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PERSISTENCE OF INCLUSION BODY DISEASE OF CRANES VIRUS

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ABSTRACT: Persistence of inclusion body disease of cranes virus (IBDCV) was determined by monitoring virus shedding, serum antibody and in vitro cultivation of trigeminal ganglia from cranes. Samples were collected from captive cranes surviving the outbreak in 1978 and from cranes inoculated with the virus. Tissues and fluids from eggs of cranes that survived the outbreak were also tested for virus. Latent IBDCV was found in the trigeminal ganglion of one crane that was exposed to the virus in 1978. Spontaneous or induced (cyclophosphamide and dexamethasone) reactivation of viral shedding was not detected in any cranes tested. Five of six experimentally inoculated cranes died with lesions of an inclusion body disease, but virus was isolated from only three of them. One crane shed detectable levels of IBDCV prior to death. The surviving crane developed a transient antibody response without evidence of viral shedding, after five exposures to the virus. A latent infection was not detected in this crane. Serum antibody titers of cranes that survived the outbreak declined from 1980–1982. No virus was isolated from the eggs. Although IBDCV is capable of persisting in a latent form in the trigeminal ganglia of cranes, the low frequency of viral shedding suggests that this virus may be only a sporadic problem.

INTRODUCTION

Inclusion body disease of cranes virus is a herpesvirus that was isolated in 1978 during an outbreak of a fatal disease at the International Crane Foundation (ICF) in Baraboo, Wisconsin (Docherty and Henning, 1980). A herpesvirus designated crane herpesvirus was isolated from captive cranes in Austria in 1975 (Burtcher and Grunberg, 1979). The relationship of these two viruses has not been determined (Kaleta et al., 1980). Because seven of the world’s 15 species of cranes are rare or endangered (Archibald et al., 1981), zoos and other specialized breeding centers are attempting to prevent their extinction by artificial propagation. If IBDCV is shed by apparently healthy persistently infected individuals, as are most herpesviruses, the virus could threaten the existence of crane breeding programs (Larue, 1981). There is also a potential for vertical transmission of the virus through eggs. The international exchange of cranes, their eggs and offspring, and reintroduction of captive cranes to their native habitats could facilitate transmission of IBDCV to susceptible cranes and result in epornitics in both captive and wild crane populations. This study was initiated 30 mo after the outbreak at the ICF and was undertaken to determine the persistence, shedding and potential for vertical transmission of IBDCV in captive cranes. Surveillance of cranes that survived the outbreak included monitoring virus shedding and antibody status, the use of sentinel cranes and attempts to chemically induce activation of virus shedding from cranes having antibody to IBDCV. Since several herpesviruses are known to persist in the trigeminal ganglion, this tissue was examined by explant cultivation for evidence of latent IBDCV. In an attempt to produce a persistent infection, cranes without antibody to IBDCV were inoculated with the virus. Eggs were tested for the presence of vertically transmitted virus.

MATERIALS AND METHODS

Stock virus

The virus was obtained from Wallace Hansen, National Wildlife Health Laboratory, Madison, Wisconsin 53711, USA. The virus had
undergone one passage in a muscovy duck and five passages in mallard embryo fibroblasts. The working stock, used for animal inoculations and virus neutralization tests, received an additional passage in muscovy embryo fibroblasts. This stock virus contained $5 \times 10^4$ plaque forming units (PFU) per ml in chicken embryo fibroblast cell culture.

**Cell cultures**

Chicken eggs were obtained from Sunnyside Hatchery, Oregon, Wisconsin 53575, USA and incubated at 39 C in a humidified incubator for 10 days. Primary embryo fibroblasts were prepared by a modification of the method of Rovozo and Burke (1973). The cells were mechanically mixed for 20–40 min in a final solution of 0.025% trypsin. The cells were washed three times in PBS and recovered by low speed (200 g) centrifugation. All tests involving chicken embryo fibroblasts (CEF) were conducted at 40 C and in 5% CO$_2$.

The growth medium consisted of filter sterilized M199/Earle's salts supplemented with 10% heat-inactivated (56 C for 30 min) gamma or newborn calf serum, 0.18 mg/ml tricine, 0.1 mg/ml gentamicin, 100 IU/ml penicillin and 100 mg/ml streptomycin. The pH was adjusted to 7.2 with 1 M NaHCO$_3$. The maintenance medium was the growth medium with calf serum reduced to 5%. The overlay medium consisted of an equal volume of a 2x maintenance medium and 1% type 3 agarose.

