Epidemiological Aspects of an Infection by *Brucella abortus* in Risk Occupational Groups in the Microregion of Araguaína, Tocantins

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The aim of this paper was to study some epidemiological aspects of the infection by *Brucella abortus* in risk occupational groups in the microregion of Araguaína, Tocantins. For antibody research, 645 serum samples were analyzed by the complement fixation test (CF). A 4.0% frequency was found (26/645) in patients' serum and among those 4.1% (23/551) were slaughterhouses employees and 8.1% (3/37) rural workers. Of the total positive samples, three (2.0%) were women and 23 (4.7%) men; ten (2.9%) were between the ages of 18 and 30, six (3.4%) between 31 and 40, and nine (8.0%) were above 41 years of age. Risk factors for brucellosis in the study groups were age, background (OR = 2.45; CI 95% = 0.98 to 6.10) and previous work conducted with production animals (OR 2.36; CI 95% = 0.95 to 6.02). It was concluded that the infection by *Brucella abortus* is found in some risk occupational groups in the microregion of Araguaína, Tocantins, and control and prophylactic measures must be implemented emphasizing risk factors identified in the study.

Key-Words: Brucellosis, epidemiology, humans, risk factors.

Brucellosis is a zoonosis found worldwide. All infections in humans are due to the direct or indirect contact with infected animals [1]. In humans, the incidence of brucellosis is directly related with the density of cattle, sheep and goat herds, degree of endemics, socioeconomic level and eating habits [2].

Humans usually get infected by *Brucella abortus*, *Brucella melitensis*, *Brucella suis* and *Brucella canis*, however, infections caused by *B. mellitensis* are the most severe as they account for most cases recorded in the world, particularly in developing countries [3,4]. Infections in humans by *B. abortus* can also seriously damage human health mainly in cases of low resistance associated with other diseases and in case of malnourishment [5].

Brucellae are found in a great number in the milk and abortive products of infected animals and thus brucellosis has become an occupational disease for farmers, veterinarians, slaughterhouse workers and lab technicians [6]. For humans, the main forms of transmission include contact with infected animals' secretions by means of cutaneous continuity solutions, aerosols, conjunctival sac inoculation or ingestion of non-pasteurized products [7]. The use of blood and blood derivatives (bone marrow) [8,9] as well as meat ingestion [10] are less commonly referred as ways of transmission.

It is a multisystemic disease with an unspecified clinic. Its clinical signs, in general, are fever, followed or not by shudders, headaches, sudoresis, anorexia, fatigue, weight loss, arthritis, spondylitis, hepatosplenomegaly, neurological symptoms [11], glomerulonephritis and orchitis [4]. Asymptomatic infections have also been recorded often in veterinarians, milk cow farmers and meat processing plant workers, which show a poor correlation between the levels of antibodies and symptomatology [12].

Brucellosis is a disease that reaches mainly individuals who work directly in contact with animals [13-15]. Its incidence has been decreasing in countries that have been able to control the disease in animals. However, every year 0.5 million new cases are recorded, among which most are caused by *B. mellitensis*, considered endemic in the Mediterranean, Latin America and Asia [12]. The disease shows serious consequences for public health, determining temporary inability for work for relatively long periods, long and expensive treatments, slow recovery and very often serious sequelae in the locomotive and nervous system [16].

Considering the importance of brucellosis for public health, our aim with this study was to conduct a research on *Brucella abortus* antibodies and to identify risk factors associated with the infection in risk occupational groups in the microregion of Araguaína, Tocantins.

Material and Methods

This study was conducted in the microregion of Araguaína, located in northern Tocantins, between March 2005 and February 2006, involving four occupational groups thus distributed: Group 1 – Individuals who worked in slaughterhouses that had an inspection service; Group 2 – Rural workers (cowboys); Group 3 – Veterinarians in field activities, and Group 4 – senior students of veterinary of the Federal University of Tocantins – UFT.

As joining the study was voluntary, four (80.0%) cattle slaughterhouses were researched among the five found in the regions, amounting to 645 samples.

