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Case Report—

Inclusion Body Disease of Cranes: A Serological Follow-up to the 1978 Die-off

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SUMMARY

A herpesvirus was isolated from captive cranes involved in a 1978 die-off. Neutralizing antibody to this virus was detected in this captive population as early as 1975 and consistently thereafter through 1979. Exposure to the virus evidently occurred at least 2½ years before the die-off, without causing any mortality diagnosed as being caused by inclusion body disease of cranes (IBDC). Overcrowding and environmental conditions in 1978 may have contributed to the deaths of certain species of cranes in one area and not in another. Mortality ratios and serological data suggest that crane species vary in their response to IBDC virus.

INTRODUCTION

In March 1978, a die-off of captive cranes occurred at the International Crane Foundation (ICF) near Baraboo, Wisconsin. All of the deaths occurred in a 4-ha nonbreeder area (Fig. 1) containing a mixed population of 50 cranes. Eighteen dead cranes were submitted to the National Wildlife Health Laboratory (NWHL) for study, comprising seven sandhill (*Grus canadensis*), five Manchurian (*G. japonensis*), four Stanley (*Anthropoides paradisea*), and two hooded cranes (*G. monacha*). The disease was tentatively called inclusion body disease of cranes (IBDC). A previously undescribed herpesvirus was reported to have been isolated from cranes involved in this die-off (1).

To determine the status of this disease for ICF management purposes, we collected sera and cloacal swabs from all cranes in 1978 and 1979. To determine whether the virus associated with IBDC was present before 1978, we tested sera obtained from routine health checks in 1975 and 1976 for antibody.

MATERIALS AND METHODS

Sera and cloacal swabs were taken from all cranes housed at the ICF in the fall of 1975, 1976, and 1978 and in the spring and fall of 1979.

Cloacal swabs were placed in a viral transport media consisting of Hanks' balanced salt solution (BSS), 0.5% gelatin, 100 μg gentamicin, 1,500 IU penicillin, 1,500 μg streptomycin, and 50 IU mycostatin/ml. These swabs were stored at -70 C until tested.

Monolayers of duck embryo cells were prepared in 24- and 96-well plates (Linbro) by standard laboratory methods (3) using minimum essential media with 10% gamma-globulin-free fetal bovine sera. The 24-well plates were inoculated with 0.2 ml per well of the cloacal swab fluid. The plates were then incubated at 37 C for 7 days and examined every other day for cytopathic effect.

Harvested sera were stored at -15 C, diluted 1:4 in Hanks' BSS, and heat-inactivated for 30 min at 56 C before testing. A tissue-culture-neutralization test

