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Detecção de anticorpos anti - *Rickettsia* spp. em galinhas domésticas de criação extensiva em uma área endêmica para febre maculosa no estado do Rio Grande do Sul, Brasil

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ABSTRACT

*The goal of this study was to investigate anti-**Rickettsia** spp. antibodies in sera of domestic chickens (**Gallus gallus domesticus**) of extensive breeding in Cerro Largo county, considered an endemic area for spotted fever in the State of Rio Grande do Sul, Brazil. Three hundred blood samples were collected and anti-**Rickettsia** spp. antibodies were evaluated by indirect immunofluorescence assay (IFA) in the sera obtained. The occurrence of anti-**Rickettsia** spp. antibodies detected in this study was 1.33% (4/300), with endpoint titers ranging from 64 to 256 for **Rickettsia rickettsii**, **R. parkeri** and/or **R. bellii**. The results suggest these domestic chickens do not participate as a reservoir and/or amplifying host in the epidemiology of spotted fever in that endemic area. The present study consists in the first serological survey in **Gallus gallus domesticus** to rickettsiae-spotted fever group in Brazil.*

Key words: *indirect immunofluorescence assay, antibody, rickettsiosis, domestic chicken.*

RESUMO

*Este estudo teve como objetivo pesquisar anticorpos anti-**Rickettsia** spp. em soros de galinhas domésticas (**Gallus gallus domesticus**) de criação extensiva, provenientes de área considerada endêmica para febre maculosa no estado do Rio Grande do Sul. Foram coletadas 300 amostras de sangue e os soros obtidos foram testados para anticorpos anti-**Rickettsia** spp. pela Reação de Imunofluorescência Indireta (RIFI). A ocorrência de anticorpos anti-**Rickettsia** spp. observada foi de 1,33% (4/300), com títulos variando de 64 a 256 para **Rickettsia rickettsii**, **Rickettsia parkeri** e/ou **Rickettsia bellii**. Os resultados sugerem que essas galinhas domésticas não participam como reservatório e/ou hospedeiro amplificador na epidemiologia da febre maculosa na área endêmica. O presente estudo consiste na primeira pesquisa sorológica em **Gallus gallus domesticus** para rickettsia do grupo da febre maculosa no Brasil.*

Palavras-chave: *reação de imunofluorescência indireta, anticorpos, rickettsioses, galinhas domésticas.*

INTRODUCTION

The genus ***Rickettsia*** belongs to the order Rickettsiales, it is composed of different obligate intracellular bacteria species which are important causative agent of human and animal infectious diseases. An important zoonotic infection characterized by acute rash illnesses spread by ticks around the world, globally called spotted fever, is caused by ***Rickettsia*** spp. (PAROLA et al., 2005). In Brazil, the main etiological agent of Brazilian Spotted Fever (BSF) is ***Rickettsia rickettsii***, considered the most pathogenic species of the genus for humans and some animals. Furthermore, this bacterium is transmitted by ***Amblyomma cajennense*** and ***Amblyomma aureolatum*** ticks (PINTER & LABRUNA 2006; SOARES et al., 2012). The occurrence of ***R. rickettsii*** has been confirmed in the Southeastern Brazilian region, where it was isolated and/or detected by molecular methods in humans, animals and ticks (LABRUNA et al., 2011).

Other reports of tick-borne rickettsial pathogens in South America refer to ***Rickettsia parkeri***, the etiologic agent of a spotted fever associated to skin rashes and swollen lymph nodes. This agent is transmitted by ***Amblyomma triste*** in

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Brazil, Uruguay and Argentina (VENZAL et al., 2004; SILVEIRA et al., 2007; NAVA et al., 2008; CONTI-DÍAZ et al., 2009; ROMER et al., 2011). In Brazil, the occurrence of a new strain of ***Rickettsia***, genetically similar to ***R. parkeri***, was diagnosed in humans in São Paulo and Bahia states (SPOLIDORIO et al., 2010; SILVA et al., 2011). This new strain is called "Atlantic" and it is transmitted by ***Amblyomma ovale***, ***Amblyomma aureolatum*** and ***Rhipicephalus sanguineus*** (SABATINI et al., 2010; MEDEIROS et al., 2011). In other Brazilian regions, human cases of BSF were diagnosed mainly by indirect serological methods, which did not allow the identification of ***Rickettsia*** species. Moreover, cross-reactions are very often among almost all ***Rickettsia*** spp. In South of Brazil, in Santa Catarina state, there were reports of human cases of BSF confirmed only by serology; however, ***Rickettsia*** sp. strain "Atlantic" was detected in ticks in this location (MEDEIROS et al., 2011).