After each group was explained the importance of the topic and formal authorizations were obtained, an individual

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research questionnaire was given containing objective questions related to some factors involving the epidemiology of the disease such as sex, age group, background, occupation, ingestion of milk or derivatives *in natura*, whether they had previously worked with production animals showing symptoms suggestive of brucellosis, whether they were aware of the disease (concept and means of contamination) and what is the specific occupation of each slaughterhouse worker. The questionnaires were given by a person previously trained to conduct this task.

Samples were collected by a nursing technician and consisted in radial venipuncture leading to approximately 10 mL of blood and using a disposable syringe and a hypodermic needle (25 x 8 mm). Blood sera were centrifuged at 900 turns for ten minutes. They were eventually conditioned in *Eppendorf* tubes, received the same numbering as the questionnaire given and were kept at -20ºC until a serological test was performed at the National Livestock and Agriculture Laboratory of Pernambuco (LANAGRO-PE).

For the research of anti-*Brucella sp.* agglutinins, the technique of complement fixation test (FC) [17] and the *B. abortus* sample 1119-3 antigen were used, being the latter produced by the Paraná Institute of Technology (TECPAR). Any reaction with one to four crosses starting at 1:5 dilution was considered positive and the final title was given according to the last dilution, in which erythrocyte sedimentation was observed; the samples showing full hemolysis (100.0%) were classified as negative [18].

For data evaluation, a descriptive statistical analysis was used by means of absolute and relative distributions, besides the technique of inferential statistics using a chi-square of independence or Fisher's exact test when conditions for a chi-square test were not met. Significance level used in the decision of statistical tests was 5%.

For the study of risk factors associated with the seropositivity of *B. abortus*, a univaried analysis by means of poin and interval estimate of odds ratio (OR). The result was significant when the trust interval did not include the value 1. The program used to obtain statistical analyses was EpiInfo version 6.04 [19]. The variables studied showed variations as to the total number of individuals (n), since some interviewees did not give enough information to all the questions in the questionnaire.

Results and Discussion

Among the 645 people studied, 551 (85.4%) were slaughterhouse workers, 37 (5.7%) rural workers, 30 (4.7%) veterinary doctors and 27 (4.2%) veterinary medicine students; among these 152 were women (23.8%) and 486 were men (76.2%). Approximately half of the researched people had already worked directly or indirectly with production animals (49.6%) and 80.0% reported having ingested milk and/or derivatives *in natura* (curd 58.0%, cheese 48.8% and milk 39.4%).

Regarding background, 28.1% (153/545) of the people interviewed came from the countryside and 71.9% (392/545) from cities. Regarding age group, 4.4% (28/632) of the individuals were below 20 years of age, 49.5% (313/632) were between 21 and 30 years of age, 28.3% (179/632) between 31 and 40 years of age, 12.7% (80/632) between 41 and 50 and 5.1% (32/632) above 51 years of age.

When we asked about the forms of contagion of the disease, we found that 15.8% (92/583) of the people had never heard about the disease and that 78.3% (457/583) were not aware of its forms of transmission.

It was observed that individuals working in slaughterhouses were distributed in the following sectors: gut + giblets – 156 (28.5%), production – 115 (21.0%), deboning + cow hoof stew + carcass – 106 (19.3%), inspection – 65 (11.9%), grease – 36 (6.6%), slaughter + bleeding – 26 (4.8%), packaging + storing – 19 (3.5%), leather – 15 (2,7%) and pen – 9 (1,7%).

It was shown that from the 645 interviewees, 26 (4.0%) were serum patients for *B. abortus* and 619 (96.0%) showed a negative result. Among the individuals who showed positive serological reactions, three (2.0%) were females and all worked in slaughterhouses and 23 (4.7%) were males. Among these, 20 worked with animal slaughter and three were rural workers. Table 1 shows the results of the association between seropositivity for *B. abortus* with people's sex, age group and background. There was no significant association ($p>0.05$) between sex and seropositivity in the individuals analyzed even though this variability has been found to be a risk factor for this disease [20,21], which is perhaps justified by the greater presence of men in the work done at slaughterhouses and with cattle handling, favoring thus the infection dynamics in this group of individuals.

Among serum patients, 10 (2.9%) were between 18 and 30 years of age, six (3.4%) between 31 and 40 years and nine (8.0%) were above 41 years of age. There was a significant association ($p<0.05$) between age group and seropositivity for this disease, with individuals above 40 years of age more predisposed to being infected, which corroborates the findings of Bigler et al. [22] and Feliciano & Catarino [23], who have described a higher frequency of seropositivity in individuals between 20 and 64 years of age, probably due to a higher time of exposure to the agent.

