

Serum proteinogram in mules naturally infected by the *Burkholderia mallei*

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Abstract

The *Burkholderia mallei* is the causing bacterium of glanders, illness of high morbidity and lethality for the equides, and also one zoonosis. Recently diagnostic in the Pernambuco and Alagoas States/Brazil, it comes decimating populations of equides in the Zone of Forest Region of these states, causing great damages to the sugar cane plantation activity that it uses such animals as traction. The objective this work was to investigate the proteins alterations caused by glanders. Had been studied ninety adult mules, of different races, destined to the work, proceeding from the sugar cane plantation region, Zone of Forest, in the Pernambuco State/Brazil. These were divided in three groups: G1: thirty animals with negative serology for glanders; G2: thirty animals with positive serology for glanders and without apparent symptoms and G3: thirty animals with positive serology for glanders and with apparent symptoms. The gotten results, referring the medium of the parameters studied respectively for G1, G2 and G3 had been: total serum protein 7.33; 7.73 and 7.46g/dl; albumin 2.57; 2.43 and 1.81g/dl; globulins 4.37; 4.86 and 5.64g/dl; albumin/globulins ratio 0.55; 0.47 and 0.34g/dl; alpha-globulin 1.06; 1.33 and 1.33g/dl; beta-globulin 1.10; 1.21 and 1.80g/dl and gamma-globulin 2.21; 2.32 and 2.51g/dl. It is concluded that the variations for the studied parameters were significant; the increase of the globulins characterizes an antigenic stimulus in the sick animals, as well as an inversion in the albumin/globulins ratio for the animals with apparent clinic in relation to the excessively animal ones. These findings could be considered in the diagnosis, prognostic and in future research that they aim at to study forms of immunization against this illness.

Key-words:
 Mules.
 Glanders.
 Serum proteins.

Introduction

Glanders is one of the oldest diseases of the equides. The etiologic agent is a bacterium that to the long of the years received different denominations. In 1980 the bacterium was enclosed in “Approved Lists Names”; as *Pseudomonas mallei*. Yabuuchi¹, based in the sequence of rRNA, molecular biology, composition of lipid acids and phenotype characteristics, transferred this bacterium to the genus *Burkholderia*, passing then to specie *B. mallei*. Infect-contagious disease, of acute or

chronic character, that attack mainly the equides, also being able to attack the human, the carnivores and eventually the small ruminants. Responsible for high morbidity and lethality, it occurs in different parts of the world².

The illness was described for the first time in Brazil in 1811, introduced probably for infected animals imported of the Europe³. After the period of approximately 40 years without registers of the illness in the Country, Mota et al.⁴ had related the microbiologicals, epidemiologicals, physicians, pathologicals and of diagnosis aspects in

equides of Pernambuco and Alagoas States.

The contagious occurs mainly for the digestive, or still for the respiratory route, genital and for injured skin⁵. The epidemiology of this disease, amongst other factors, is related directly with the handling, what incriminates the collective stables as potential focus of dissemination of the infection between the animals¹². The age is an important factor and is directly related to the appearance of the clinical form in the natural infection, which in the majority of the cases occurs in aged and weak animals for the bad conditions of handling and creation⁴.

The animals without symptoms, in the acute phase or periods of convalescence, play important role in the direct and indirect transmission of the agent; therefore they present the bacterium in cutaneous and respiratory secretions⁴.

The chronic form of the illness can be presented of three forms: nasal, pulmonary and cutaneous, however these are not distinct, being able the same animal to present all the forms². The clinical signs in the sick animals include fever, pulmonary difficulty, with noisy breath and pulmonary boisterous; in the inspection pus nasal dump is observed, nasal ulcers resulting in irregular scars and apathy; in the lymphatic system observes high nodules of firm consistency and the many times fistula already, draining pus secretion yellowish; beyond edemas in the ventral parts of the body, as in the prepuce and members^{2,4,7,8,9,10,11}.

