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Immunoglobulin G Class Identification from Wild Ungulates by Cross-reactivity with Antisera to Domestic Animals

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With 2 figures

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Summary

Seven species of Spanish ungulates were tested for the presence of homologous immunoglobulin G (IgG) with a gel-diffusion test using bovine, ovine, caprine and porcine IgG antisera. Homologous ovine and caprine IgG were detected in sera from chamois (*Rupicapra pyrenaica*), Spanish ibex (*Capra pyrenaica hispanica*), mouflon (*Ovis orientalis musimon*), red deer (*Cervus elaphus*), fallow deer (*Dama dama*) and roe deer (*Capreolus capreolus*). Homologous porcine IgG was detected in wild boar (*Sus scrofa*) serum. Immunoelectrophoretic assays were performed to compare the electrophoretic mobility of IgG from domestic and wild species.

Introduction

In recent years interest in the physiological condition of wild ungulates has increased. Although haematological and biochemical data are available, little is known about their immune system. Development of immunoglobulin quantification assays in these animals will provide an additional indication of their overall health. It is also important to detect evidence of different infections in the wild species and to compare them to the known domestic ones. However, the detection and measurement require a species-specific antibody to immunoglobulins, which is typically not available for the wide range of non-domestic ungulates.

Serological cross-reactivity has been successfully employed for identification of immunoglobulin classes in a variety of mammalian species (Nash et al., 1969; Pahud and Mach, 1970; Nash and Mach, 1971; Vaerman and Heremans, 1971; Neoh et al., 1973). Use was made of the ability of specific antisera against an immunoglobulin class to cross-react with a homologous protein in different species. These antisera could be used therefore in the immunoglobulin quantification assays or in the detection of different antibodies of cross-reacted species.

The aim of this study was to determine if available antisera against bovine, ovine, caprine and porcine immunoglobulin G (IgG) could be used to quantify IgG in the sera of the wild ungulates of Catalonia (north east Spain).

Materials and Methods

Blood samples were taken from captured animals in the Catalonia region (Spain) during the course of investigation for disease. The species tested were chamois (*Rupicapra pyrenaica*), Spanish ibex (*Capra pyrenaica hispanica*), mouflon (*Ovis orientalis musimon*), red deer (*Cervus elaphus*), fallow deer (*Dama dama*), roe

deer (*Capreolus capreolus*) and wild boar (*Sus scrofa*). The samples were collected from the external jugular vein and the sera were stored without preservatives at –20°C.

Gel-diffusion (Ouchterlony) tests were performed in 1 % agarose gel (Sigma Chemical Co., St. Louis, MO) made up in phosphate-buffered saline. The agarose was melted and poured onto a plate to form a uniform thickness of 2 mm. Hexagonal patterns of wells were cut in the gels, distances between central antiserum and peripheral antigen wells were 10 mm and the well diameters were 3 mm. Antisera to bovine, ovine, caprine and porcine IgG (polyclonal antibodies, IgG fractions; The Binding Site, Birmingham, UK) were used. Each antiserum was loaded into the central well of an Ouchterlony plate and tested against undiluted and diluted sera (1 : 2, 1 : 4, 1 : 8) from six chamois, five Spanish ibex, six mouflon, five red deer, three fallow deer, three roe deer and three wild boar. Positive and negative (distilled water) controls were used for each assay. The plates were incubated at room temperature for 24 h and examined for the presence of immunoprecipitin lines.

Immunoelectrophoretic assays (Barta and Barta, 1993) were performed in 1 % agarose gel made up in barbital buffer (sodium veronal 0.04 M – sodium diethyl barbiturate; Atom, Barcelona, Spain). The agarose was melted and poured onto microscope slides (3.75 ml for 75 mm × 25 mm microscope slide). The required pattern of holes (usually 2-mm diameter) and troughs (2 mm wide) was cut. The slides were placed between two buffer vessels (barbital buffer) in such a position that holes containing the serum were nearer to the cathode than to the anode. The buffer in the vessels was connected to the end of the slides using a stack of four filter paper strips saturated with buffer. The wells were filled with 3 μg of the serum samples for the seven species of wild ungulates tested and with caprine, ovine and porcine sera. Two individuals were studied for each species of wild ungulate. The power supply for electrophoresis (GD 61D, Sebia, Issy-les-Moulineaux, France) was set at 300 V for 3 h. At the end of the electrophoretic separation, the trough was filled with antiserum against ovine/caprine or porcine IgG depending on the species tested and the results of the Ouchterlony tests.

Results and Discussion

Representative examples of cross-reactivity are illustrated in Figs 1 and 2. The specific antisera for the IgG of domestic species were found to precipitate with the sera of wild species in the double diffusion Ouchterlony test, giving only a single precipitin line. Sera from chamois, Spanish ibex, mouflon, red deer, fallow deer and roe deer cross-reacted against ovine and caprine IgG antisera. Serum from wild boar cross-reacted against porcine IgG antiserum. No positive reaction was found between sera from any of the wild species and the bovine IgG antiserum. The precipitin lines fused with those obtained with domestic species sera IgG (positive controls) and the binding curves showed good parallelism. The complete fusion between the precipitin lines from the wild species and the positive controls was indicative of the presence of common antigenic determinants between IgG from domestic and wild species.