A significant association was observed ($p<0.05$) between background and seropositivity. Individuals born in the countryside have a higher probability of being infected with *Brucella abortus* (OR 2.45; IC 95%= 0.98 to 6.10) than those born in the cities. Such a condition favors direct contact with the main source of contamination, which is the animal. Feliciano & Catarino [23] have also found that infection by contact with animals accounted for 68.1% of the infections observed in humans.

Table 2 shows the data related with association between seropositivity for *B. abortus* and the factors: previous work with direct contact with production animals, type of activity developed, time of work with animals and consumption of milk and derivative *in natura*.

Table 1. Association between sex, age group and background variables of individuals with seropositivity for *Brucella abortus* at the Complement fixation exam in the microregion of Araguaína, Tocantins, 2006

Variable	Brucellosis						p value	OR (IC 95%)
	Positive		Negative		Total group			
	N	%	N	%	N	%		
Sex								
Male	23	4.7	463	95.3	486	100.0	$p^{(1)} = 0.133$	2.47 (0.73 to 8.33)
Female	3	2.0	149	98.0	152	100.0		
Total	26	4.1	612	95.9	638	100.0		
Age group								
18 to 30	10	2.9	331	97.1	341	100.0	$p^{(1)} = 0.049$*	**
31 to 40	6	3.4	173	96.6	179	100.0		
41 and above	9	8.0	103	92.0	112	100.0		
Total	25	4.0	607	96.0	632	100.0		
Background								
Countryside	11	7.2	142	92.8	153	100.0	$p^{(1)} = 0.035$*	2.45 (0.98 to 6.10)
Cities	12	3.1	380	96.9	392	100.0		
Total	23	4.2	522	95.8	545	100.0		

* Significant association at 5.0%. ** It was not possible to determine due to occurrence. No or very low frequencies. 1 – By Pearson's chi-square test. 2 – By Fisher's exact test.

Table 2. Association between the variables previous work in direct contact with production animals, kind of work done, time of work with animals and consumption of milk and/or derivatives *in natura* and seropositivity for *B. abortus* at the Complement fixation exam in the microregion of Araguaína, Tocantins, 2006

Variable	Brucellosis						p value	OR (IC 95%)
	Positive		Negative		Total group			
	N	%	N	%	N	%		
Previous work in direct contactwith production animals								
Yes	18	6.1	297	93.9	315	100.0	$p^{(1)} = 0.041$*	2.36 (0.95 to 6.02)
No	8	2.5	312	97.5	320	100.0		
Total	26	4.1	609	95.9	635	100.0		
Kind of work done								
Slaughterhouse worker	23	4.2	528	95.8	551	100.0	$p^{(1)} = 0.134$	**
Cowboy	3	8.1	34	91.9	37	100.0		
Veterinarian/Student	-	-	57	100.0	57	100.0		
Total	26	4.0	619	96.0	645	100.0		
Time of work with animals								
<1 year	3	2.4	124	97.6	127	100.0	$p^{(1)} = 0.456$	**
1 – 10 years	17	4.9	331	95.1	348	100.0		
>10 years	3	5.3	54	94.7	57	100.0		
Total	23	4.3	509	95.7	532	100.0		
Consumption of milk and/or derivatives *in natura*								
Yes	19	3.7	492	96.3	511	100.0		
No	7	5.5	120	94.5	127	100.0	$p^{(1)} = 0.360$	0.66 (0.26 to 1.91)
Total	26	4.1	612	95.9	638	100.0		

* Significant association at 5.0%. ** It was not possible to determine due to the occurrence of very low frequencies. 1 – By Fisher's exact test. (2) – By Pearson's chi-square test.

A significant association ($p<0.05$) was found between having previously worked in direct contact with production animals and seropositivity for infection (OR 2.36; IC 95%= 0,95 to 6.02). According to Marques et al.[24], contact with animals, main host of the disease, and the ingestion of non pasteurized milk were shown to be the most likely source of contagion of the disease, which was found in that study since most serum patients have already handled the animals directly (cowboys).