**Sample collection and test procedures**

Cecal and oropharyngeal samples were collected with sterile dacron swabs and transported to the laboratory in 2 ml of growth medium supplemented with 0.005 M HEPES buffer. Samples were centrifuged at 1,500 g at 4 C for 10 min to pellet debris. The clarified supernatant was stored at −75 C for 1 wk to 6 mo prior to testing. Samples were tested by inoculating 0.1 or 0.2 ml of the swab supernatant onto CEF suspensions or monolayers respectively, in 24-well Linbro plates. The inoculum remained with the cell suspensions for 24 hr and was then replaced with 3 ml of agarose overlay medium. The inoculum was removed from cell monolayer cultures after 1 hr, and the monolayers were then washed with PBS and covered with the agarose overlay medium.

Liver, spleen, brain, lung, gonad, kidney and bone marrow for IBDCV isolation were collected in Whirlpak bags and stored at −75 C. The tissues were prepared as 10% w/v suspensions in growth medium. Serial 10-fold dilutions of 0.05 ml of tissue suspension were inoculated in quadruplicate into 96-well Falcon plates and 0.15 ml containing $1.86 \times 10^4$ CEF cells were added to each well. Plates were incubated for 6 days. Typical cytopathic effect (cell rounding and clumping) appeared 3–4 days postinoculation (PI) and was maximal at 5–6 days PI. Isolation of IBDCV was confirmed by neutralization of the inoculum with an equal volume of a 1:10 dilution of a convalescent crane anti-IBDCV serum (serum neutralization titer of 1:128), incubated for 1 hr at 40 C prior to addition of the CEF cells.

Blood was collected from cranes by jugular venipuncture with 20-ga needles and a syringe or a Vacutainer collection tube. Whole blood for viremia titration was mixed with an equal volume of growth medium and stored at −75 C 10–40 days prior to testing. Viremia testing was carried out as for tissue suspensions. Serum antibody to IBDCV was quantified by a micro-neutralization titration using the constant virus-varying serum method, established by Docherty and Henning (1980), with the following modifications. The test dose of IBDCV was 10 TCID$_{50}$ in CEF cells. The sera and virus were dispensed in 0.025 ml aliquots and incubated at 40 C for 1 hr and 0.15 ml of 1.86 $\times 10^4$ CEF cells were then added. Titer of 1:8 or greater were considered positive.

Trigeminal ganglia for in vivo cultivation (explants) were minced in PBS to pieces of less than 1 mm diameter. Tissue pieces were washed in PBS and collected in a minimum volume of PBS with a 5 ml syringe. Up to 20 pieces per well were placed in six-well Linbro plates with 1.5 ml of growth medium. Crane embryo explants were prepared without washing by aspirating tissues (liver, spleen and trigeminal ganglia) or whole embryos, together with 2 ml of PBS through a 22-ga needle into a 5 ml syringe. A 0.2-ml aliquot of each embryo suspension was placed in three wells of a six-well plate. Tissue pieces were undisturbed for $\frac{1}{2}$–1 hr before 2 ml of growth medium were gently added to each well. Explant medium was replaced twice a week; aliquots of the used medium were stored at −75 C. Explants were incubated at 40 C for a minimum of 45 days. The cells were collected with the final aliquot of medium. All aliquots were tested in quadruplicate for IBDCV in 196-well Linbro plates by adding 0.05 ml of inoculum and 0.15 ml of 1.86 $\times 10^4$ CEF cells to each well. Virus isolation was confirmed by neutralization with the reference IBDCV antisera.

Liver, spleen, thymus, bone marrow, lung, heart, kidney and adrenal for histological study were fixed in 10% buffered formalin. Brain and
TABLE 1. Experimental attempts to reactivate inclusion body disease of cranes virus infection in cranes having antibody.

<table>
<thead>
<tr>
<th>Experimental number</th>
<th>Species</th>
<th>Antibody* titer</th>
<th>Sex</th>
<th>Type*</th>
<th>Dose (mg/kg)</th>
<th>No. days admin.</th>
<th>Virus isolation</th>
<th>Tri-geminal ganglion explants</th>
<th>Days after last injection</th>
<th>Time to death</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Common crane</td>
<td>8</td>
<td>m</td>
<td>CY</td>
<td>50</td>
<td>1</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>10</td>
</tr>
<tr>
<td>9</td>
<td>Sarus crane</td>
<td>8 f</td>
<td></td>
<td>CY</td>
<td>25</td>
<td>4</td>
<td>no</td>
<td>no</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Sandhill crane</td>
<td>32 m</td>
<td></td>
<td>CY</td>
<td>25</td>
<td>4</td>
<td>no</td>
<td>no</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Common crane</td>
<td>64 f</td>
<td></td>
<td>CY</td>
<td>25</td>
<td>4</td>
<td>no</td>
<td>yes</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Common crane</td>
<td>8 f</td>
<td></td>
<td>DX</td>
<td>0.2</td>
<td>5</td>
<td>no</td>
<td>no</td>
<td>14*</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Common crane</td>
<td>16 m</td>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>no</td>
<td>no</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

* Reciprocal of neutralizing antibody titers to IBDCV.
* CY = cyclophosphamide, DX = dexamethasone.
* Injection repeated after a 2-wk rest period.
* Euthanized.

