goat flocks. Small Ruminant Research. 65: 170-175

Clark, T. G. and Cassidv-Hanley, D. (2005) Recombinant subunit vaccines: potentials and constrain. Europe PMC. 121: 153-163

Clayton, D. J., Bowen, A. J., Hulme, S. D., Buckley, A. M., Deacon, V. L., Thompson, N. R., Barrow, P. A., Morgan, E., Jones, M. A., Watson, M. and Steven, M. P. (2008) Analysis of the role of 13 major fimbrial subunits in colonization of the chicken intestines by *Salmonella enterica* serovar Enteritis reveals a role for a novel locus. BMC Microbiology. 18: 228. DOI 10.1186/1471-2180-8-228

Colell, A., Grenn, D. R., Ricci, J. E. (2009) Novel roles for GAPDH in cell death and carcinogenesis. Cell Death and Differentiation. 16:1573-1581

Colom-Cadena, A., Velarde, R., Salinas, J., Borge, C., Garca-Bocanegra, I., Serrano, E., Gass, D., Bach, E., Casas-Daz, E., Lpez-Olvera, J. R., Lavn, S., Len-Vizcano, L. and Mentaberre, G. (2014) Management of a caseous lymphadenitis outbreak in a new Iberian ibex (*Capra pyrenaica*) stock reservoir. Acta Veterinaria Scandinavica. 56: 83-93

Connor, K. M., Fontaine, M. C., Rudge, K., Baird, G. J. and Donachie, W. (2007) Molecular genotyping of multinational ovine and caprine *Corynebacterium pseudotuberculosis* isolates using pulse-field gel electrophoresis. Veterinary Research. 38: 613-623

Connor, K. M., Quirie, M. M., Baird, G. and Donachie, W. (2000) Characterization of United Kingdom isolates of *Corynebacterium pseudotuberculosis* using pulse-field gel electrophoresis. Journal of Clinical Microbiology. 38(7): 2633-2637

Costa, M. P., McCulloch, J. A., Almeida, S. S., Dorella, F. A., Fonseca, C. T., Oliveira, D. M., Teixeira, M. F. S., Laskowska, E., Lipinska, B., Meyer, R., Portela, R. W., Oliveira, S. C., Miyoshi, A. and Azevedo, V. (2011) Molecular characterization of the *Corynebacterium pseudotuberculosis hsp60-hsp10* operon, and evaluation of the immune response and protective efficacy induced by *hsp60* DNA vaccination in mice. BMC Research Notes. 4: 243-252

Cuevas, W. A. and Songer, J. G. (1993) *Arcanobacterium haemolyticum* phospholipase D is genetically and functionally similar to *Corynebacterium pseudotuberculosis* phospholipase D. Infection and Immunity. 61(10): 4310-4316

D'Afonseca, V., Moraes, P. M., Dorella, F. A., Pacheco, L. G.C., Meyer, R., Portela, R. W., Miyoshi, A. and Azevedo, V. (2008) A description of genes of *Corynebacterium pseudotuberculosis* useful in diagnostics and vaccine applications. Genetics and Molecular Research. 7(1): 252-260

De Rose, R., Tennent, J., McWaters, P., Chaplin, P. J., Wood, P. R., Kimpton, W., Cahill, R. and Scheerlinck, J. Y. (2002) Efficacy of DNA vaccination by different routes of immunization in sheep. Veterinary Immunology and Immunopathology. 90: 55-63

Dorella, F. A., Pacheco, L. G. C., Oliveira, S. C., Miyoshi, A. and Azevedo, V. (2006a) *Corynebacterium pseudotuberculosis*: microbiology, biochemical properties, pathogenesis and molecular studies of virulence. Veterinary Research. 37: 201-218

Dorella, F. A., Estevam, E. M., Pacheco, L. G. C., Guimaraes, C T., Lana, U. G. P., Gomes, E. A., Barsante, M. M., Oliveira, S. C., Meyer, R., Miyoshi, A. and Azevedo, V. (2006b) *In vivo* insertional mutagenesis in *Corynebacterium pseudotuberculosis*: an efficient means to identify DNA sequences encoding exported proteins. Applied and Environmental Microbiology. 72(11): 7368-7372

Dorella, F. A., Gala-Garcia, A., Pinto, A. C., Sarrouh, B., Antunes, C. A., Ribeiro, D., Arbujaile, F. F., Fiaux, K. K. Guimaraes, L. C., Seyffert, N., El-Aouar, R. A., Hassan, S. S., Castro, T. L. P., Marques, W. S., Ramos, R., Carneiro, A., Sa, P., Miyoshi, A., Azevedo, V. and Silva, A. (2013) Progression of 'OMICS' methodologies for understanding the pathogenicity of *Corynebacterium pseudotuberculosis*: The Brazilian experience. Computational and Structural Biotechnology Journal. 6(7): e201303013

Dorella, F. A., Pacheco, L. G. C., Seyffert, N., Portella, R. W., Meyer, R., Miyoshi, A. and Vasco, A. (2009) Antigen of *Corynebacterium pseudotuberculosis* and prospects for vaccine development. Expert Review on Vaccine. 8(2): 205-213