Regarding the occupational groups under investigation, it was further observed that 4.1% (23/551) of slaughterhouse workers were seropositive. There was no significant association ($p<0.05$) between their activity and seropositivity for this disease. The result of the prevalence found in this

Table 3. Association between the variable sector where work is done in cold storage plants and seropositivity for *B. abortus* at the Complement fixation exam in the microregion of Araguaína, Tocantins, 2006

Variable	Brucellosis						p value	OR (IC 95%)
	Positive		Negative		Total group			
	N	%	N	%	N	%		
Sector of cold storage plants								
Slaughter + bleeding	2	7.7	24	92.3	26	100.0	p(1) = 0.200	*
Production (Assistant)	6	5.2	109	94.8	115	100.0		
Leather	1	6.7	14	93.3	15	100.0		
Pen	-	-	9	100.0	9	100.0		
Deboning + cow hoof stew + carcass	6	5.7	100	94.3	106	100.0		
Packaging + storing	1	5.3	18	94.7	19	100.0		
Grease	4	11.1	32	88.9	36	100.0		
Inspection	1	1.5	64	98.5	65	100.0		
Guts + giblets	2	1.3	154	98.7	156	100.0		
Total	26	4.8	521	95.2	547	100.0		

* It was not possible to determine due to very low frequencies. 1 – By Fisher's exact test.

Table 4. Association of the symptoms evaluated in serum patients for brucellosis for the Complement fixation exam in the microregion of Araguaína, Tocantins, 2006

Variable	Brucellosis						p value	OR (IC 95%)
	Positive		Negative		Total group			
	N	%	N	%	N	%		
Do you have a health problem?								
Yes	6	3.6	161	96.4	167	100.0	p(1) = 0.713	0.84 (0.27 to 2.22)
No	20	4.2	451	95.8	471	100.0		
Total	26	4.1	612	95.9	638	100.0		
Fever								
Yes	1	3.4	28	96.6	29	100.0	p(2) = 0.531	1.41 (0.18 a 11.03)
No	25	4.1	591	95.9	616	100.0		
Total	26	4.0	619	96.0	645	100.0		
Shudders								
Yes	1	33.3	2	66.7	3	100.0	p(2) = 0.116	**
No	25	3.9	617	96.1	642	100.0		
Total	26	4.0	619	96.0	645	100.0		
Constant headaches								
Yes	4	3.8	101	96.2	105	100.0	p(2) = 1.000	1.07 (0.36 to 3.18)
No	22	4.1	518	95.9	540	100.0		
Total	26	4.0	619	96.0	645	100.0		
Insomnia								
Yes	1	7.7	12	92.3	13	100.0	p(2) = 0.417	2.02 (0.25 to 16.18)
No	25	4.0	607	96.0	632	100.0		
TOTAL	26	4.0	619	96.0	645	100.0		
Joint pain								
Yes	4	4.6	83	95.4	87	100.0	p(2) = 0.768	1.17 (0.40 to 3.49)
No	22	3.9	536	96.1	558	100.0		
Total	26	4.0	619	96.0	645	100.0		

** It was not possible to determine due to the occurrence of very low frequencies. 1 – By Fisher's exact test. 2 – By Pearson's chi-square test.

Table 5. Association between specific variables of disease awareness in serum patients for brucellosis at the Complement fixation exam in the microregion of Araguaína, Tocantins, 2006

Variable	Brucellosis						p value	OR (IC 95%)
	Positive		Negative		Total group			
	N	%	N	%	N	%		
Heard about brucellosis?								
Yes	20	4.1	471	95.9	491	100.0	$p^{(2)} = 0.213$	0.61 (0.23 to 1.91)
No	6	6.5	86	93.5	92	100.0		
Total	26	4.5	557	95.5	583	100.0		
Knows how to get infected								
Yes	5	4.0	121	96.0	126	100.0	$p^{(1)} = 0.762$	0.86 (0.25 to 2.40)
No	21	4.6	436	95.4	457	100.0		
Total	26	4.5	557	95.5	583	100.0		

1 – By Pearson's chi-square test. 2 – By Fisher's exact test.

study was higher than the one reported by Coelho et al. [25] for slaughterhouse workers in São Luís, Maranhão, where a prevalence of 2.2% was reported by the Complement fixation test. Spinola & Costa [26] in Salvador, Bahia, have studied human brucellosis under the serological, occupational and clinic perspective in 128 cold storage plant workers and reported a prevalence of 10.6% in the test of fast and slow seroagglutination. In general, it is considered that individuals working in slaughterhouses and cold storage plants, mainly those involved in evisceration, become more easily infected [27], since the gravid uterus, the fetal membranes and annexes are the places where *Brucella sp.* are most often lodged [16] and thus manipulation of these tissues can favor the infection in humans.