Immunoelectrophoresis showed that IgG from domestic species (goat/sheep or swine) and IgG from wild species (ruminants or wild boar, respectively) were not distinctive in their electrophoretic mobility. It was shown that the IgG from wild species formed precipitin arcs in a manner similar to those of related domestic species.

A study of cross-reactivity is a practical alternative for the demonstration of structural similarities between immunoglobulins of several mammalian species. The presence of protein homologous to human immunoglobulins has been reported in many primate and non-primate species. Neoh et al. (1973) demonstrated the broad homology of the major immunoglobulin classes in the blood plasma from 90 species of mammals and Nash and Mach (1971) identified three immunoglobulin classes homologous to human IgG, IgM and IgA in two species of aquatic mammals. Caprine and ovine IgA were identified by the same method by Pahud and Mach (1970). However, it has proved difficult to demonstrate cross-reactivity of immunoglobulins of non-primate species with human immunoglobulins (Neoh et al., 1973).

The studies of cross-reactivity reported here utilized domestic species IgG antisera and therefore, homology between domestic and wild species was found. The fusion arc of complete identity does confirm the presence of common determinants, but it should not be used as the sole scientific basis for establishing quantitative assays using these antisera. For IgG the possibility of multiple subisotypes which may be expressed in different proportions between species must be taken into consideration. In this context, immunoelectrophoretic analyses were

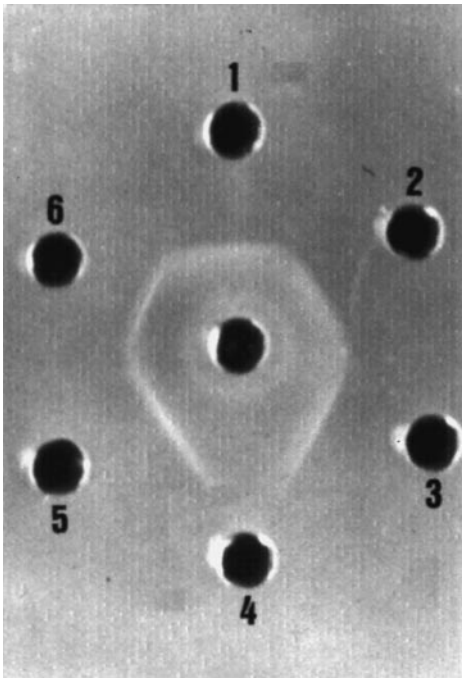

Fig. 1. Ouchterlony test showing reactivity of antiserum to caprine IgG with serum from chamois: 1, goat serum; 2, undiluted serum from chamois; 3, 1 : 2 diluted serum from chamois; 4, distilled water; 5, 1 : 4 diluted serum from chamois; 6, 1 : 8 diluted serum from chamois; centre well, anti-goat IgG.

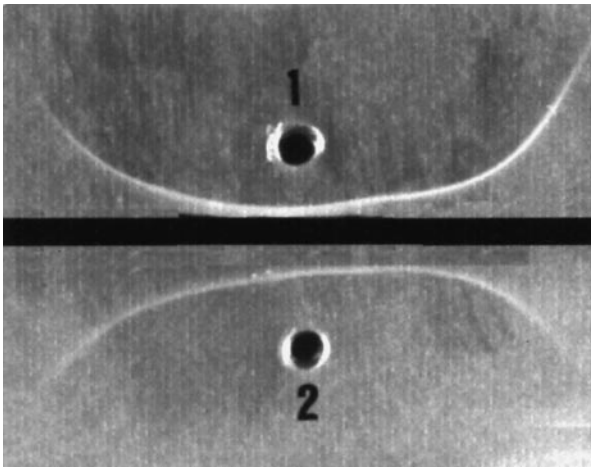

Fig. 2. Immunoelectrophoresis indicating electrophoretic mobility of IgG from wild species: 1, goat serum; 2, serum from chamois.

performed. The cross-reacting antigen from non-domestic species was shown to behave as a poorly migrating γ-globulin.

Serological tests of sera from wild animals have been limited due to the lack of suitable

detection reagents. This has restricted the repertoire of assays for diagnosis as well as determination of species relatedness. Since these antisera are usually not commercially available, they must be prepared for each individual species and the procedure for this is very time-consuming (Kania et al., 1997). Chimeric proteins that combine the IgG binding capacities of Protein A and Protein G have been used to detect immunoglobulins from a wide variety of African wild animal species. They may thus be of great value in seroepidemiological investigations of these animal populations (Kelly et al., 1993).

It is concluded that the anti-goat/sheep IgG and the anti-porcine IgG contained antibody to a protein from non-domestic ruminants and wild boar, respectively, which has antigenic structures in common with ovine/caprine IgG and porcine IgG. This protein is homologous to the domestic species IgG. It seems reasonable to assume that it is easier to demonstrate protein homologous between phylogenetically related domestic and wild ungulate species. This would allow the use of antisera from domestic ungulates for immunological determination in wild ungulates. The possibility of using these cross-reacted antisera is the first step towards immunoglobulin quantification (specifically IgG quantification) in wild ungulate species to establish the immunoglobulin values and thus monitor health and immunological status in wild ungulate populations.

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