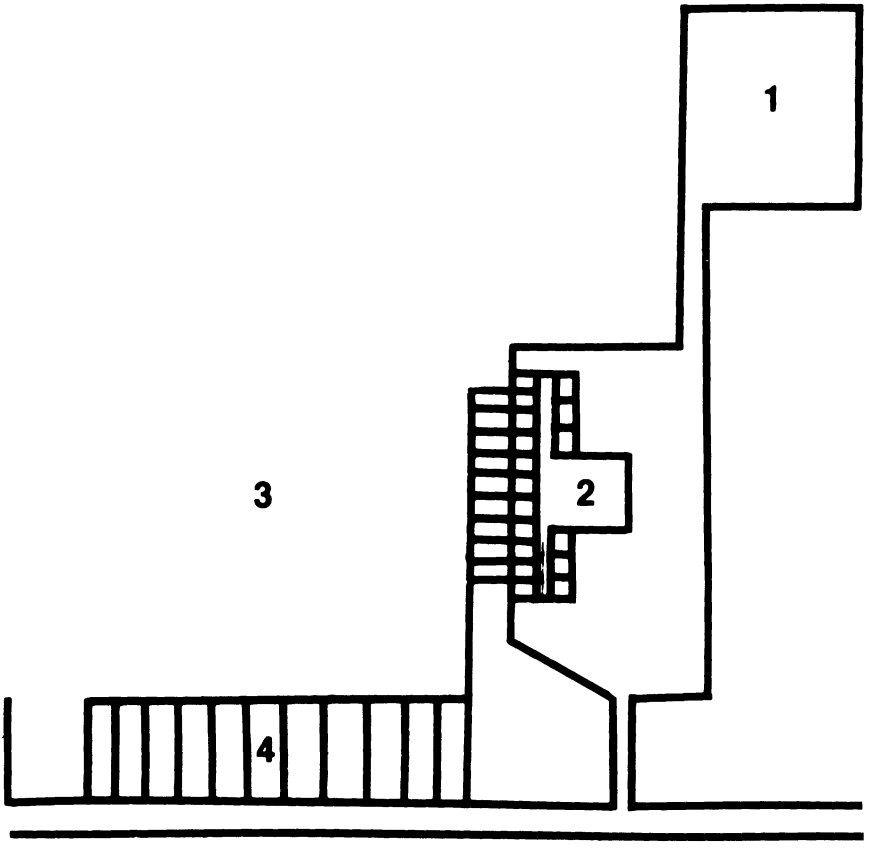

Fig. 1. Grounds of the International Crane Foundation, 1978. Key to Areas: 1. Breeder, 2. Horsebarn, 3. Nonbreeder, 4. Roadside.

Table 1. Effect of inclusion body disease in terms of mortality and antibody response of various species of cranes housed in the nonbreeder and adjacent areas of the International Crane Foundation during 1978.

Crane	Nonbreeder field		Horse barn		Roadside pen	
	Mortality	Antibody-positive	Mortality	Antibody-positive	Mortality	Antibody-positive
Stanley *(Anthropoides paradisea)*	4/4[A]	0/0	-[B]	-	-	-
Sandhill *(Grus canadensis)*	7/8	0/1	-	-	0/10	2/10
Manchurian *(G. japonensis)*	5/8	3/3	-	-	-	-
Hooded *(G. monacha)*	2/4	2/2	0/11	10/11	-	-
Sarus *(G. antigone)*	0/4	4/4	-	-	-	-
Common *(G. grus)*	0/3	3/3	-	-	0/10	7/10
White-naped *(G. vipio)*	0/2	0/2	-	-	-	-
Demoiselle *(A. virgo)*	0/6	1/6	-	-	-	-
Brolga *(G. rubicunda)*	0/2	0/2	-	-	-	-

[A] No. positive/no. tested.

[B] (-) None present.

incorporating constant-virus varying-serum dilution in 24- and 96-well plates was used. The test dose of IBDC virus was made by dilution in Hanks' BSS with 0.5% gelatin to yield $10^{1.3}$ to $10^{1.7}$ median tissue culture infectious doses calculated by the Kärber method (2). All crane sera were screened at 1:4 for neutralizing antibody. Any sera found to be antibody-positive were tested further by using twofold serial dilutions to determine endpoints. The endpoints were expressed as the reciprocal of the highest serum dilution that inhibited cytopathic effect. To avoid the effect of any nonspecific inhibitors in lower serum dilutions, only crane sera with titers $\geq$ 1:8 were considered significant. A fourfold change in titer from one sampling period to another was considered to be a significant rise or decline.

RESULTS AND DISCUSSION

A total of 46 cranes, representing 9 of the 14 species present, were antibody-positive over the 5-year period. Twenty-three cranes had detectable antibody before the die-off. This suggests that exposure to the virus occurred at least 2½ years before the die-off without causing any mortality that could be diagnosed as IBDC-induced. The remaining 23/46 antibody-positive cranes were detected in 1978–1979. These were cranes that survived the die-off and newly acquired cranes that may have been exposed before or after their arrival at ICF.

Antibody titers to the virus were maintained consistently in most of the 46 positive cranes, with 10 having titer for only 1 year, 20 for up to 2 years, 6 for up to 4 years, and 10 for up to 5 years.

