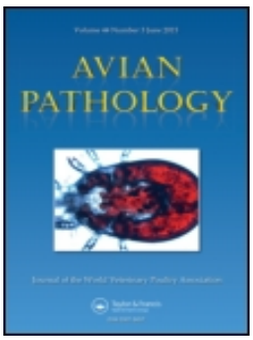

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SHORT COMMUNICATION

Virus serological diagnosis in two avian species based on binding of human C1q to avian antibody

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SUMMARY

Diagnosis of infectious diseases in birds using direct assays such as conventional ELISA or immunofluorescence require antibody directed to IgG of each avian species. In this study, we tested binding of human C1q to different antigen-antibody complexes of two avian species in a sandwich immunofluorescent complement fixation test (S-ICFT). The reaction was as follows: virus (in cells) + decomplemented avian serum + human-C1q + goat anti-human-C1q + fluorescein isothiocyanate rabbit anti-goat-IgG. Positive and negative chicken (order Galliformes) sera against chicken anaemia virus (CAV) and sera against avian pox virus as well as positive and negative sera against chicken pox virus raised in a milvago chimango (order Falconiformes) were used. Positive sera of either avian species demonstrated clear fluorescent staining of infected cells while negative sera did not show any reaction. This demonstrated that both chicken and milvago chimango antibodies were able to bind human C1q. Since both avian species tested belonged to different orders, we believe that antibodies of other avian species will also bind human C1q allowing serological surveys in feral birds through S-ICFT.

INTRODUCTION

Wild living birds are susceptible to many avian pathogens that affect the domestic fowl and they may constitute reservoirs of economically important avian pathogens. Furthermore, specific pathogens of feral bird are becoming increasingly important themselves because of their ecological impact. Therefore, there is a need for reliable serological techniques for epidemiological surveys in avian species.

Although IgG belonging to different avian species exceptionally cross-react, serological diagnosis of infectious diseases in birds using direct assays such as conventional ELISA or immunofluoresence require antibody directed specifically

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to the epitopes of the IgG molecules of each avian species. Producing these antibodies, considering the wide variety of avian species, is difficult and time consuming.

As shown by other authors, human C1q binds to different mammalian antigen-antibody complexes. Based on this binding capability of human C1q, an immunofluorescent complement fixation test (ICFT) has been used to diagnose trypanosomal infections in different mammals (Perie *et al.*, 1975).

In this study, we tested binding of human C1q to antigen-antibody complexes of two avian species using a sandwich immunofluorescent complement fixation test (S-ICFT). The reaction was as follows: virus (in cells) + decomplemented avian antiserum + human-C1q + goat anti-human-C1q + fluorescein isothiocyanate rabbit anti-goat-IgG.

MATERIALS AND METHODS

Virus antigens and biological substrates

Chicken anaemia infected cell source

Slides with chicken anaemia virus (CAV) acetone-fixed infected MDCC-MSB1 cell smears kindly supplied by Dr M. S. McNulty (Department of Agriculture, Northern Ireland, Belfast, UK) were used.

Fowl pox virus infected cell source

Twelve-day-old embryonated chicken eggs (ECE) from a commercial source were inoculated via the chorioallantoic membrane (CAM) with a live embryo-adapted vaccine strain of avian pox virus from a commercial source (Intervet, Boxmeer, The Netherlands). This vaccine had a titre of 7.0 log EID50% according to the producers' specifications. Five days after inoculation, CAMs showing plaque formations were harvested and dissociated using trypsin according to procedures described for avian cell cultures (Schat & Purchase, 1989) to obtain pox virus infected epithelial cells.

Chicken sera

Ten negative control sera were obtained from CAV-negative SPF chickens (Spafas, USA). Ten CAV-antibody positive chicken sera from commercial layer breeders were used. The positiveness of these sera was previously established and published elsewhere (Toro *et al.*, 1994). Chicken sera positive to pox virus antibodies were raised by vaccination of three 20-week-old White Leghorn fowls from a commercial source. These birds were simultaneously immunized by the wing-web method and intramuscularly with the same vaccine strain mentioned above. Sera were collected 15 days after viral exposure.

Wild living bird serum

Positive serum against chicken pox virus was raised in one milvago chimango (order Falconiformes) by inoculation via the wing-web method and intramuscularly. A negative serum from the same species was obtained from another milvago chimango. Both birds were patients in a Releasing Center for Birds of Prey of the Association of Chilean Ornithologists.

