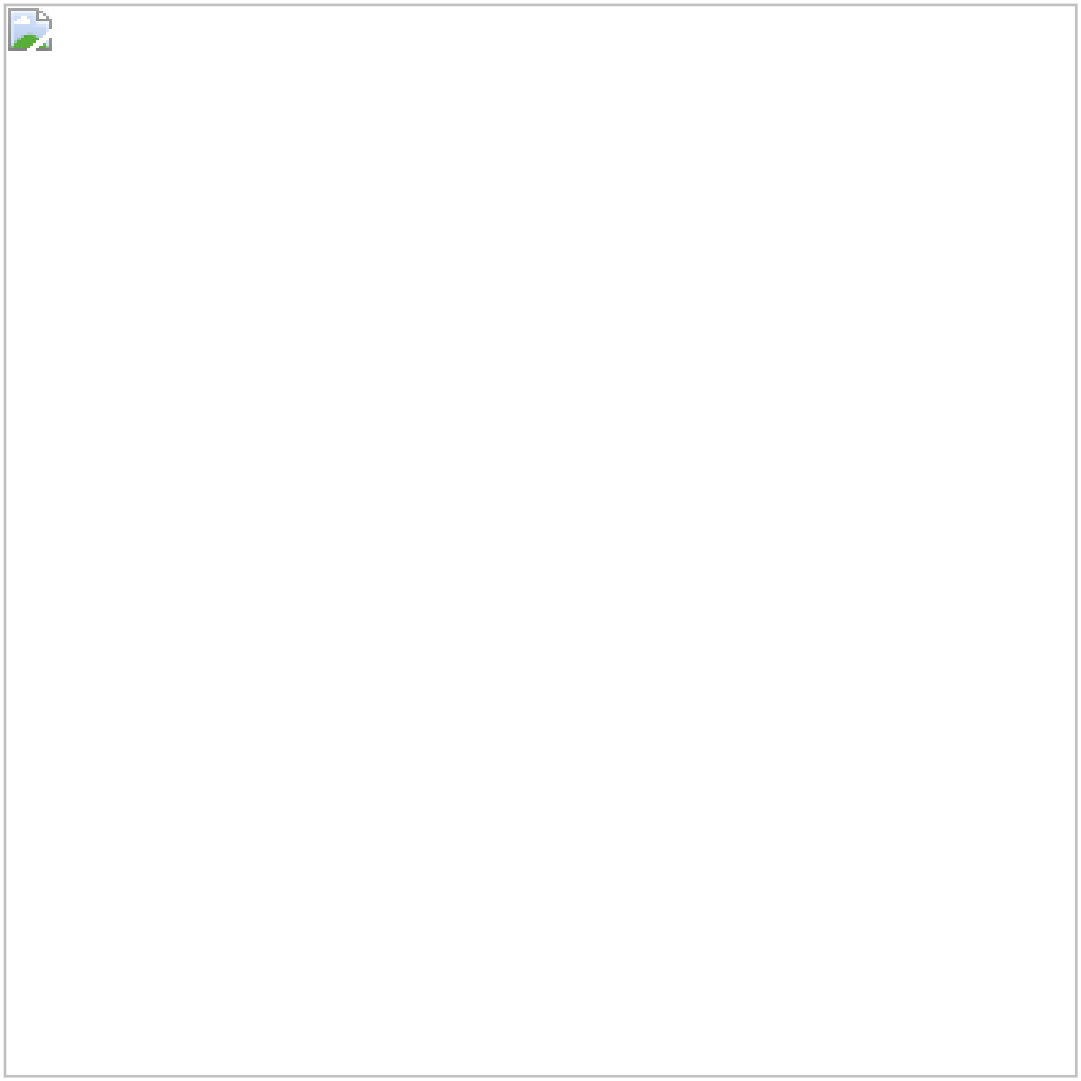

MARBLE SPLEEN DISEASE IN RING-NECKED PHEASANTS: DEMONSTRATION OF AGAR GEL PRECIPITIN ANTIBODY IN PHEASANTS FROM AN INFECTED FLOCK

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MARBLE SPLEEN DISEASE IN RING-NECKED PHEASANTS: DEMONSTRATION OF AGAR GEL PRECIPITIN ANTIBODY IN PHEASANTS FROM AN INFECTED FLOCK

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Abstract: Agar gel precipitin (AGP) antibody was detected in 31 of 283 (11%) pheasant *(Phasianus colchicus)* serum samples using antigen prepared with spleen tissue from a pheasant dying of marble spleen disease (MSD). These same serum samples failed to react with normal pheasant spleen or antigens of chick embryo lethal orphan (CELO) virus or Marek's disease virus.