In 1975, antibody to IBDC virus was detected in cranes housed in the horsebarn and breeder areas. There were no conversions from known antibody-negative to antibody-positive between the falls of 1975 and 1976. In 1976, many antibody-positive cranes in the breeder areas were moved to the nonbreeder and roadside areas. Since no health check was performed in 1977, it was impossible to determine the antibody status of new cranes added between the falls of 1976 and 1978. In 1978 and 1979, antibody-positive cranes were located in the horsebarn, nonbreeder, and roadside areas.

Fourfold changes in titer were noted in 27/46 cranes. Most of the rises (11/13) occurred between the falls of 1976 and 1978. The declines were somewhat more evenly distributed throughout the 5-year period. Four cranes experienced both a rise and fall in titer. No evidence of significant change in titer was noted in 18/46, with one antibody-positive crane available for testing only once during the 5-year period.

During March 1978, 50 cranes representing nine species were housed in the 4-ha nonbreeder area. Snow cover and limited

availability of water and shelter restricted crane movement to a relatively small site. Crowded conditions produced hygiene problems, increased interaction between species of cranes, and increased the chances for disease transmission. These conditions did not exist before 1978 and may have been factors contributing to the die-off. Since 11 of the 13 known fourfold rises occurred between the falls of 1976 and 1978, it appears that more cranes experienced contact with virus during this period than any other. Perhaps the conditions during the die-off increased the chances of contracting the virus.

Exposure to cold, wet conditions, and direct contact with infected cranes could have contributed to the mortality of a given species in the nonbreeder area and not in other areas (Table 1). Sandhill and hooded cranes experienced mortality in the nonbreeder area. In the horsebarn or roadside area, these two species showed evidence of exposure to the virus by detectable antibody levels but experienced no mortality. This suggests that the virus is most lethal when accompanied by conditions similar to those in the nonbreeder area in March 1978.

The species surviving the 1978 die-off in the nonbreeder area can be divided into four fairly distinct groups in terms of mortality experienced and antibody response. The Stanley and sandhill cranes are characterized by high mortality and no antibody response in the single survivor. All of the sandhill and two of the Stanley cranes had no detectable antibody to IBDC in 1976. The two remaining Stanley cranes were added between 1976 and 1978 and therefore were not tested before death. This group was made up of adults and appears to be the most susceptible, since all but one of its members died.

In another group, represented by the Manchurian and hooded cranes, there was limited mortality (62 and 50%, respectively), and all survivors had detectable antibody. Most of these cranes were added to the nonbreeder area between 1976 and 1978. Two cranes that were present in 1976 had no antibody to IBDC. This group was made up of immature cranes and seems to be somewhat less susceptible than the Stanley and sandhill cranes.

A third group, characterized as having experienced no mortality and yet responding to the virus by antibody production, includes the sarus (*G. antigone*) and common cranes (*G. grus*). Although the sarus cranes were added between 1976 and 1978 and were not sampled before the die-off, all had antibody in the fall of 1978. The common cranes demonstrated pre-existing antibody in 1976 and may have survived the die-off owing to protective antibody levels.

The last group, represented by the white-naped (*G. vipio*), demoiselle (*A. virgo*), and brolga (*G. rubincunda*) cranes, is characterized by no mortality and antibody response in only 1/10. Perhaps these species were more resistant to the virus or more tolerant of the conditions existing in March 1978, or their habits prevented exposure to the virus.

The herpesvirus associated with IBDC was not isolated from any of the cloacal swabs. Perhaps the virus is shed intermittently, at very low titers, or not at all via the cloaca. Since we were able to isolate IBDC virus from the tissues of dead cranes, our cell-culture system appears to be sensitive to the virus.

The results of this serological follow-up indicate that exposure to IBDC occurred at ICF in 1975 or earlier. A comparison of antibody levels and mortality ratios suggests that the crane host reaction varies by species. Differential crane mortality among ICF areas appears to be due to overcrowding and the unfavorable environmental conditions of March 1978. Until additional studies on virus pathogenesis and animal susceptibility are completed, the reasons for survival of certain species remains speculative.

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