Sandwich-immunofluorescent complement fixation test (S-ICFT)

The S-ICFT was conducted mainly as described by Perie *et al.* (1975) with modifications. Virus infected cells were incubated for 35 min with either positive or negative decomplemented avian antisera. Decomplementation of all avian sera was carried out by 56°C for 60 min in a water bath. Avian antisera were diluted 1:200 in PBS before adding them to the cells. All cells were then washed rigorously with PBS pH 7.2 four times. Normal human serum was diluted 1:30 in veronal-buffered-saline pH 7.4 to which Ca^{2+} and Mg^{2+} was added (Perie *et al.*, 1975). This C1q source was added to each cell substrate, and incubated for 60 min in a humid chamber at room temperature. After a washing step, an anti-human-C1q IgG (Sigma, St Louis, USA) diluted 1:300 was then incubated for 30 min at room temperature. After a washing step a goat-anti human C1q (Sigma, St Louis, USA) was then added and incubated for 30 min. Finally, after a washing step, a rabbit anti-goat-IgG conjugated with fluorescein isothianate was then added (1:300), and incubated for 30 min. Reactions were observed in a fluorescent microscope.

Two methods were employed depending on the biological substrate:

1. In the case of acetone fixed MDCC-MSB1 cell smears infected with CAV, the whole reaction was conducted directly on the glass slides. Incubations were carried out in a humid chamber.
2. The S-ICFT reaction with pox infected CAM epithelial cells was conducted in suspension in 4-ml tubes with either chicken sera or milvago chimango sera respectively. Each reactant was added according to the scheme described above. In this case, cells were incubated with each reactant by suspension of the cell pellet in approximately 300 μl of each reactant. After the incubation period, tubes were centrifuged at 1000 *g*. The supernatant was then discarded, and cells were resuspended in PBS and subsequently centrifuged again for a washing step. Washing was repeated three times. After the last washing step, cells were suspended in PBS containing 20% glycine and mounted onto glass slides prior to observation.

RESULTS

The S-ICFT resulted in clear fluorescent staining of MSB1 cells infected with

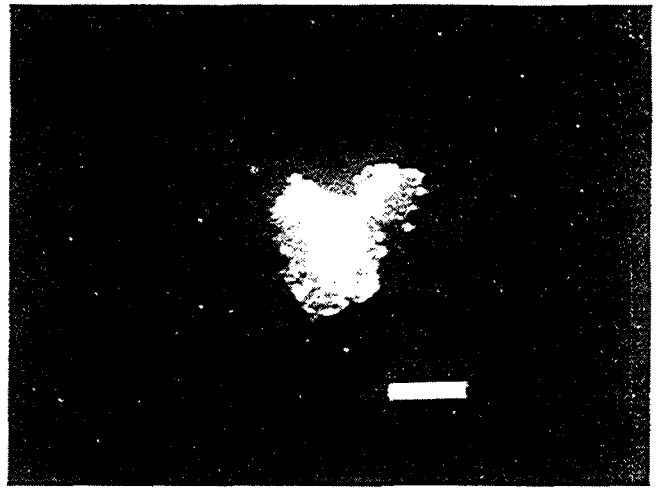

Figure 1. *Fluorescent cell cluster from fowlpox virus infected chorioallantoic membrane after the sandwich-immunofluorescent complement fixation test based on binding of human C1q to specific chicken antibody. Bar = 22 μm.*

CAV when CAV-positive chicken sera were used. SPF chicken CAV negative sera gave no reaction. Some minor background staining was evident.

As shown in Figure 1, single CAM fowlpox virus-infected epithelial cells obtained after trypsinization demonstrated a clearly distinguishable reaction of positive and negative sera against poxvirus from both chicken and milvago chimango. No background staining could be detected when the tube methodology was used.

DISCUSSION

Sera of only two falcon specimens could be included in this study because difficulties in working with feral birds. Despite the fact that more serum samples would have been desirable to obtain more conclusive results, positive sera of both avian species tested in S-ICFT resulted in a positive fluorescent reaction of homologous virus infected cells. Controls did not show any reaction. This demonstrated that both avian antibodies, chicken and milvago chimango, were able to bind human C1q. Antibodies of different mammal species have proved to be capable of binding human C1q (Perie *et al.*, 1975). The results of this study demonstrated that antibodies from two avian species are also able to bind human C1q in S-ICFT assays. Since both avian species tested belonged to different, and phylogenetically distant, avian orders, we believe that antibodies of other avian species will also bind human C1q. Consequently, the S-ICFT employed here may be a promising method for serological surveys in feral birds.

In our experience the selection of an appropriate cell substrate is important. For example, when testing for fowlpox antibodies, infected cell sources, such as

infected skin and/or whole CAMs (data not shown) resulted either in background staining or in non-specifically stained particles that hindered a diagnosis. Therefore, an uncontaminated cell source should be chosen for the best results. Finally, the best results were obtained when the reaction was conducted in tubes. In this case, neither background staining nor non-specifically stained particles were detected.