INTRODUCTION

Marble spleen disease (MSD) is a peracute, fatal disease of ring-necked pheasants in which splenic necrosis and amyloidosis with pulmonary edema are the outstanding findings. The lesions and evidence for possible viral etiology have been reported previously[5].

The agar gel precipitin (AGP) test has been used to demonstrate antibody in many viral diseases, including infectious bronchitis, laryngotracheitis, infectious bursal (Gumboro) disease, and Marek's disease in avian species[1,2,3,4].

Splenic reticulum cells of pheasants dying of MSD contain large amounts of intranuclear virus as evidenced by virions seen with electron microscopy[5]. Splenic antigen, prepared from a case of MSD, was used in this study to test serum from pen-reared pheasants surviving an outbreak with 2% mortality from MSD several months prior to test bleeding.

MATERIALS AND METHODS

Preparations of antigens. MSD antigen was prepared from a spleen of a pheasant with histologically confirmed MSD. A 1:1 tissue suspension was made by grinding 5 g of spleen tissue in 5 ml phosphate buffered saline (pH 7.2). This crude suspension was sonicated for 3 minutes at 70 watts (Sonifier Cell Disruptor, Model W185, Heat Systems-Ultrasonic, Inc., Plainview, Long Island, New York), centrifuged for 15 monutes at 300 x g, and the supernatant fluid removed and retained as antigen for subsequent tests. Control antigen was prepared in a similar manner using normal pheasant spleens.

The preparation of CELO and MD antigens and antiserums used in this study have been described previously[1,4].

Serum samples. Two hundred and eighty-three blood samples were collected from a flock of 9-month-old pheasants by the State of Connecticut Testing Laboratory for pullorum testing. All birds were bled by wing vein puncture. The flock had experienced 2% mortality from MSD 3 to 4 months earlier. Serums, donated by the State Testing Laboratory, were kept frozen at —20 C until the time of testing.

Preparation of agar. A 1% agar (Ionagar No. 2., Colab Laboratories Inc., Chicago Heights, Illinois 60411)

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was prepared in 0.05M phosphate buffer (pH 7.4), with the addition of 8% sodium chloride. This medium was kept refrigerated at 4 C in 5 ml aliquots in screw cap tubes. Slides were prepared by pouring 2 ml of hot agar on conventional 1 x 3 inch glass microslides. After chilling for 2 to 3 hours at 4 C, wells were cut with a commercial die (Gel Punch, Gelman Instrument Co., Ann Arbor, Michigan). Wells were filled using a plain microhematocrit tube with an attached small rubber bulb. The outer wells received serum and antigen was deposited in the central well. All slides were incubated at 25 C in a moist chamber and observed for precipitin bands after 24 to 48 hours.

RESULTS

Of 283 serum samples tested against the MSD spleen antigen, 31 (11%) responded with a single precipitin line during the 24 to 48 hour incubation period (Fig. 1). These same 31 samples failed to show bands when tested against normal pheasant spleen antigen. When MSD positive serums were tested against CELO and Marek's disease antigens there were no cross reactions while CELO and Marek's disease antiserums developed the expected AGP bands (Fig. 2).

DISCUSSION

The finding of AGP antibody of spleen homogenate heavily laden with virus antigen in 11% of 283 pheasant serums, while not conclusive, further substantiates a viral etiology for MSD. When 53 serum samples from another pheasant flock were tested with the same spleen antigen, 4 (7.5%) were found to give precipitin bands. No information on mortality rate due to MSD was available for this flock however.