Dorneles, E. M. S., Santana, J. A., Andrade, G. I., Santos, E. L. S., Guimaraes, A. S., Mota, R. A., Santos, A. S., Miyoshi, A., Azevedo, V., Gouveia, A. M. G., Lage, A. P. and Heinemann, M. B. (2012) Molecular characterization of *Corynebacterium pseudotuberculosis* isolated from goats using ERIC-PCR. Genetic and Molecular Research. 11(3): 2051-2059

Dorneles, E. M. S., Santana, J. A., Ribeiro, D., Dorella, F. A., Guimaraes, A. S., Moawad, M. S., Selim, S. A., Garaldi, A. L. M., Miyoshi, A., Ribeiro, M. G., Gouviea, A. M. G., Azevedo, V., Heinemann, M. B. and Lage, A. P. (2014) Evaluation of ERIC-PCR as genotyping method for *Corynebacterium pseudotuberculosis* isolates. PLoS ONE. 9(6): e98758. (1-10)

Droppa-Almeida, D., Vibas, W. L. P., Silva, K. K. O., Rezende, A. F. S., Simionatto, S., Meyer, R., Lima-Verde, I. B., Delagostin, O., Borsuk, S. and Padilha, F.F. (2016) Recombinant CP40 from *Corynebacterium pseudotuberculosis* confers protection in mice after challenge with a virulent strain. Vaccine. 34: 1091-1096

Droppa-Almeida, D., Vivas, W. L. P., da Silva, J. W. R., Ferreira, C. S., Oliveira, K. K., Brito, I., Diz, F. M., Borsuk, S., Lima-Verde, I. B., Azevedo, V., Meyer, R. and Padilha, F.F. (2014) Potential immune of recombinant serine protease of *Corynebacterium pseudotuberculosis*. BMC Proceedings. 8(suppl 4): P143

Dunham, S. P. (2002) The application of nucleic acid vaccines in veterinary medicine. Research in Veterinary Science. 73: 9-16

Ellis, J. A., Hawk, D. A., Mills, K. W. and Pratt, D. L. (1991a) Antigen specificity and activity of ovine antibodies induced by immunization with *Corynebacterium pseudotuberculosis* culture filtrate. Veterinary Immunology. 28: 303-316

Ellis, J. A., Hawk, D. A., Mills, K. W. and Pratt, D. L. (1991b) Antigen specificity of antibody responses to *Corynebacterium pseudotuberculosis* in naturally infected sheep with caseous lymphadenitis. Veterinary Immunology and Immunopathology. 28: 289-301

Ellis, R. W. (1999). New technologies for making vaccines. Vaccine. 17: 1596-1604

Ellis, R. W. (2001). Technologies for the design, discovery, formulation and administration of vaccines. Vaccine. 19: 2681-2687

Emerson, D., Agulto, L., Liu, H. and Liu, L. (2008) Identifying and characterizing bacteria in an era of genomics and proteomics. BioScience. 58 (10): 925-936

Findik, A. and Ciftci, A. (2012) Bacterial DNA vaccines in veterinary medicine: A review. Journal of Veterinary Advances. 2(4): 139-148

Foley, J. E., Spier, S. J., Mihalyi, J., Drazenovich, N. and Leutenegger, C. M. (2004) Molecular epidemiologic features of *Corynebacterium pseudotuberculosis* isolated from horses. American Journal of Veterinary Research. 65(12): 1734-1737

Fontaine, M. C. and Baird, G. J. (2008). Caseous lymphadenitis. Small Ruminant Research. 76: 42-48

Fontaine, M. C., Baird, G., Connor, K. M., Rudge, K., Sales, J. and Donachie, W. (2006) Vaccination confers significant protection of sheep against infection of virulent United Kingdom strain of *Corynebacterium pseudotuberculosis*. Vaccine. 24: 5986-5996

Garcon, N., Leroux-Roels, G and Cheng, W. F. (2011) Vaccine adjuvants. Understanding modern vaccines: Perspective in Vaccinology. 1(1): 89-113
Ghannoum, M. A. (2000) Potential role of phospholipase in virulence and fungal pathogenesis. Clinical Microbiology Review. 13(1): 122-143

Giombini, E., Orsini, M., Carrabino, D. and Tramontano, A. (2010) An automatic method for identifying surface proteins in bacteria: SLEP. BMC Bioinformatics. 11: 39-44

Griffith, A. J. F., Wessler, S. R., Lewontin, R. C., Gelbart, W. M., Suzuki, D. T and Miller, J.H. (2005) An introduction of genetic analysis (8th edition). New York: W. H. Freeman

Guimaraes, A. S., Dorneles, E. M. S., Andrade, G. I., Lage, A. P., Miyoshi, A., Azevedo, V., Gouveia, A. M. G. and Heinemann, M. B. (2011a) Molecular characterization of *Corynebacterium pseudotuberculosis* isolates using ERIC-PCR. Veterinary Microbiology. 153: 299-306

