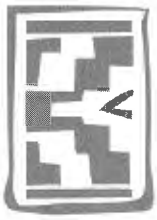

The binding potential of commercial antibody conjugates with sera of various small terrestrial mammals

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ABSTRACT

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Infectious diseases of wild animals are of increasing importance, both from an economic viewpoint and because several of these diseases are pathogenic to man. However, serosurveys to determine the circulation of infectious organisms in wildlife are complicated by the fact that antibodies to species-specific immunoglobulins are not available for use in serological assays such as enzyme-linked immunosorbent assays (ELISAs) or immunofluorescence assays. To determine the binding potential of four commercially available antibody conjugates with the sera of wild animals, sera from 27 species of small terrestrial mammals were allowed to react with alkaline phosphatase-labelled protein A, anti-rabbit IgG, anti-mouse IgG and anti-human IgG by by the use of an ELISA. It was found that sera from some species of the order Lagomorpha bound optimally to anti-rabbit IgG, while anti-mouse IgG could be used for most species of Rodentia. For all Carnivora, Insectivora, Macroscelidea, Hyracoidea and other Rodentia, staphylococcal protein A demonstrated optimal binding. None of the sera that was tested bound to anti-human IgG. These results demonstrate that commercial conjugates can be used in serological assays in which wild animal sera are used, and should be useful for future serosurveys to determine the circulation of infectious agents in small terrestrial mammals.

Keywords: Antibody conjugates, binding potential, commercial cojugates, ELISA, serosurvey

INTRODUCTION

The increase of eco-tourism worldwide has led to an enormous increase in the importance of wild animals and their diseases, owing to the increased contact of humans with these animals. Wildlife may act as potential reservoirs of infections transmissible not only to man, but also to domestic animals. Serosurveys to determine exposure to infectious diseases in these animals are, however, hampered by the fact that no commercial conjugated antisera to species-specific immunoglobulins are available for use in serological assays such as enzyme-linked immunosorbent assays (ELISAs) or immunofluorescence assays. The preparation of such antibodies for each animal species is difficult and time-consuming, and also requires rigorous quality assurance to ensure that there is no cross-reactivity due to infections in the animals used for preparation of the antibodies (Kelly, Tagwira, Matthewman, Mason & Wright 1993). Little is known regarding the binding of available antibody conjugates with sera from wild animals. Several studies have shown that sera from diverse animal species bind staphylococcal protein A with varying efficiency (Richman, Cleveland, Oxman & Johnson 1982; Oelofsen 1988; Kelly *et al.* 1993). However, protein A binds relatively poorly to sera of some rodent species (Richman *et al.* 1982). In this study, we determined the binding potential of four commercially available antibody conjugates with sera from 27 different species of small terrestrial mammals from southern Africa.

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MATERIALS AND METHODS

Specimen collection

Small mammals were trapped on farms and nature reserves in the Free State and Northern Cape provinces of South Africa by means of Sherman mouse traps. Captured animals were anaesthetized with ether and bled by cardiac puncture. Larger animals were shot by officials from the Department of Nature Conservation and then bled by cardiac puncture. Permission for all animal experiments was obtained from the Department of Nature Conservation and the Ethical Committee for Animal Experimentation of the University of the Free State. Sera were centrifuged and kept at –20 °C until transported to the laboratory where they were stored at –70 °C until use.

Enzyme-linked immunosorbent assay

The sera of two animals from each species were diluted 1:20 in coating buffer [1,59 g of Na_2CO_3 and 2,93 g of $NaHCO_3$ in 1 ℓ of H2O (pH = 9,6)], and added to 96-well microtitre plates (Maxisorp, Nunc) at 100 µℓ per well. Wells containing only coating buffer were included as negative controls. Plates were incubated overnight at 4 °C in a humid atmosphere and washed three times with phosphate-buffered saline containing 0,05 % Tween-20 (PBS-T). Subsequently, the remaining binding areas on the plates were blocked by addition of 200 µℓ of PBS containing 5 % bovine serum albumin to each well, followed by incubation at 37 °C for 60 min. After three washings with PBS-T, 100 µℓ of a commercial conjugate was added to the wells. The following alkaline phosphatase-labelled

TABLE 1 Binding of sera of small terrestrial mammals with four commercial conjugates