Virus particles found in the nuclei of splenic reticuloendothelial cells in pheasants dying of MSD were morphologically similar to both adenovirus and herpesvirus groups. Since MSD spleen antigen positive serum had no cross reactivity with either CELO or Marek's disease virus antigens (representatives

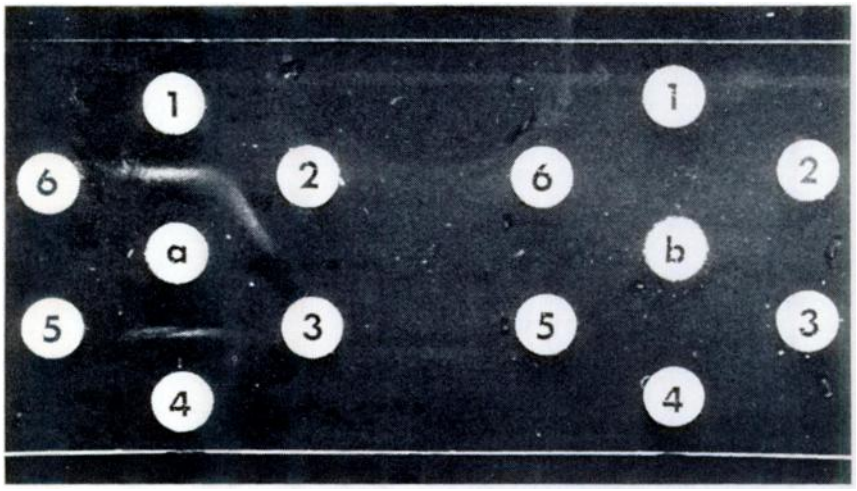

FIGURE 1. Central wells (a) and (b) contain MSD spleen and normal spleen suspensions, respectively. Outer wells in both series contain the same serums: wells 1, 2, and 4, MSD spleen antigen positive pheasant serum; well 6, negative pheasant serum; well 3, Marek's disease positive serum; and well 5, CELO virus positive serum.

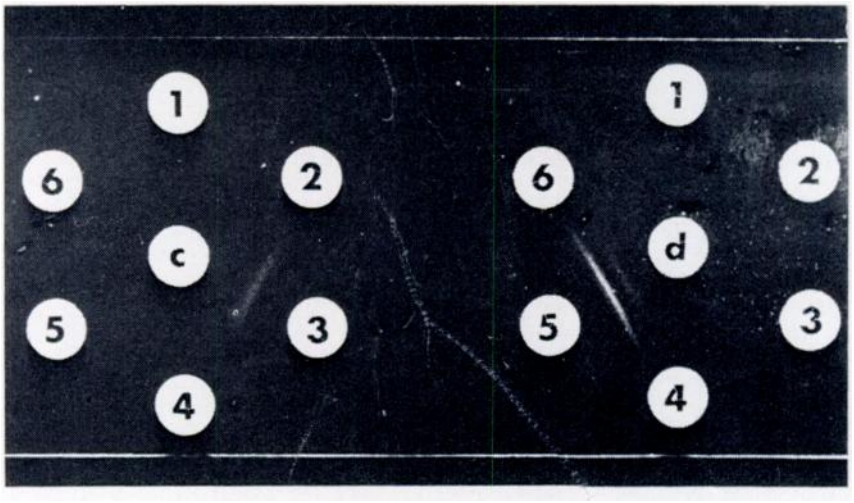

FIGURE 2. Central wells (c) and (d) contain Merek's disease virus antigen and CELO virus antigen, respectively. Outer wells have some serums described in Figure 1.

from adenovirus and herpesvirus groups), it is unlikely that either of these agents are responsible for MSD.

While birds clinically affected with MSD die suddenly, presuably from pulmonary edema, the presence of amyloid in the spleen, in addition to a large quantity of virus, would indicate a rather lengthy prodromal period. The finding of AGP antibody in a flock with MSD mortality would support the hypothesis that birds may be infected with the MSD agent without subsequent mortality. Whether this antibody is protective cannot be stated at present since serum from pheasants dying of MSD have not been tested. These findings would indicate that more pheasants are exposed to the agent of MSD than is apparent from birds showing clinical signs and postmortem lesions.

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