Srinivasan et al.¹² had reported a human case of the illness, occurred in the United States in the year of 2000, a patient whom worked in the microbiological inquiry of the *B. mallei*, and described persistent fever, respiratory difficulty and in the computerized scan they had observed hepatic and multiples splenium abscesses. Of this form, the people who manipulate the bacterium in laboratories and those that have direct contact with the sick animals are displayed to the infection.

The total serum protein includes all

the proteins of the blood, with exception of the fibrinogen that suffers to together retraction with the cellular elements from the blood¹³. With relation to proteins occurs in all the animals with the advance of the age; a decrease of the albumin and an increase of the globulins and total protein, and in the very old animals the total proteins decline again¹⁴.

The proteins are substances that discharge the biggest number of functions in the animal organism, essential in the maintenance of the osmotic pressure of the blood, and others functions as carrier of hormones, minerals and lipids. These suffer alterations from clinical importance, mainly in inflammatory processes (bacterial and immunology), parasitic and metabolic^{13,15}.

Considering the importance of this illness for the equides and the scarcity of data on the alterations in animals with this disease, the aim of this work was to study the serum proteins (total protein, albumin, globulin, albumin:globulins ratio and globulins fractions) in mules with glanders.

Materials and Methods

Animals:

Had been studied ninety adult mules of different races, destined to the work, proceeding from the sugar cane plantation region, Zone of Forest, of Pernambuco State, that were divided in three groups: G1: thirty animals negative serology for glanders; G2: thirty animals with positive serology and without apparent symptoms and G3: thirty animals with positive serology and with apparent symptoms. These animals were previously submitted to the fixation of the complement test for formation of the groups.

Attainment of the samples of serum:

After of the detailed clinical examination in the animals with glanders and the healthy animals, all were contained individually and was carried through the collection of blood through punch of the

jugular vein, having used itself dismissable needles (40x12mm). The collected volume was of approximately 20ml, placed in tubes of assay barren and inclined 45° to facilitate the coagulation process; 8 hours after, it was proceeded the clearing from the sanguine clot and had gotten the samples of serum, that duly had been identified and kept cooled to the temperature of 8°C until processing.

Local of processing of the samples the serum:

The samples were processed in the Laboratory of Immunopatology of the Hemocenter Foundation of Pernambuco – HEMOPE, in the city of Recife – Pernambuco State.

Serum proteins:

The dosage of the total serum protein was determined by the device photocolorimeter COBAS MIRA 200, by biuret method using commercial kit. The dosage of albumin and the splitting of the serum protean fractions of the globulins had been carried through electrophoresis in cellulose acetate¹⁴. The globulins and albumin/globulins ratio had been estimated using mathematical calculations¹⁶.

Statistical analysis:

The gotten average values for each

group had been tested statistical between itself for the accomplishment of the test of Tukey, and also the analysis of association between quantitatives variable (correlation and linear regression)¹⁷.

Results and Discussion

The gotten results of the 90 animals, referring to the medium of the parameters studied respectively for G1, G2 and G3 had been: total serum protein 7.33; 7.73 and 7.43g/dl; albumin 2.57; 2.43 and 1.81g/dl; globulins 4.37; 4.86 and 5.64g/dl and albumin/globulins ratio 0.55; 0.47 and 0.34g/dl. In the same way for the serum protein fractions of the globulins: alpha-globulin 1.06; 1.33 and 1.33g/dl; beta-globulin 1.10; 1.21 and 1.80g/dl and gamma-globulin 2.21; 2.32 and 2.51g/dl (Table 1).

The averages gotten for the total serum protein in G2 and G3 had been bigger and significant in relation to the G1. How much the fraction albumin, it was observed that the average gotten for G3 significantly lesser when was compared with the G2, and the same happening between G2 and G1 (Table 1).