Some rickettsiae, such as ***Rickettsia bellii***, ***Rickettsia amblyommii***, ***Rickettsia rhipicephali,*** and ***Rickettsia monteiroi*** are considered nonpathogenic or unknown pathogenicity. They have been reported infecting ***Amblyomma*** spp., ***Haemaphysalis*** spp. and ***Ixodes*** spp. in Brazil (LABRUNA et al., 2011). Concerning to these microorganisms, only ***R. bellii*** and ***R. monteiroi*** are not genetically and serologically related to the spotted fever group species (PAROLA et al., 2005).

The birds are among the preferred hosts of the immature stages of many species of ***Amblyomma*** spp. ticks in Brazil (ARZUA et al., 2003; OGRZEWALSKA et al., 2010, 2011, 2012; LUZ et al., 2012). The ticks which are parasitizing these hosts also take advantage of their mobility to the geographical dispersion. Epidemiologically, this spread becomes more important if these ticks are vectors harboring infectious agents (MOVILA et al., 2012). Studies on the prevalence of ***Rickettsia*** spp. in ticks of parasitic life in birds have been performed in various locations around the world, including Brazil (LABRUNA et al., 2007; OGRZEWALSKA et al., 2010, 2011; LUZ et al., 2012). All of these studies were carried out in wild birds, especially in Passeriformes. Furthermore, there is a lack of serological studies of rickettsiosis in wild or domestic birds as well as the demonstration of their role as an amplifier or reservoir host in the epidemiology of the BSF (OGRZEWALSKA et al., 2012).

The aim of this study was to determine the occurrence of anti-***Rickettsia*** spp. antibodies in domestic chicken (***Gallus gallus domesticus***) from extensive breeding farms and verify epidemiological significance as reservoir and/or amplifying hosts of the etiological agent of spotted fever in an area considered endemic for BSF in Rio Grande do Sul state (RS), Brazil.

MATERIAL AND METHODS

Endemic area

This study was performed from May to November 2011 and included 29 farms in the municipality of Cerro Largo, northwest of RS (28° 08'49 "S, 54º 44'17" W). Human cases of BSF were reported in rural areas of this town; however the etiological agent associated with the disease has not been isolated (SANGIONI et al., 2011). These farms of this study were small areas, employing family labor, breeding extensively various domestic animal species, including domestic chicken. All the farms were located adjacent to native forest areas.

Sampling

In this research it was collected a total of 300 blood samples from chickens (***Gallus gallus domesticus***). This sampling was established based on calculus of infinite population in according to THURSFIELD (2004). All of the poultry were breeding extensively and potentially exposed to tick parasitism, being observed the presence of Argasidae in the majority of these birds. To procedure of the blood samples collection, the birds were properly contained and carried out the ulnar venipuncture without anti-clotting. Following, the blood samples were placed in 10mL-fresh tubes and kept refrigerated in cool boxes for shipping. The samples were sent to the Laboratory of Parasitic Diseases at Universidade Federal of Santa Maria (UFSM), RS, Brazil. In the lab, the blood sera were obtained and kept frozen at -20°C until the serological tests were performed.

Indirect fluorescent antibody assay

The serum of each chicken was tested by indirect immunofluorescence (IFA) using crude antigens of different species of ***Rickettsia***, as previously described (HORTA et al., 2004). Serum samples were initially tested at 1:64 dilution in PBS for ***R. rickettsii*** (strain Taiaçu) and ***R. parkeri*** (strain AT24); both species are known as pathogenic and transmitted by ticks in Brazil. If any serum sample was reactive at least one of these two antigens, following titrations were performed for both antigens and for the other species of ***Rickettsia*** occurring in Brazil, such as ***R. amblyommii*** (strain Ac37), ***R. rhipicephali*** (HJ5 strain), ***R. felis*** (Pedreira strain)

and ***R. bellii*** (strain Mogi) in order to verify the possible antigen involved in seropositive cases, as previously established (HORTA et al., 2004, 2010; PIRANDA et al., 2008). In all reactions, it was used positive and negative control sera. The negative control serum was originated from a SPF chicken. To obtain the positive control, the same chicken was inoculated intramuscularly with an inoculum consisting of approximately $5x10^5$ VERO cells infected with ***R. rickettsii***, and clinically monitored, especially by rectal temperature, until the 21st day after inoculation. Later, the chicken was properly submitted to euthanasia and the blood serum was collected. In all reactions, the secondary antibody used was anti-chicken IgY produced in rabbit and labeled with fluorescein isothiocyanate (Sigma Diagnostics, St. Louis, MO) in a dilution at 1:3,000.