Guimaraes, A. S., Carmo, F. B., Pauletti, R. B., Seyffert, N., Ribeiro, D., Lage, A. P., Heinemann, M. B., Miyoshi, A., Azevedo, V. and Gouveia, A. M. G. (2011b) Caseous lymphadenitis: Epidemiology, diagnosis and control. The Institute of Integrative Omics and Biotechnology Journal. 2(2): 33-43

Hansson, M., Nygren, P. A. and Stahl, S. (2000) Design and production of recombinant subunit vaccines. Biotechnology and Applied Biochemistry. 32: 95-107

Hard, G. C. (1969) Electron microscopic examination of *Corynebacterium ovis*. Journal of Bacteriology. 97(3): 1480-1485

Hard, G. C. (1972) Examination by electron microscopy of the interaction between peritonel phagocytes and *Corynebacterium ovis*. Journal of Medical Microbiology. 5: 483-491

Haynes, J. A., Tkalcevic, J. and Nisbet, I. T. (1992) Production of enzymatically analog of phospholipase D from *Corynebacterium pseudotuberculosis*. Gene. 119: 119-121

Heggelund, L., Gaustad, P., Havelsrud, O. E., Blom, J., Borgen, l., Sundset, A., Sorum, H. and Froland, S. S. (2015) *Corynebacterium pseudotuberculosis* in a veterinary student infected during laboratory work. Open Forum Infectious Disease. 2: 1-6

Hengen, P. N. (1995). Purification of His.Tag fusion proteins from *Escherichia coli*. Trends in Biochemical Sciences. 20: 285-286

Hodgson, A. L. M. Tachedjian, M., Corner, L. A. and Radford, A. J. (1994) Protection of sheep against caseous lymphadenitis by use of single oral dose of live recombinant *Corynebacterium pseudotuberculosis*. Infection and Immunity. 62(12): 5275-5280

Hodgson, A. L. M., Carter, K., Tachedjian, M., Krywult, J., Corner, L. A., McColl, M. and Cameron, A. (1999) Efficacy of an ovine caseous lymphadenitis vaccine formulated using a genetically inactive form of *Corynebacterium pseudotuberculosis* phospholipase D. Vaccine. 17: 802-808

Hodgson, A. L. M., Krywult, J., Corner, L. A., Rothel, J. S. and Radford, A. J. (1992) Rational attenuation of *Corynebacterium pseudotuberculosis*: Potential cheesy gland vaccine and live delivery vehicle. Infection and Immunity. 60(7): 2900-2905

Ibrahim, A. K., El-Seedy, F. R. and Hassan, W. H. (2007) The role of corynebacterial phospholipase D vaccine in activation of macrophages. Beni-Suef Veterinary Medical Journal. 17(1): 50-55

Ilhan, Z. (2013) Detection of *Corynebacterium pseudotuberculosis* from sheep lymph nodes by PCR. Revuede Medecine Veterinaire. 164(2): 60-66

Ina-Salwany, M. Y., Sabri, M. Y., Zamri, S. M. and Effendi, A. W. M. (2011) Efficacy of inactivated recombinant vaccine encoding a fimbrial protein of *Pasteurella multocida* B:2 against hemorrhagic septicaemia in goats. Tropical Animal Health Production. 43: 179-187

Ivanovic, S., Zutic, M., Pavlovic, I. and Zujovic, M. (2009) Caseous lymphadenitis in goats. Biotechnology in Animal Husbandry. 25(5-6): 999-1007

Izgur, M., Akan, M., Ilhan, Z. and Yazicioglu, N. (2010) Studies on vaccine development for ovine caseous lymphadenitis. Veterinary Journal of Ankara University. 57: 161-165

Jeong, H., Barbe, V., Lee, C. H., Vallenet, D., Yu, D. S., Choi, S. H., Couloux, A., Lee, S. W., Yoon, S. H., Cattolico, L., Hur, C. G., Park, H. S., Segurens, B., Kim, S. C., Oh, T. K., Lenski, R. E., Studier, F. W., Daegelen, P and Kim, J. F. (2009) Genome sequences of *Escherichia coli* B strains REL606 and BL21 (DE3). Journal of Molecular Biology. 394: 644-652

Join-Lambert, O. F., Ouache, M., Canioni, D., Beretti, J. L., Blanche, S., Berche, P. and Kayal, S. (2006) *Corynebacterium pseudotuberculosis* necrotizing lymphadenitis in a twelve-year-old patient. The Pediatric Infectious Disease Journal. 25(9): 848-851

Jolly, R. D. (1965) The pathogenic action of the exotoxin of *Corynebacterium ovis*. Journal of Comparative Pathology. 75: 417-431

Jolly, R. D. (1966). Some observations on surface lipids of virulent and attenuated strains of *Corynebacterium ovis*. Journal of Applied Bacteriology. 29: 189-196

Komala, T. S., Ramlan, M., Yeoh, N. N., Surayani, A. R. and Sharifah Hamidah, S. M. (2008). A survey of caseous lymphadenitis in small ruminant farms from two districts in Perak, Malaysia- Kinta and Hilir Perak. Tropical Biomedicine. 25(3): 196-201