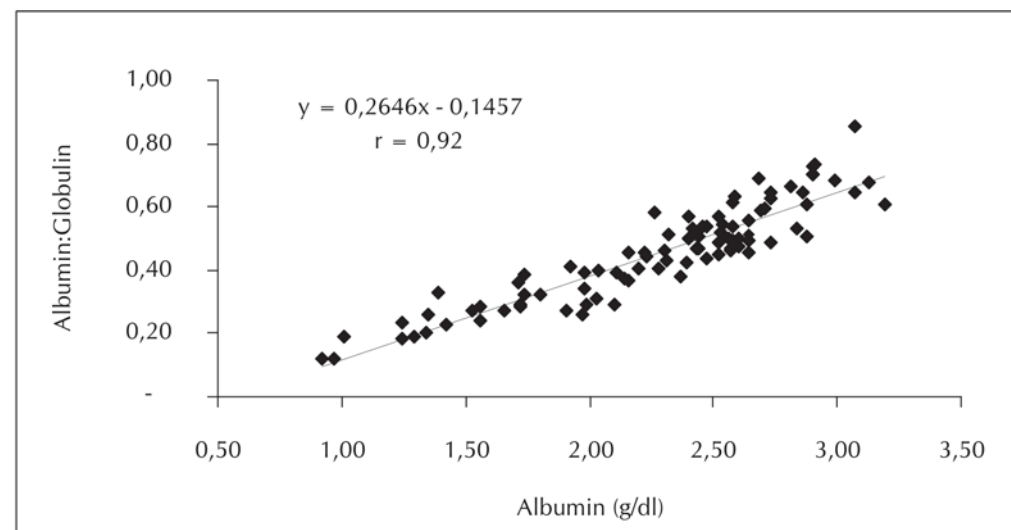


Figure 1 - Ratio between the serum albumin and the albumin:globulin relation of mules with glanders, from Pernambuco State/Brazil - 2003

Table 1 - Average values, shunt standard, medium and percentiles (P25; P75) of the serum proteinogram of negative mules and positive mules with and without symptoms for glanders, from Pernambuco State/Brazil - 2003

Parameters	Control	Clinical conditions	
		+ glanders - symptoms	+ glanders + symptoms
Total protein(g/dl)	7,33 ^b (± 0,53)	7,73 ^a (± 0,66)	7,46 ^{ab} (± 0,89)
Albumin (g/dl)	2,57 ^a (± 0,28)	2,43 ^a (± 0,33)	1,81 ^b (± 0,61)
Globulins (g/dl)			
α – Globulins	1,06 ^b (± 0,23)	1,33 ^a (± 0,31)	1,33 ^a (± 0,27)
β1 – Globulins	1,10 ^b (0,92; 1,22)	1,21 ^a (1,01; 1,54)	0,90 ^b (0,65; 1,12)
β2 – Globulins	0 ^b (0; 0,87)	0 ^b (0; 0,63)	0,90 ^a (0; 1,12)
γ – Globulins	2,21 ^a (± 0,51)	2,32 ^a (± 0,52)	2,51 ^a (± 0,79)
A:G*	0,55 ^a (± 0,13)	0,47 ^b (± 0,11)	0,34 ^c (± 0,15)

* Ratio Albumin: Globulins

Small Letters in the same line not coincident differs statistical to $p < 0,05$

The main causes of hypoalbuminemia include the illnesses chronic and the inflammatory processes. The clinical condition of the animals justifies such findings for the serum albumin. Another aspect that must be considered is the possibility of the animals of G3 to present chronic liver illness or renal under obligation that could contribute for the hypoalbuminemia¹⁴, for the reduction of synthesis in the first case and for the relative loss in the second case¹².

In this study it was observed that the electrophoretic serum protein technique used was capable to separate 5 serum protean

fractions in three animals of G1 (10%), in six animals of G2 (20%) and in eighteen animals of G3 (60%). The remaining animals had presented 4 serum protean fractions.

The results gotten for several electrophoretic techniques can differ significantly in relation of the some protean fractions, making it difficult the matching of the data gotten for different techniques¹⁵. The electrophoretic profile of the serum of equides can vary with the used technique of processing of the samples, ambient conditions and state of health of the animals¹⁹.