RESULTS AND DISCUSSION

The SPF chicken inoculated with ***R. rickettsii*** showed no clinical signs during the 21 days post-inoculation. Additionally, the serum collected before inoculation demonstrated no reaction to rickettsial antigens. The highest antibody titer detected in the positive control serum was 4,096 in both ***R. rickettsii*** and ***R. parkeri***. The SPF chicken serum was used at 1:64 dilution as positive control in all reactions.

In the 300 chickens sera initially tested by IFI for both ***R. rickettsii*** and ***R. parkeri***, only four birds (1.33%) were reactive to ***R. rickettsii*** and/or ***R. parkeri***. Later on, these four samples were titrated for different rickettsiae and the titers were 64 to 128 to ***R. rickettsii*** and ***R. parkeri***. In addition, no sample reacted to ***R. amblyommii***, R. ***rhipicephali*** or ***R. felis***. However, only one sample showed titer of 256 to ***R. bellii*** (Table 1).

Cerro Largo was chosen as the target area for this research due to a case of BSF in human reported in 2007. Subsequently, an epidemiological study demonstrated antibody reagents (titer ≥64) to ***R. rickettsii*** and ***R. parkeri*** in equines 51.6% (16/31), dogs 22.3% (6/27) and healthy human 29.6% (8/27). Two dogs showed ***R. parkeri*** antibody titers at least four times greater than the titers for other ***Rickettsiae*** tested, suggesting that ***R. parkeri*** (or a similar genotype) was circulating in that region (SANGIONI et al., 2011).

In the current study, it was observed that the domestic chickens were breeding freely in the farms. For this reason, they were potentially exposed to parasitism by ticks, and consequently to rickettsial infection. Furthermore, the poultry in this traditional breeding system have ingested a diversified natural diet such as a number of invertebrates (arthropods and annelids), which were infected by different species of rickettsiae, including ***R. bellii*** (KIKUCHI & FUKATSU, 2005; WEINERT et al., 2009). However, in this research, only 1.33% of the chickens showed spotted fever group anti-***Rickettsia*** spp. antibodies. The highest antibody titer was 128 and it is considered less than the previously anti-***Rickettsia*** spp. antibodies found in horses, dogs and humans in that endemic region (SANGIONI et al., 2011). These findings suggest that chickens have no epidemiological significance as reservoirs and/or amplifying hosts of the etiological agent of BSF in Cerro Largo. Probably the birds can be refractory to infection, or they are not being parasitized by the tick vectors of Rocky Mountain spotted fever. Nevertheless, these hypotheses need to be verified in further studies. Although the SPF chicken experimentally inoculated with ***R. rickettsii*** exhibited no clinical signs, it seroconverted and displayed elevated titer (4,096). LUNDGREN et al. (1966) inoculated ***R. rickettsii*** in different species of birds, including domestic fowls. The authors not detected antibodies titers by complement fixation test in domestic fowls; however it was demonstrated rickettsemia in these birds.

Table 1 - Anti-rickettsial antibodies titration in four serum-positive domestic chickens to R. ***rickettsii*** and/or R. ***parkeri*** from Cerro Largo, RS, Brazil.

Species	Chicken A	Chicken B	Chicken C	Chicken D
R. rickettsii	128	64	--	128
R. parkeri	64	--	64	64
R. felis	--	--	--	--
R. bellii	--	256	--	--
R. amblyommi	--	--	--	--
R. rhipicephali	--	--	--	--

--: Not reactive serum to antibody titer ≥ 64.

In this study, a single chicken showed titer up to 256 to ***R. bellii***, interestingly this species does not belong to the spotted fever group. Although ***R. bellii*** has been reported infecting a wide variety of ticks in Brazil (LABRUNA et al., 2011), species genetically similar to ***R. bellii*** have been isolated from a variety of free-living invertebrates in the soil (KIKUCHI & FUKATSU, 2005; WEINERT et al., 2009). Thus, the occurrence of a chicken with anti-***R. bellii*** antibody titer up to 256, at least four times higher than those of group rickettsiae spotted fever (Table 1), suggests that chicken may have been infected by ticks from different sources, including ingestion of some invertebrate infected by a rickettsia similar to ***R. bellii***.

CONCLUSION

This study is the first serological survey of domestic chickens of extensive breeding to rickettsiae of the spotted fever group. The results suggest that these chickens have slight epidemiological significance as reservoir and/or amplifying hosts of the etiological agent of spotted fever in Cerro Largo. Further studies must be performed in order to verify the role of chickens in the life cycle of rickettsias.

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ETHICS COMMITTEE AND BIOSAFETY

Regarding to all animal experimentation procedures, this study was approved by the ethical committee on animal experimentation of UFSM (Protocol number: 009/2011).

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