

NOTES AND COMMENTS

DETERMINATION OF DUFFY PHENOTYPE OF RED BLOOD CELLS IN *Dasypus novemcinctus* AND *Cabassous* sp.

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Received June 9, 2003 – Accepted January 6, 2004 – Distributed August 31, 2005

SHORT COMUNICACION

The *Dasypus novemcinctus* (Linnaeus, 1758) is an armadillo, a primitive mammal belonging to the superorder Edentata, the order Xenarthra, and the Dasypodidae family that has eight genera. Two of these are the *Dasypus*, a native of Mexico and found exclusively on the American continent, and the *Cabassous* sp., which is found in South America. There are about 20 species of *Dasypus* living mainly in South and Central America. *D. novemcinctus*, the only widely-distributed one, also occurs in North America (Talmage & Buchanan, 1954; Storrs, 1971; Romer & Parsons, 1985).

The peculiar biological aspects of the armadillo have aroused the curiosity of researchers in several areas of biology. Biomedical interest increased after Kirchheimer & Storrs (1971) and Storrs (1978) reported experimental reproduction of leprosy in *D. novemcinctus*, an important discovery mainly because it yielded the only scientifically controlled method for large-scale reproduction of *Mycobacterium leprae* (Job, 1991). Walsh *et al.* (1975) reported that macrophages with bacilli resembling *M. leprae* and bacilli found in experimentally inoculated animals had been observed in wild armadillos with lesions in the liver, spleen, and lymph nodes.

In addition to these microorganisms, studies have demonstrated that the *D. novemcinctus* is a natural reservoir of *Trypanosoma* sp. (Lainson *et al.*, 1979a), *Paracoccidioides brasiliensis* (Vidal *et al.*, 1995), and *Leishmania* sp. (Lainson *et al.*, 1979b).

Other studies of the biology of the armadillo have been conducted since those of Kirchheimer & Storrs (1971), though many questions remain unanswered. This is the case with certain aspects of the blood systems in the armadillo.

Among the few studies found on the subject is the research of Lewis & Doyle (1964) who observed that erythrocytes of a *D. novemcinctus* armadillo were agglutinated with anti-A, anti-B, and anti-D serum. They concluded that these belonged to the AB blood group and Rh positive phenotype. Similar results were obtained by Souza *et al.* who 1987 reported the presence of the AB blood group in two armadillos and the B blood group in seven armadillos of the same species.

But the quantitative inadequacy of these results due to the small number of animals used in previous studies made evident a need to amplify the investigation into the blood groups of armadillos. This led us to carry out a study on the distribution of the Duffy blood system in the *D. novemcinctus* and *Cabassous* sp. as part of a broad line of research developed in the Instituto Lauro de Souza Lima to provide answers to questions related to the biology of these species.

Thus, 21 adult *D. novemcinctus* (10 males and 11 females) and 3 *Cabassous* sp. (one male and two females) were used in this study. They were all captured in the outlying regions of the city of Bauru, São Paulo State, Brazil (License n. 459 – IBAMA).

Blood samples were obtained by femoral vein puncture and placed in sterile glass vials with the anticoagulant EDTA. The red cells were washed and diluted to 5% in saline solution and tested with the polyclonal rare anti-sera Fy^a and Fy^b for the Duffy blood system.

The blood typing was carried out through a tube agglutination technique in accordance with the anti-serum manufacturer's recommendations (Biotest AS) when used for typing human red cells. The result was considered positive when the red cells agglutinated with the respective anti-serum and negative in the absence of agglutination.

In the present study, all *D. novemcinctus* analyzed presented the same Duffy Fy (a+ b+) phenotypes. On the other hand, the *Cabassous* sp. erythrocytes, which did not react with the human Duffy antibodies, presented the phenotype Fy (a- b-). Thus, by using the same methodology used for humans, both *D. novemcinctus* and *Cabassous* sp. could be classified according to the Duffy blood system.

The direct Coombs' test was done on erythrocytes of three animals, with negative results in all.

Agglutination of antigens Fy^a and Fy^b in *D. novemcinctus* may be considered a false positive reaction because these sera present non-specific reactivity between species. However, the absence of agglutination in the same antigens when tested in *Cabassous* sp. led us to think that the results obtained were not false positives.

In order to determine whether *D. novemcinctus* actually has the Duffy (a+ b+) phenotype, we would have had to perform the absorption and elution test. This was not done because of the reduced volume of the samples collected.

The association between blood group and disease has been widely studied. Amongst the most important results of these studies has been the demonstration of a relationship between the Duffy blood system and malaria in humans (Miller *et al.*, 1976). Another was that the Duffy protein belongs to the superfamily of chemokine receptors (Luo *et al.*, 2000).

In another study of this blood system in animals, Colauto (1991) detected the Fy^a antigen while working with red cells of the fish *Synbranchus marmoratus*, which had been parasitized by *Cyrlia gomesi*. He concluded that the participation of this antigen was fundamental in the success of the invasion mechanism of this parasite with respect to those red cells.

In studying the red cells of two different bovine breeds, Nakamoto *et al.* (1998) concluded that the animals most resistant to infection by *Babesia* spp. had a high frequency of the Fy (a- b-) phenotype, and that the most susceptible (86% of the cattle) were carriers of Fy^a and/or Fy^b antigens.

We strongly suggest the extension of the present study to other species of armadillo, in addition to further research on the hemoparasites

to which they are vulnerable. This could further our understanding about the mechanisms involved in the host-parasite relationship found in human infections, in addition to expanding our understanding of the biology of these animals.

Moreover, further detailed studies of the molecular biochemistry of erythrocyte structures through sequencing and amplification of DNA isolated from animal tissues are required to establish the specificity of the blood group antigens in armadillos and their possible role as parasite receptors.

Acknowledgements — The authors wish to thank Mr. Vasos Korkou for reviewing the English version of this manuscript.

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