ELSEVIER

Preventive Veterinary Medicine 21 (1994) 103–106

PREVENTIVE VETERINARY MEDICINE

Short communication

Detection of chicken anemia virus antibodies in four poultry operations in Chile

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Accepted 9 February 1994

Abstract

Broiler and layer chicken sera obtained from four different farms in Chile were tested by indirect immunoperoxidase assay for the presence or absence of antibodies against chicken anemia virus (CAV). Tests were conducted in acetone fixed CAV-infected MDCC-MSB1 cells. From a total of 64 sera, 39 samples representing all four different commercial farms were found to be positive for CAV antibodies. These results demonstrate that CAV is present in some poultry operations in Chile.

1. Introduction

Chicken anemia virus (CAV) was first reported by Yuasa et al. (1979) in Japan. CAV has also been isolated in Germany, England, USA, Canada, Sweden and other countries (McNulty, 1991). Serological surveys conducted in several European, Asian and African countries have also demonstrated that antibody to CAV is widespread. Vertical transmission through the hatching egg is an important means of dissemination (Yuasa and Yoshida, 1983; Chettle et al., 1989). Therefore, countries importing breeders from infected areas could become infected. Little information is available on the presence of CAV in South American countries.

Virus neutralization tests have been used to detect antibody to CAV, but until recently most large-scale serologic studies were undertaken using immunofluorescence (IFA) (Yuasa et al., 1985). Lamichhane et al. (1992) developed an ELISA and an indirect immunoperoxidase assay (IIP), using CAV-infected

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0167-5877/94/$07.00
SSDI 0167-5877(94)00369-T

MDCC-MSB1 cells, and compared them with the IFA test for detecting antibody to CAV. They reported 91–93% agreement between IIP and the results of ELISA and IFA. However, IIP showed the lowest number of positive results indicating that it may lack sensitivity. These authors concluded that the overall average agreement of 84% among the three tests was sufficient to indicate that all three tests were measuring CAV antibody and that any of the three could be used for serological screening applications.

This paper describes the detection by IIP of specific antibodies against CAV in four poultry operations in Chile.

2. Material and methods

2.1. Sera

Specific pathogen free (SPF) embryonated eggs (SPAFAS Inc., USA) were incubated until hatched. Birds were bled at 1 day of age to provide negative control sera.

An anti-CAV monoclonal antibody produced as previously described (McNulty et al., 1990) was used as positive control.

Sixty-four serum samples obtained from four different commercial chicken farms were included in this study. No specific criterion for sample selection was used and no sera were obtained with the specific objective of testing for CAV antibodies originally. In all cases, birds were captured (broilers and pullets) or taken from the cages (layers) by the respective poultry operator in each farm either for routine serology or for other specific aims. Sample 1 included 24 sera obtained from a 10 000 replacement pullet flock of a layer farm. In this case 12 sera were obtained from the same pullet flock at two different ages (10 and 35 days). A second sample was represented by ten sera from a 15 000 broiler flock, aged 45 days, suffering from inclusion body hepatitis (IBH). The third sample included 11 21-day-old broiler (belonging to a 12 000 bird flock) and ten 21-day-old layer pullet samples (belonging to a 10 000 bird flock) from the same company. Broilers of this origin were also suffering from IBH. A fourth sample included nine sera from a 12 000 laying hen flock, aged 55 weeks, submitted to the laboratory for routine serology.

2.2. Indirect immunoperoxidase assay

MDCC-MSB1 cells, a cell line derived from a Marek's disease lymphoma, were kindly supplied by Professor V. von Bülow, Free University of Berlin, Germany. Preparation of CAV infected MDCC-MSB1 cells on multispot slides was carried out in the laboratories of the Veterinary Sciences Division, Department of Agriculture, Belfast, Northern Ireland, as described (McNulty, 1989).

The IIP assay was conducted in the laboratory of Avian Pathology, College of Veterinary Sciences, University of Chile, Santiago, Chile. Briefly, test and nega-

tive control sera were diluted 1:200 in phosphate buffered saline (PBS) pH 7.2 and added in 50-μl volumes to the wells of slides that contained acetone fixed CAV-infected MDCC-MSB1 cells. Uninfected cells from the same line were also used as controls. After 60 min incubation at room temperature, wells were drained employing a vacuum pump and washed by immersion in PBS containing 0.05% Tween 20 with continuous stirring. Wells were then reacted with 50 μl of a rabbit anti-chicken IgG (Nordic Immunological Labs., Tilburg, The Netherlands) for 40 min. After washing, 50 μl of a goat anti-rabbit IgG horseradish peroxidase conjugate (5 μg ml^{-1}) from the same source was added. After 40 min wells were washed again and the enzyme substrate 3,3-diaminobenzidine (DAB) (Sigma Chemical Co., St. Louis, USA) was added to detect the reaction. This substrate was freshly prepared by dissolving one tablet (10 mg of DAB) in 10 ml of PBS and subsequent adding 50 μl of 30% hydrogen peroxide (Merck, Darmstadt, Germany). After 15 min incubation, the reaction was stopped by washing with PBS.

The positive controls were treated in a similar manner but incubation was carried out using a goat anti-mouse IgG conjugated with horseradish peroxidase (Sigma, St. Louis, USA).

3. Results

A positive IIP reaction was characterized by brown staining of the nucleus and cell membrane. Negative control sera yielded no staining.

From 64 samples tested, 39 (61%) serum samples, representing all four commercial poultry farms studied, were positive for CAV antibodies. In Sample 1, 12 replacement layer pullet sera tested, showed negative results at 10 days of age and nine birds from the same group showed positive results at 35 days of age. Sample 2, consisting of ten 5-week-old chicks from the broiler flock suffering from IBH, was positive for CAV antibodies. Sample 3, consisting of 11 21-day-old broilers and ten layer pullets from the same company, showed positive results in all 11 broiler samples tested. Sera from the ten layer pullets were negative for CAV antibodies. Sample 4, consisting of nine sera from laying hens with no clinical signs, was also shown to be positive.

4. Discussion

CAV has been isolated in the USA (Goodwin et al., 1989; McNulty et al., 1989), the UK (Chettle et al., 1989), the Netherlands (De Boer et al., 1989) and Germany (Bülow et al., 1983). CAV readily spreads horizontally among chickens in a group but vertical transmission is also an important means of dissemination (Yuasa and Yoshida, 1983). Until now there was little information on the presence of CAV in South America. Considering many countries, including Chile, import breeding stock from CAV infected areas it was important to determine if CAV is present in Chile.

All field serum samples tested here were obtained from two poultry producing areas in Chile. The detection of antibodies in these sera demonstrates exposure of chicken to CAV. The number of sera tested here does not allow estimation of the prevalence of CAV in this country. Furthermore, an unequivocal diagnosis of CAV infection requires the isolation of the causative agent. Investigations are currently being carried out on these subjects.

Acknowledgment

The international cooperation required to conduct this work was financed by the International Foundation for Science (IFS), Stockholm, Sweden.

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