ACKNOWLEDGEMENTS

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RESUME

Diagnostic sérologique viral chez deux espèces aviaires, basée sur la liaison du C1q humain aux anticorps aviaires

Le diagnostic des maladies infectieuses des volailles utilisant des tests directs telle l'ELISA conventionnelle ou l'immunofluorescence requiert des anticorps dirigés contre les IgG de chaque espèce aviaire. Dans cette étude, il a été étudié la liaison du C1q humain à différents complexes antigène-anticorps de deux espèces aviaires à l'aide d'un test sandwich de fixation du complément par immunofluorescence (S-ICFT). La réaction a été la suivante: virus (dans les cellules) + sérum aviaire décomplémenté + C1q humain + anti C1q humain de chèvre + IgG anti-chèvre de lapin à l'isothiocyanate de fluorescéine. Des sérums de poulets positifs et négatifs (ordre des Galliformes) contre le virus de l'anémie du poulet et des sérums contre le virus de la variole aviaire aussi bien que des sérums positifs et négatifs contre le virus de la variole du poulet obtenus sur Milvago Chimango (ordre des Falconiformes) ont été utilisés. Les sérums positifs de chaque espèce aviaire ont montré une fluorescence nette des cellules alors que les cellules négatives n'ont montré aucune réaction. Ces résultats démontrent qu'à la fois les anticorps de poulets et de Milvago Chimango sont capables de se lier au C1q humain. Bien que ces deux espèces aviaires appartiennent à des ordres différents, il est probable que les anticorps des autres espèces aviaires se lient également au C1q humain permettant des enquêtes sérologiques chez les oiseaux sauvages à l'aide du S-ICFT.

ZUSAMMENFASSUNG

Serologische Virusdiagnostik bei zwei Vogelspezies auf der Grundlage der Bindung von Human-C1q an aviäre Antikörper

Die Diagnose von Infektionskrankheiten bei Vögeln mit Hilfe direkter Nachweisverfahren wie dem konventionellen ELISA oder der Immunfluoreszenz erfordert Antikörper gegen IgG jeder Vogelspezies. In der vorliegenden Studie prüften wir die Bindung von Human-C1q an verschiedene Antigen-Antikörper-Komplexe von zwei Vogelarten in einer Sandwich-Immunfluoreszenz-Komplementbindungsreaktion (S-IKBR). Die Reaktion war folgendermaßen: Virus (in Zellen) + dekomplementiertes Vogelserum + Human-C1q + Ziegenantikörper gegen Human-C1q + Fluorescein-markierte Kaninchenantikörper gegen Ziegen-IgG. Positive und negative Seren vom Huhn (Ordnung Galliformes) gegen Hühneranämievirus (CAV) und Seren gegen Vogelpockenvirus sowie in einem Chimango (Ordnung Falconiformes) produzierte positive und negative Seren gegen Hühnerpockenvirus wurden verwendet. Die positiven Seren beider Vogelarten ergaben eine deutliche Fluoreszenzfärbung infizierter Zellen, während negative Seren keinerlei Reaktion bewirkten. Das zeigte, daß sowohl Hühner- als auch Chimango-Antikörper in der Lage waren, Human-C1q zu binden. Da die beiden untersuchten Vogelspezies zu unterschiedlichen Ordnungen gehörten, glauben wir, daß Antikörper anderer Vogelarten ebenfalls Human-C1q binden und somit serologische Erhebungen bei Wildvögeln durch die S-IKBR zulassen werden.

RESUMEN

Dignóstico serológico de virus de dos especies aviares basado en la unión C1q humano a anticuerpos de aves

El diagnóstico de enfermedades infecciosas aviaros empleando métodos directos como un ELISA convencional o inmunofluorescencia requiere anticuerpos para IgG de la especie aviar en particular. En este estudio comprobamos la unión de C1q humano a diferentes inmunocomplejos de dos especies aviares empleando un método de sandwich de inmunofluorescencia-fijación de complemento (S-ICFT). El método es el siguiente: virus (en células) + suero aviar libre de complemento + C1q humano + suero de cabra anti C1q humano + suero de conejo anti IgG de cabra marcado con fluoresceina. Se emplearon sueros de gallina (orden Galliforme) positivos y negativos para el virus de la anemia de los pollos (CAV) y sueros frente al virus de la viruela aviar en un halcón chimango (orden Falconiforme). Los sueros positivos de ambas especies dieron una reacción de inmunofluorescencia clara en células infectadas mientras que los sueros negativos no. Estos resultados demostraron que tanto el suero de las gallinas como el del halcón fueron capaces de unirse a C1q humano. Puesto que ambas especies aviares pertenecen a órdenes diferentes consideramos que los anticuerpos de otras especies aviares se unirán también a C1q humano con lo que se podrán realizar estudios serológicos en aves salvajes mediante el método S-ICFT.