Animal species	Conjugates			
	Protein A	Anti-rabbit	Anti-mouse	Anti-human
Carnivora				
Canis mesomelas	++	–	–	–
Cynictis penicillata	++	+	+	–
Felis lybica	++	+	+	–
Genetta genetta	+	+–	–	–
Herpestes pulverulentus	+	+	+	–
Ictonyx striatus	++	–	–	–
Otocyon megalotis	++	–	+	–
Proteles cristatus	+	–	–	–
Vulpes chama	+	–	–	–
Hyracoidea				
Procavia capensis	+	+–	+	–
Insectivora				
Crocidura cyanea	+	–	+	–
Elephantulus myurus	++	–	+	–
Lagomorpha				
Lepus capensis	+	++	–	–
Lepus saxatilis	+	++	–	–
Pronolagus rupestris	–	++	–	–
Rodentia				
Aethomys namaquensis	–	–	++	–
Cryptomys hottentotus	+	–	–	–
Desmodilus auricularis	–	–	+	–
Gerbillurus paeba	–	–	+	–
Hystrix africae-australis	+	–	+	–
Pedetes capensis	+	–	–	–
Mastomys natalensis	–	–	++	–
Rhabdomys pumilio	+	–	–	–
Saccostomus campestris	+	–	–	–
Tatera leucogaster	+	–	–	–
Xerus inauris	++	–	+	–

++ = OD > 1
\+ = OD 0,3–1
+/– = OD 0,05–0,299
– = OD < 0,05

conjugates were used at different dilutions ranging from 1:1000–1:2000; protein A (BioMakor), goat anti-rabbit IgG (Bio Yeda, Israel), goat anti-mouse IgG (Atlantic Antibodies, USA) and goat anti-human IgG (Behring, Germany). The plates were incubated for 60 min at 37 °C and washed, then 100 µℓ of p-nitrophenyl phosphate substrate (Behring, Germany) was added to each well. After a 30-min incubation period at 37 °C in the dark, 50 µℓ of stopping solution (2N NaOH) was added to each well. The optical density (OD) was read at a wavelength of 405 nm with a reference wavelength of 492 nm. The true OD was defined as the OD of the test sample minus the average OD of the negative controls. All tests were done in duplicate and the average OD of two duplicate wells were used to determine the binding strength of a conjugate with the animal serum used to coat the wells.

RESULTS

The binding potential of sera from 27 different animal species with four commercial conjugates was evaluated in an ELISA. The differential bindings of sera from each species to the four conjugates tested are shown in Table 1.

The results demonstrated that anti-rabbit IgG bound strongly to sera from all Lagomorpha tested, and should be the conjugate of choice for serological studies in which sera of these species are used. The optimal dilution was 1:2000. For Rodentia, except *Rhabdomys, Saccostomus, Cryptomys, Tatera* and *Pedetes*, optimal binding was observed with anti-mouse IgG at 1:1000. Staphylococcal protein A at 1:1000 was the best conjugate for use with sera from all Carnivora tested, as well as for Insectivora, Macroscelidea, Hyracoidea and the Rodentia for which anti-mouse IgG could not be used. None of the animal sera tested showed any binding with anti-human IgG.

DISCUSSION

In this study it was demonstrated that commonly available commercial conjugates can be used for most serological assays in which sera from wild animals are used, obviating the need for the preparation of species-specific antibodies. In accordance with earlier studies (Richman *et al.* 1982; Oelofsen 1988; Kelly *et al.* 1993), staphylococcal protein A also bound to most of the animal sera tested in this study. However, protein A bound only weakly, or not at all, to sera from several Rodentia, while it also did not bind to serum from one of the Lagomorpha species tested. As rodents are important vectors for the transmission of several infectious agents such as Hantaviruses (Lee, French, Lee, Back, Tsuchiya & Foulke 1981) and Lassa virus (McCormick, Webb, Krebs, Johnson & Smith 1987; Monath, Newhouse, Kemp, Setzer & Cacciapuoti 1974), it is important that serological assays in these animals be carried out for epidemiological purposes. Anti-mouse IgG, however, bound to all sera from Rodentia that were not bound by protein A, demonstrating that in cases where protein A does not bind to the serum of a specific rodent species, anti-mouse IgG can most likely be used. This study, furthermore, shows that commercial anti-rabbit IgG bound strongly to all three Lagomorpha species tested, indicating that this could be used as secondary antibody in serological assays for studies on Lagomorpha. Sera from each of the 27 species tested, bound to at least one of the conjugates. It should therefore be possible to use commercial conjugates for the majority of serosurveys in wild animals. It should be noted that streptococcal protein G also binds very well to sera from several animal species (Akerstrom, Brodin, Reis & Bjore 1985) and should also be evaluated for its potential to bind to sera from wild animals.

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