The electrophoresis of serum proteins has wide application and can considered in

Table 2- Levels of coefficient of correlation (r) between variables of the serum proteinogram of mules with and without glanders, from Pernambuco State/Brazil - 2003

Variables	PT	ALB	α	β-1	β-2	γ	Alb:Glo
PT	1	0.02 ^{nsa}	0.60 ^{****}	0.44 ^{****}	0.31 ^{***}	0.15 ^{ns}	-0.31 ^{****}
ALB		1	-0.50 ^{****}	0.07 ^{ns}	-0.50 ^{****}	-0.15 ^{ns}	0.92 ^{****}
α			1	0.44 ^{****}	0.25 [*]	-0.05 ^{ns}	-0.63 ^{****}
β-1				1	-0.22 [*]	-0.35 ^{***}	-0.11 ^{ns}
β-2					1	-0.31 ^{**}	-0.50 ^{****}
γ						1	-0.22 [*]
Alb:Glo							1

* $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$; **** $p < 0,0001$; ns not significative

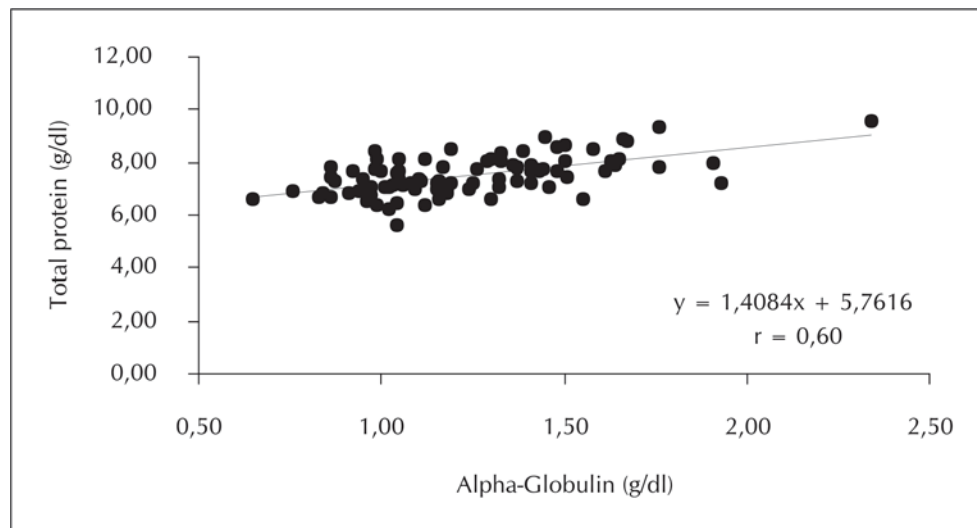


Figure 2 - Ratio between the globulin alpha and the total serum protein of mules with glanders, from Pernambuco State/Brazil - 2003

the diagnosis, demonstrating qualitative and quantitative alterations in the protean fractions, however they are not conclusive information. But the values of serum proteins associates to the clinical picture found are important in the diagnosis, prognostic and course of large quantity pathological conditions¹⁴.

The positive animals with apparent symptoms (G3) presented compatible clinical pictures with those told by Al-Ani et al.⁷, Bazargani et al.⁸, Blancou⁹, Jubb, Kennedy and Plummer², Krishna, Gupta and Masand¹⁰, Mota et al.¹¹, Mota et al.¹³. The presence of edemas observed in some acute cases, occurs due to the fluid extravasation of the compartment vascular for the extra vascular space in an attempt of the organism to revert the osmotic disequilibrium caused by the protean losses, mainly the albumin¹⁵.

In the set of relative of the variables to the serum globulins, mark contrast was verified in the parameter alpha-globulin between the three studied groups, that revealed increased gradual and significantly for G2 and G3 in relation to the G1 (Table 1). Clinically, the rise of the fraction alpha-globulin can be explained by the proteins of acute phase of the illness that run in this protein fraction, being increased in

consequence of acute hepatitis and/or glomerulonephritis¹⁴. Coles¹⁶ describes the alpha-globulin magnified in inflammatory conditions, had the protein increase of acute phase, being the component alpha-2 of the alpha-globulins significantly magnified in bacterial infections.