Krizek, M. and Krizek, P. (2012) Why has nature invented three stop codons of DNA and only one start codon? Journal of Theoritical Biology. 304: 183-187

Kuria, J. K., Mbuthia, P. G., Kang'ethe, E. K. and Wahome, R. G. (2001) Caseous lymphadenitis in goats: the pathogenesis, incubation period and serological esponse after experimental infection. Veterinary Research Communication. 25: 89-97

Lan, D. T. B., Taniguchi, S., Makino, S., Shirata, T. and Nakane, A. (1998) Role of endogenous tumor necrosis factor alpha and gamma interferon in resistance to *Corynebacterium pseudotuberculosis* infection in mice. Microbiology and Immunology. 42(12): 863-870

Lipford, B. G., Wagner, H. and Heeg, K. (1994) Vaccination with immunodominant peptides encapsulated in Quil A-containing liposomes induces peptide-specific primary CD8+ cytotoxic T cells. Vaccine. 12(1): 73-80

Lobanov, A. V., Turanov, A. A., Hatfield, D. L. and Gladyshev, V. N. (2010) Dual functions of codons in the genetic code. Critical Review in Biochemistry and Molecular Biology. 45(4): 257-265

Madureira, P., Baptisa, M., Vieira, M., Magalhaes, V., Camelo, A., Oliveira, L., Ribeiro, A., Tavares, D., Trieu-Cuot, P., Vilanova, M and Ferreira, P. (2006) *Streptococcus agalactiae* GAPDH is a virulence-associated immunomodulatory protein. Journal of Immunology. 178: 1379-1387

Magi, B. and Liberatori, S. (2005) Immunoblotting technique. In Burns, R. (Ed.). Immunochemical protocols (pp. 227-253). New Jersey: Humana Press

McNamara, P. J., Cuevas, W. A. and Songer, J. G. (1995) Toxic phospholipase D of *Corynebacterium pseudotuberculosis*, *C. ulcerans* and *Arcanobacterium haemolyticum*: cloning and sequence homology. Gene. 156: 113-118

Menzies, P. I., Muckle, C. A., Brogden, K. A. and Robinson, L. (1991) A field trial to evaluate a whole cell vaccine for the prevention of caseous lymphadenitis in sheep and goat flock. Canadian Journal of Veterinary Research. 55: 362-366

Miroux, B. and Walker, J. E. (1996) Over-production of proteins in *Escherichia coli*: Mutant hosts that allow synthesis of some membrane proteins and globular proteins at high levels. Journal of Molecular Biology. 260: 289-298

Mittal, D., Filia, G., Singh, Y. and Singh, J. (2010) Caseous lymphadenitis in Beetle goats at an organized farm. Haryana Veterinary. 46: 66-67

Mohan, P., Vathsala, M. and Jayaprakasan, V. (2008) Comparative characterization of *Corynebacterium pseudotuberculosis* from goats in Kerala, India and reference strain. Small Ruminant Research. 74: 226-230

Moore, R., Miyoshi, A., Pacheco, L. G. C., Seyffert, N. and Azevedo, V. (2010) *Corynebacterium* and *Arcanobacterium*. Gyles, C. L., Prescott, J. F., Songer, G. and Thoen, C. O (Ed.), Pathogenesis of bacterial infections in animals (pp 133-147). United States: Blackwell Publishing

Moreira, L. O., Andrade, A. F. B., Vale, M. D., Souza, S. M. S., Hirata Jr, R., Asad, L. M. O. B., Asad, N. R., Monteiro-Leal, L. H., Previato, J. O. and Mattos-Guaraldi, A. L. (2003) Effects of iron limitation on adherence and cell surface carbohydrates of *Corynebacterium diptheriae* strains. Applied and Environmental Microbiology. 69(10): 5907-5913

Moussa, I. M., Hessain, A. M., Hemeg, H. A. and Selim, S. A. (2014) Single-point mutation as a molecular tool for preparation of recombinant vaccine against caseous lymphadenitis. Journal of Food Agriculture and Environment. 12(2): 626-629

Mowat, N. (1999). Vaccine manual: the production and quality control of veterinary vaccines for use in developing countries. Daya Publishing House

Muckle, C. A. and Gyles, C. L. (1982) Characterization of strains of *Corynebacterium pseudotuberculosis*. Canadian Journal of Comparative Medicine. 46: 206-208

Muckle, C. A., Menzies, P. I., Li, Y., Hwang, Y. T. and Wesenbeek, M. (1992) Analysis of the immunodominant antigens of *Corynebacterium pseudotuberculosis.* Veterinary Microbiology. 30: 47-58

Nascimento, I.P. and Leite, L. C. C. (2012) Recombinant vaccines and the development of new vaccine strategies. Brazillian Journal of Medical and Biological Research. 45: 1102-1111

Navarre, C. B. and Gill, M. S. (2010) Caseous lymphadenitis. Louisiana Agriculture Centre Research and Extension. Retrieve from www.lsuagcentre.com