In Group 2, the prevalence of seropositive individuals was 8.1% (3/37). Regarding this risk group, Moura et al. [28] in a serological study conducted on 33 rural workers of Pedra and Ventura, Pernambuco, have reported a prevalence of 21.1%. The authors claim that the ingestion of milk and raw derivatives from infected animals as well as the manipulation of aborted fetuses, placenta and uterus and vaginal secretions may be considered sources of infection for the referred agent. In this work the study of prevalence in cattle was done on the farms where these individuals worked and it was found that 4.1% of the milk matrices studied were infected. This fact must contribute to the cowboys' infection, since there were reports of the ingestion of raw milk and its derivatives as well as manipulation of fetuses and placenta. Schein [29] has also observed a significant association ($p<0.05$) for brucellosis and the presence of positive cattle.

In this study positive reactions were not found for *B. abortus* in veterinary medicine students. This probably arises from their little contact with sick animals and their infected products and also by that group's awareness about the zoonotic potential of brucellosis. A similar result has been found by Vasconcelos [30] in veterinary students of the Botucatu Faculty in São Paulo, although for *B. canis* and *B. ovis* there was a 6.3% positivity for the population studied by the agarose gel immunodiffusion tests (AGID).

For the group of veterinary doctors in this study, no serum patient was found. In this group, the likelihood of infection is higher because people are more often exposed to the agent, mainly at manipulation of fetuses and placenta [32-33].

The habit of ingesting milk and/or its derivatives *in natura* was reported by 511 (80.9%) of the interviewees. Although there is no significant association ($p>0.05$) between this variable and seropositivity, it is known that cheese, curd, cream cheese, butter and other derivatives, when eaten raw, may be vehicles of infection. That is one of the principal means of transmission of this disease from animals to humans [30,33], which has also been proved by Langoni et al. [34] and Moura et al. [28], who have isolated *Brucella sp.* from milk samples of seropositive cows and discussed the milk's share in transmitting the agent.

Table 3 shows the inexistence of significant association ($p>0.05$) between sectors where work is done in cold storage plants and seropositivity for *B. abortus*, although the sectors guts [26], slaughter and evisceration [35] presumably have a high risk of infection due to exposure to organs and viscera of infected animals.

Table 4 shows the results of the association study between the symptoms evaluated such as fever, shudders, headaches, insomnia and joint pain in serum patients for this disease. No significant association ($p>0.05$) was reported between the factors analyzed and seropositivity for *B. abortus*. The 26 seropositive people did not make any complaints suggestive of brucellosis and only four of them said they had headaches (3.8%) and joint pain (4,6%), unspecific symptoms for brucellosis. The disease in humans can be subclinical and is characterized by the existence of positive serology without evidence of clinic symptoms for the disease [24], or with slight alterations that can go unnoticed [33]. Keane [36] mentions that the asymptomatic infection is frequent in slaughterhouse workers, veterinarians and cattle breeders.

Table 5 shows the data related to the association between seropositive individuals for the disease and there awareness of it. There was no significant association ($p>0.05$) between the factors analyzed although 15.7% (91/581) of the

interviewees claim that they have never heard about the disease and 77.9% (443/569) say they are not aware of its forms of transmission.

Regarding the results obtained in this study that demonstrate seropositivity for *B. abortus* in some risk groups studied and considering that brucellosis is a disease that is not very much known by health professionals, educational campaigns about it and other zoonoses must be set up to reduce the risks of transmission of the agent to these occupational groups.

Conclusion

The infection by *Brucella abortus* is found in some risk occupational groups in the microregion of Araguaína, Tocantins, and control and prophylactic measures must be implemented emphasizing risk factors related to age group, background and previous work in direct contact with production animals, which were the factors associated with the infection by *B. abortus* in the risk groups researched.

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