The results of the analysis of correlation coefficient (Table 2), show to exist high degree of association enter the fraction alpha-globulin with the total serum protein ($r = 0,60$) (Figure 1) and with relation A:G ($r = 0,63$) (Figure 2), and also the albumin with relation A:G ($r = 0,92$) (Fig. 3), with $p < 0,0001$.

The fractions beta and gamma-globulins had been presented statistical high for G3 in relation to the group have controlled (Table 1). It was still observed, that it had separation of the fraction globulin beta-2 in 60% of the animals of G3 (Figure 4). Two proteins compose the Beta-globulin zone in the electrophoretic tracing with cellulose acetate: the transferrin and the C3 component of the system complement. The meaning functional of the C3 component of the complement (75-150 mg/dl) is well known; therefore it acts as measuring in numerous immune reactions: its reduction is the expression of the consumption of

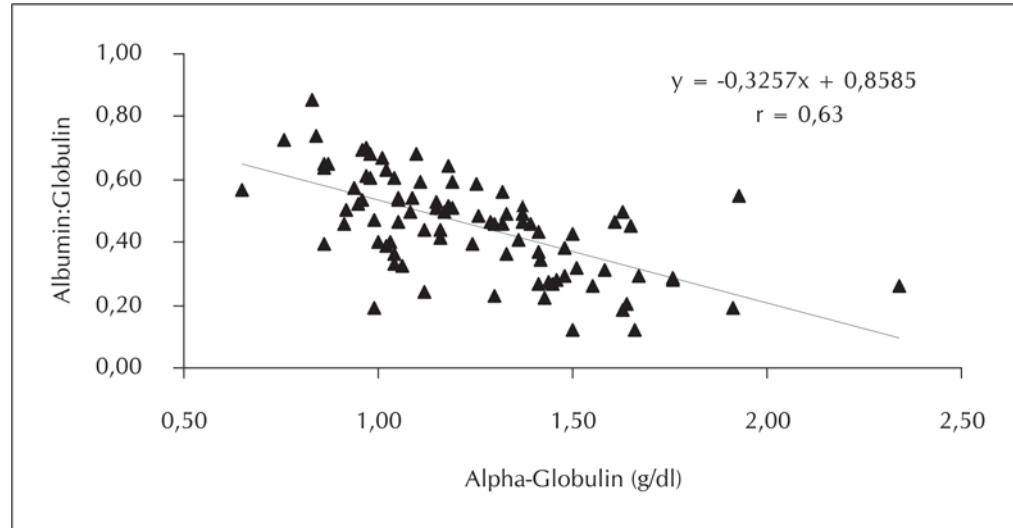


Figure 3 - Ratio between the alpha-globulin and the albumin:globulin relation of mules with glanders , from Pernambuco State/Brazil - 2003

activation of the sequence of complementary factors. The C3 also participates of the reply to the acute inflammatory processes with increase of its concentration in the lateness phase of these processes²⁰. How much to the meaning of the appearance of this fraction only in the animals of the G3, it can be estimated that it is related with the clinical condition of observed acute phase in some animals and that has contributed for this condition, therefore as Kaneko¹⁴ the imunoglobulins produced in the acute phase of the illness (IgA or IgM or both) run in the fraction beta-2.

The increase of the gamma-globulin

fraction could be characterized a specific group of diseases by highly levels of immunoglobulins in blood¹⁸. The gammopathies are classified as monoclonal or polyclonal on the basis on the relative spreading of the g-globulin zone on serum electrophoresis. Polyclonal gammopathies are seen in chronic infections, chronic inflammatory diseases, immune-mediated diseases, and occasionally in lymphoma. What it was evidenced in the present study for G3 (Figure 4), suggestive of a gammopathy of polyclonal character, generally characteristic of benign plasma cell proliferation in response to a persistent antigenic stimulation.