Nowicki, M., Kaba, J., Mosiej, E., Lutynska, A., Augustynowicz, E., Rzewuska, M., Binek, M., Szalus-Jordanow, O., Stefanska, I. and Frymus, T. (2011) Fingerprinting of *Corynebacterium pseudotuberculosis* strains by random amplified polymorphic DNA and amplified fragment length polymorphism techniques. Bulletin of the Veterinary Institute in Pulawy. 55: 403-409

Oliveira, L., Madureira, P., Andrade, E. B., Bouabod, A., Morello, E., Ferreira, P., Poyart, C., Trieu-Cout, P. and Dramsi, S. (2012) Group B Streptococcus GAPDH is released upon cell lysis, associated with bacterial surface and induces apoptosis in murine macrophages. PLoS ONE. 7(1): e29963. Doi: 10.1371/journal.pone.0029963

Pacheco, L. G. C., Pena, R. R., Castro, T. L. P., Dorella, F. A., Bahia, R. C., Carminati, R., Frota, M. N. L., Oliveira, S. C., Meyer, R., Alves, F. S. F., Miyoshi, A. and Azevedo, V. (2007) Multiplex PCR assay for identification of *Corynebacterium pseudotuberculosis* from pure cultures and for rapid detection of this pathogen in clinical samples. Journal of Medical Microbiology. 56: 480-486

Pacheco, L. G. C., Slade, S. E., Seyffert, N., Santos, A. R., Castro, T. L. P., Silva, W. M., Santos, A. V., Santos, S. G., Farias, L. M., Carvalho, M. A. R., Pimenta, A. M. C., Meyer, R., Silva, A., Scrivens, J. H., Oliveira, S. C., Miyoshi, A., Dowson, C. G. and Azevedo, A. (2011) A combined approach for comparative exoproteome analysis of *Corynebacterium pseudotuberculosis*. BMC Microbiology. 11:12

Pany, S. S., Mohanty, N. N., Sarangi, L. N., Mohapatra, J. K. and Shivachandra, S. B. (2015) Vaccination: A future perspective. Advances in Animal and Veterinary Sciences. 3(4): 1-8

Paton, M. W., Walker, S. B., Rose, I. R. and Watt, G. F. (2003) Prevalence of caseous lymphadenitis and usage of caseous lymphadenitis vaccines in sheep flocks. Australian Veterinary Journal. 81: 91-95

Paule, B. J. A., Azevedo, V., Regis, L. F., Carminati, R., Bahia, C. R., Vale, V. L. C., Moura-Costa, L. F., Freire, S. M., Nascimento, I., Schaer, R., Goes, A. M. and Meyer, R. (2003) Experimental *Corynebacterium pseudotuberculosis* primary infection in goats: kinetic of IgG and interferon-γ production, IgG avidity and

antigen recognition by Western blotting. Veterinary Immunology and Immunopathology. 96: 129-139

Paule, B. J. A., Azevedo, V., Moura-Costa, L. F., Freire, S. M., Regis, L. F., Vale, V. L. C., Bahia, R. C., Carminati, R., Nascimento, I. and Meyer, R. (2004) SDS-PAGE and Western blot analysis of somatic and extracellular antigens of *Corynebacterium pseudotuberculosis*. Journal of Medical and Biological Science. 3(1): 44-52

Peel, M. M., Palmer, G. G., Stacpoole, A. M. and Kerr, T. G. (1997) Human lymphadenitis due to *Corynebacterium pseudotuberculosis*: report of ten cases of Australia and review. Clinical Infectious Diseases. 24: 185-191

Pepin, M., Pardon, P., Marly, J., Lantier, F. and Arrigo, J. L. (1993) Acquired immunity after primary caseous lymphadenitis in sheep. American Journal of Veterinary Research. 54: 873-877

Pinho, J. M. R., Dorella, F. A., Coelho, K. S., Fonseca, C. T., Cardoso, F. C., Meyer, R., Portela, R. W. D., Oliveira, S. C., Miyoshi, A. and Azevedo, V. (2009) Immunization with recombinant *Corynebacterium pseudotuberculosis* heat-shock protein (Hsp)-60 to induce an immune response in mice, but fails to confer protection against infection. The Open Veterinary Science Journal. 3: 22-27

Puspitasari, Y., Zamri-Saad, M., Sabri, M. Y. and Zuki, A. B. (2012) Humoral and cellular immune responses in goats exposed to recombinant cells expressing Omp 34kDa *Brucella melitensis* gene. Online Journal of Veterinary Research. 16(1): 16-25

Ribeiro, D., Rocha, F. S., Leite, K. M. C., Soares, S. C., Silva, A., Portela, R. W. D., Meyer, R., Miyoshi, A., Oliveira, S. C., Azevedo, V. and Dorella, F. A. (2014) An iron-acquisition-deficient mutant of *Corynebacterium pseudotuberculosis* efficiently protects mice against challenge. Veterinary Research. 45: 28-33