The death and sacrifice of equides with

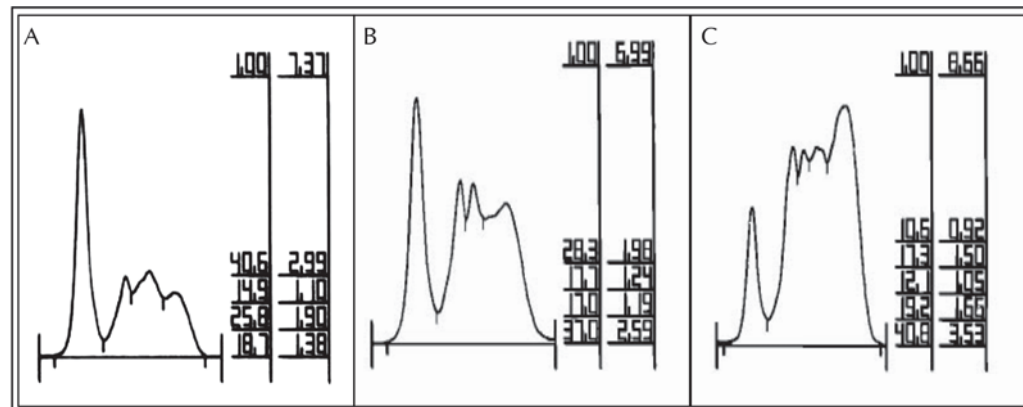


Figure 4 - Electrophoretic profile of mule without glanders (A), mule serological positive for glanders without symptoms (B) and mule serological positive for glanders with symptoms (C), from Pernambuco State/Brazil - 2003

glanders is causing great damages for the sugar cane-of-sugar production, main economic activity of the Zone of Forest of the Pernambuco State/ Brazil, moreover the illness is considered as one zoonosis. The results gotten here make of this pioneering study an important instrument better to understand the behavior of this important disease in different phases.

Conclusions

The data gotten in the serum

proteinogram of mules with glanders supply important information's to the complementary diagnostic and prognostic of the illness. The increase of the globulins characterizes an antigenic stimulation in the sick animals, as well as an inversion in the relation albumin/ globulins for the animals with apparent clinic in relation to the animal ones. These findings could be considered in future research that they aim at to study forms of immunization against this important disease.

Proteinograma sérico em muares naturalmente infectados pela *Burkholderia mallei*

Resumo

A *Burkholderia mallei* é a bactéria causadora do mormo, doença de alta morbidade e letalidade para os eqüídeos, e também uma zoonose. Recentemente diagnosticada nos estados de Pernambuco e Alagoas, vem dizimando populações de eqüídeos na Região da Zona da Mata destes estados, causando grandes prejuízos à atividade canavieira que utiliza tais animais como tração. Objetivou-se com este trabalho investigar as alterações protéicas causadas pelo mormo. Foram estudados 90 muares adultos, de diferentes raças, destinados ao trabalho, provenientes da região canavieira, Zona da Mata, do Estado de Pernambuco. Estes foram divididos em três grupos: G1: composto por trinta animais sorologicamente negativos para o mormo; G2: composto por trinta animais sorologicamente positivos e sem sintomatologia clínica aparente e G3: composto por trinta animais sorologicamente positivos e com sintomatologia clínica aparente. Os resultados obtidos, referentes à média dos parâmetros estudados para G1, G2 e G3 foram respectivamente: proteína sérica total 7,33; 7,73 e 7,46g/dl; albumina 2,57; 2,43 e 1,81g/dl; globulinas 4,37; 4,86 e 5,64g/dl; relação albumina/globulinas 0,55; 0,47 e 0,34g/dl; alfa-globulina 1,06; 1,33 e 1,33g/dl; beta-globulina 1,10; 1,21 e 1,80g/dl e gama-globulina 2,21; 2,32 e 2,51g/dl. Conclui-se que as variações para os parâmetros estudados foram significativas, o aumento das globulinas caracteriza um estímulo antigênico nos animais positivos, bem como uma inversão na relação albumina/globulinas para os animais com clínica aparente em relação aos demais animais. Estes achados poderão ser considerados no diagnóstico, prognóstico e em pesquisas futuras que visem estudar formas de imunização contra esta importante enfermidade.

Unitermos:

Muares.
Mormo.
Proteínas séricas.

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