Rogers, E. A., Das, A. and Ton-That, H. (2011) Adhesion by pathogenic *Corynebacteria*. Linke, D. and Goldman, A. (Ed.), In Bacterial adhesion: Chemistry, Biology and Physics (pp 91-103). Unites States: Springer Publishing

Ruiz, J. C., D'Afonseca, V., Silva, A., Ali, A., Pinto, A. C., Santos, A. R., Rocha, A. A. M. C., Lopes, D. O., Dorella, F. A., Pacheco, L. G. C., Costa, M. P., Turk, M. Z., Seyffert, N., Moraes, P. M. R. O., Soares, S. C., Almeida, S. S., Castro, T. L. P., Abreu, V. A. C., Trost, E., Baumbach, J., Tauch, A., Schneider, M. P. C., McCulloch, J., Cerdeira, L. T., Ramos, R. T. J., Zerlotini, A., Dominitini, A., Resende, D. M., Coser, E. M., Oliveira., L. M., Pedrosa, A. L., Vieira, C. U., Guimaraes, C. T., Bartholomeu, D. C., Oliveira, D. M., Santos, F. R., Rabelo, E. M., Lobo, F. P., Franco, G. R., Costa, A. F., Castro, I. M., Dias, S. R. C., Ferro, J. A., Ortega, J. M., Paiva, L. V., Goulart, L. R., Almeida, J. F., Ferro, M. I. T., Carneiro, N. P., Falkao, P. R. K., Grynberg, p., Teixeira, S. M. R., Brommonschenkel, S., Oliveira, S. C., Meyer, R., Moore, R. J., Miyoshi, A., Oliveira, G. C. and Azevedo, V. (2011) Evidence for reductive genome

evolution and lateral acquisition of virulence functions in two *Corynebacterium pseudotuberculosis* strains. Plos One. 6(4): e18551

Sa, M. C. A., Gouveia, G. V., Krewer, C. C., Veschi, J. L. A., Mattos-Guaraldi, A. L. and Costa, M. M. (2013) Distribution of PLD and FagA, B, C and D genes in *Corynebacterium pseudotuberculosis* isolates from sheep and goats with caseous lymphadenitis. Genetics and Molecular Biology. 36(2): 265-268

Sabri, M. Y. (2006) Identification, cloning, sequencing, expression and protective capacity of the gene encoding a 31 kilodalton outer membrane protein of *Mannheimia haemolytica*. PhD Thesis. Universiti Putra Malaysia

Safavi, F. and Rostami, A. (2012) Role of serine proteases in inflammation: Bowman-Birk protease inhibitor (BBI) as a potential therapy for autoimmune diseases. Experimental and Molecular Pathology. 93: 428-433

Shun, R. P. S., Jesse, F. F. A., Zamri-Saad, M. and Wahid, A. H. (Eds.). (2012) Proceedings from 7th Seminar in Veterinary Sciences: Clinical response and pathological changes associated with *Corynebacterium pseudotuberculosis* infection in mice. Universiti Putra Malaysia Press

Santos, A. R. Carneiro, A., Gala-Garcia, A., Pinto, A., Barh, D., Barbosa, E., Aburjaile, F., Dorella, F., Rocha, F., Guimaraes, L., Zurita-Turk, M., Ramos, R., Almeida, S., Soares, S., Pereira, U., Abreu, V. C., Silva, A., Miyoshi, A and Azevedo, V. (2012) The *Corynebacterium pseudotuberculosis* in silico predicted pan-exoproteome. BMC Genomics. 13(Suppl 5): S6

Schmiel, D. H. and Miller, V. L. (1999) Bacterial phospholipases and pathogenesis. Microbes and Infection. 1: 1103-1112

Schreuder, B. E. C., Laak, E. A. and Griesen, H. W. (1986) An outbreak of caseous lymphadenitis in dairy goats: First report of the disease in Netherlands. Veterinary Quarterly. 8(1): 61-67

Selim, S. A., Ghoneim, M. E. and Mohamed, Kh. F. (2010) Vaccinal efficacy of genetically inactivated phospholipase D against caseous lymphadenitis in small ruminants. International Journal of Microbiological Research. 1(3): 129-136

Sengupta, N., Alam, S. I., Kumar, B., Kumar, R. B., Gautam, V., Kumar, S. and Singh, L. (2010) Comparative proteomic analysis of extracellular proteins of *Clostridium perfringens* type A and type C strains. Infection and Immunity. 78(9): 3957-3968

Seyffert, N., Pacheco, L. G. C., Silva, W. M., Castro, T. L. P., Santos, A. V., Santos, A., McCulloch, J. A., Rodrigues, M. R., Santos, S. G., Farias, L. M., Carvalho, M. A. R., Pimenta, A. M. C., Silva, A., Meyer, R., Miyoshi, A. and Azevedo, V. (2011) Preliminary serological secretome analysis of *Corynebacterium pseudotuberculosis*. Journal of Intergrated OMICS. 1(2): 193-197

Shams, H. (2005). Recent developments in veterinary vaccinology. The Veterinary Journal. 170: 289-299

Shannon, A. D., Colaco, C. A. L. S and Frost, M. J. (2009) Recombinant subunit vaccine. US Patent No. US 7, 494, 785 Washington DC:US

Shi, Q and Jackowski, G. (1998) One-dimensional polyacrylamide gel electrophoresis. In Hames, B. D. (Ed.), Gel electrophoresis of proteins (pp. 1-50). New York: Oxford University Press

Silva, J. W., Droppa-Almeida, D., Borsuk, S., Azevedo, V., Portela, R. W., Miyoshi, A., Rocha, F. S., Dorella, F. A., Vivas, W. L., Padilha, F. F., Hernandez-Macedo, M. L. ans Lima-Verde, I. B. (2014) *Corynebacterium pseudotuberculosis* cp09 mutant and cp40 recombinant protein partially protect mice against caseous lymphadenitis. BMC Veterinary Research. 10: 965. DOI 10.1186/s12917-014-0304-6

Silva, W. M., Seyffert, N., Santos, A. V., Castro, T. L. P., Pacheco, L. G. C., Santos, A. R., Ciprandi, A., Dorella, F. A., Andrade, H. M., Barh, D., Pimenta, A. M. C., Silva, Miyoshi, A., and Azevedo, V. (2013a) Identification of 11 new exoproteins in *Corynebacterium pseudotuberculosis* by comparative analysis of the exoproteome. Microbial Pathogenesis. 61-62: 37-42

Silva, W. M., Seyffert, N., Ciprandi, A., Santos, A. V., Castro, T. L. P., Pacheco, L. G. C., Barh, D., Loir, Y. L., Pimenta, A. M. C., Miyoshi, A., Silva, A. and Azevedo, V. (2013b) Differential exoproteome analysis of two *Corynebacterium pseudotuberculosis* biovar ovis strains isplated from goat (1002) and sheep (C231). Current Microbiology. 67: 460-465

Simmons, C. P., Dunstan, S. J., Tachedjian, M., Krywult, J., Hodgson, A. L. M. and Strugnell, R. A. (1998) Vaccine potential of attenuated mutants of *Corynebacterium pseudotuberculosis* in sheep. Infection and Immunity. 66(2): 474-479

Simmons, C. P., Hodgson, A. L. M. and Strugnell, R. A. (1997) Attenuation and vaccine potential of *aroQ* mutants of *Corynebacterium pseudotuberculosis*. Infection and Immunity. 65(8): 3048-3056

Soares, S. C., Trost, E., Ramos, R. T. J., Carneiro, A. R., Santos, A. R., Pinto, A. C., Barbosa, E., Aburjaile, F., Ali, A., Diniz, C. A. A., Hassan, S. S., Fiaux, K., Guimaraes, L. C., Bakhtiar, S. M., Pereira, U., Almeida, S. S., Abreau, V. A., C., Rocha, F. S., Dorella, F. A., Miyoshi, A., Silva, A., Azevedo, V. and Tauch, A. (2013) Genome sequence of *Corynebacterium pseudotuberculosis* biovar equi strain 258 and prediction of antigenic targets to improve biotechnological vaccine production. Journal of Biotechnology. 167: 135-141

Songer, J. G., Beckenbach, K., Marshall, M.M., Olson, G. B. and Kelley, L. (1988) Biochemical and genetic characterization of *Corynebacterium pseudotuberculosis*. American Journal of Veterinary Research. 49(2): 223-226

Sood, N.K., Sandhu, B.S., Gupta, K., Narang, D., Vasudeva, K. and Singh, N.D. (2012) Mesenteric caseous lymphadenitis in a cow calf caused by *Corynebacterium pseudotuberculosis*: a case report. Veterinarni Medicina. 57(7): 371-375

Sorensen, H. P. and Mortensen, K. K. (2005). Advanced genetic strategies for recombinant protein expression in *Escherichia coli*. Journal of Biotechnology. 115: 113-128

Stapleton, S., Bradshaw, B. and O'Kennedy, R. (2009) Development of a surface plasmon resonance-based assay for the detection of *Corynebacterium pseudotuberculosis* infection in sheep. Analytica Chimica Acta. 651: 98-104

Stefanska, I., Rzewuska, M. and Binek, M. (2008) Evaluation of three methods for DNA fingerprinting of *Corynebacterium pseudotuberculosis* strains isolated from goats in Poland. Polish Journal of Microbiology. 57(2): 105-112

Sutherland, S. S., Hart, R. A. and Buller, N. B. (1996) Genetic differences between nitrate-negative and nitrate-positive *Corynebacterium pseudotuberculosis* strains using restriction fragment length polymorphism. Veterinary Microbiology. 49: 1-9

Tachedjian, M., Krywult, J., Moore, R. J. and Hodgson, A. L.M. (1995) Caseous lymphadenitis vaccine development: site-specific inactivation of the *Corynebacterium pseudotuberculosis* phospholipase D gene. Vaccine. 13(18): 1785-1792

Terpe, K. (2003). Overview of tag protein fusions: from molecular and biochemical fundamentals to commercial systems. Applied Microbiology and Biotechnology. 60: 523-533

Titball, R. W. (1993) Bacterial phospholipases C. Microbiological Reviews. 57(2): 347-366

Trost, E., Ott, L., Schneider, J., Schroder, J., Jaekicke, S., Goesmann, A., Husemann, P., Stoye, J., Dorella, F. A., Rocha, F. S., Soares, S. C., D'Afonseca, V., Miyoshi, A., Ruiz, J., Silva, A., Azevedo, V., Burkovski, A., Guiso, N., Join-Lambert, O. F., Kayal, S. and Tauch, A. (2010) The complete genome sequence of *Corynebacterium pseudotuberculosis* FRC41 isolated from a 12-year-old girl with necrotizing lymphadenitis reveals insights into gene-regulatory networks contributing to virulence. BMC Genomics. 11: 728-744

Vale, V. L. C., Silva, M. C., Souza, A. P., Trindade, S. C., Moura-Costa, L. F., Santos-Lima, E. K. N., Nascimento, I. L. O., Cardoso, H. S. P., Marques. E. j., Paule, B. J. A. and Nascimento, R. J. M. (2016) Humoral and cellular immune responses in mice against secreted and somatic antigens from a *Corynebacterium pseudotuberculosis* attenuated strain: Immune response against a *Corynebacterium pseudotubercuosis* strain. BMC Veterinay Research. 12: 195-202

Van der Velden, A. W., Baumler, A. J., Tsolis, R. M. and Heffron, F. (1998) Multiple fimbrial adhesins are required for full virulence of *Salmonella typhimurium* in mice. Infection and Immunity. 66: 2803-2808

Venezia, J., Cassiday, P. K., Marini, R. P., Shen, Z., Buckley, E. M., Peters, Y., Taylor, N., Dewhirst, F. E., Tondella, M. L. and Fox, J. G. (2012) Characterization of Corynebacterium species in macaques. Journal of Medical Microbiology. 61: 1401-1408

Walker, J., Jackson, H. J., Eggleton, D. G. Meeusen, E. N. T. Wilson, M. J. and Brandon, M. R. (1994) Identification of a novel antigen from *Corynebacterium pseudotuberculosis* that protects sheep against caseous lymphadenitis. Infection and Immunity. 62(6): 2562-2567

Wang, W. and Jeffery, C. J. (2016) An analysis of surface proteomics results reveals novel candidates for intracellular/surface moonlighting proteins in bacteria. Molecular Biosystem. 12(5): 1420-1431

Wilderman, P. J., Vasil, A. I., Johnson, Z. and Vasil, M. L. (2001) Genetic and biochemical analyses of a eukaryotic-like phospholipase D of *Pseudomonas aeruginosa* suggest horizontal acquisition and a role for persistence in a chronic pulmonary infection model. Molecular Microbiology. 39: 291-304

Williamson, L. H. (2001) Caseous lymphadenitis in small ruminants. Update on Small Ruminant Medicine. 17(2): 359-371

Wilson, M. J., Brandon, M. R. and Walker, J. (1995) Molecular and biochemical characterization of a protective 40-kilodalton antigen from *Corynebacterium pseudotuberculosis*. Infection and Immunity. 63(1): 206-211

Windsor, P. A. and Bush, R. D. (2016) Caseous lymphadenitis: Present and near forgotten from persistent vaccination?. Small Ruminant Research. http://dxdoi.org/10.1016/j.smallrumres.2016.03.023

Windsor, P. A. (2011) Control of caseous lymphadenitis. Veterinary Clinical Food Animal. 27: 193-202

Windsor, P. A. (2014) Managing control program for ovine caseous lymphadenitis and paratuberculosis in Australia and the need for persistent vaccination. Veterinary Medicine: Research and Reports. 5: 11-22

Xu, Y. and Hook, M. A. O. (2007) Surface proteins from Gram-positive bacteria having highly conserved motifs and antibodies that recognized them. US Patent No. US 7,195,763 B2 Texas: US

Yeruham, I.,Friedman, S., Perl, S., Elad, D., Berkovichi, Y. and Kalgard, Y. (2004) A herd level analysis of a *Corynebacterium pseudotuberculosis* outbreak in a dairy cattle. Veterinary Dermatology. 15: 315-320

Young, R. A. (1990) Stress proteins and immunology. Annual Review of Immunology. 8: 401-420

Zavoshti, F. R., Khoojine, A. B. S., Helan, J. A., Hassanzadeh, B. and Heydari, A. A. (2012) Frequency of caseous lymphadenitis (CLA) in sheep slaughter in an abattoir in Tabriz: comparison of bacterial culture and pathological study. Comparative Clinical Pathology. 21: 667-671

Zulkifli, Y., Alitheen, N. B., Son, R., Raha, A. R., Samuel, L., Yeap, S. K. and Nishibuci, M. (2009) Random amplified polymorphic DNA-PCR analysis on *Vibrio parahaemolyticus* isolated from cockles in Padang, Indonesia. International Food Research Journal. 16: 141-150