Trigeminal ganglia were fixed in formalin ammonium bromide (Luna, 1968). Fixed tissue was embedded in paraffin, cut at 6 μm and stained with hematoxylin and eosin.

**Experimental animals**

Cranes were obtained from a captive flock maintained at the ICF. The following species of cranes, identified by their vernacular name in usage at the ICF, were used in these studies: common crane (Grus grus (Linnaeus)), red-crowned crane (Grus japonensis (Muller)), white-naped crane (Grus vipio Pallas), sandhill crane (Grus canadensis (Linnaeus)), hooded crane (Grus monachus Temminck), Sarus crane (Grus antigone (Linnaeus)), Brolga crane (Grus rubicundus (Perry)), one hybrid Sarus × Brolga crane (Grus antigone × G. rubicunda), black-crowned crane (B. pavonina (Linnaeus)), gray-crowned crane (B. eneornis pavonina regulorum), Demoiselle crane (Anthropoides virgo (Linnaeus)), and Stanley crane (Ardea paradisea (Lichtenstein)). The species, age and sex of the cranes used in these studies were determined by the availability of cranes permanently rejected from the breeding program at the ICF. Ten days prior to inoculation with virus or injection with immunosuppressants, the cranes were individually acclimated in an isolation room at the University of Wisconsin—Madison Charmany Farms. During this period oropharyngeal and cloacal swab samples were obtained on alternate days. Blood was drawn during the first week to determine antibody status. Since the outbreak of IBDCV, the ICF has maintained all cranes with antibody or known exposure to IBDCV in a permanent quarantine area. Cranes housed in this area were made available for sampling.

**Experiments**

To induce viral shedding by potential latent IBDCV carriers, adult cranes with IBDCV antibody were immunosuppressed by daily intraperitoneal injections of cyclophosphamide (CY) or an intramuscular injection of dexamethasone (DX) as outlined in Table 1. Crane 10 was given two series of CY injections. Crane 7 received no immunosuppressant. Serum was collected 6–7 days after the last injection to monitor antibody levels. Oropharyngeal and cloacal swabs were obtained daily for 10 days and every second day thereafter. At necropsy, trigeminal ganglia were collected for explantation and tissues were collected for histological study.

In an attempt to establish a latent infection, six cranes without IBDCV neutralizing antibody were inoculated with the stock virus as outlined in Table 2. The common crane juvenile (crane 1) was raised for 4 wk with his parents, one of which had a titer to IBDCV. Prior to inoculation with IBDCV, crane 1 was immunosuppressed with a single 250-mg intraperitoneal injection of cyclophosphamide. Daily swab samples were collected from crane 1 for 7 days and tested for virus. After inoculation with IBDCV, daily oropharyngeal and cloacal swabs were obtained from all six cranes. Whole blood was obtained daily from three of the birds (cranes 1, 3, and 6) to test for the presence of a viremia. Serum was tested for antibody at
TABLE 2. Results of experimental inoculation of inclusion body disease of cranes virus into cranes free of specific antibody.

<table>
<thead>
<tr>
<th>Experimental number</th>
<th>Species</th>
<th>Age*</th>
<th>Sex</th>
<th>Virus</th>
<th>Dose (PFU)</th>
<th>Route*</th>
<th>Blood</th>
<th>Swabs</th>
<th>Liver and spleen homogenates</th>
<th>Trigeminal ganglion explants</th>
<th>Time to death (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Common crane</td>
<td>juv</td>
<td>m</td>
<td></td>
<td>15,000</td>
<td>IM</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>ND</td>
<td>7.5</td>
</tr>
<tr>
<td>3</td>
<td>Red-crowned</td>
<td>juv</td>
<td>m</td>
<td></td>
<td>2,000</td>
<td>IC</td>
<td>no</td>
<td>yes</td>
<td>ND</td>
<td>no</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>Red-crowned</td>
<td>ad</td>
<td>m</td>
<td></td>
<td>1,000</td>
<td>IC</td>
<td>ND</td>
<td>no</td>
<td>yes</td>
<td>ND</td>
<td>7*</td>
</tr>
<tr>
<td>5</td>
<td>Red-crowned</td>
<td>ad</td>
<td>m</td>
<td></td>
<td>1,500</td>
<td>PO</td>
<td>ND</td>
<td>no</td>
<td>no</td>
<td>ND</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>White-naped</td>
<td>ad f</td>
<td></td>
<td></td>
<td>1,000</td>
<td>IN</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>ND</td>
<td>45*</td>
</tr>
<tr>
<td></td>
<td>crane</td>
<td></td>
<td></td>
<td></td>
<td>2,500,1,000</td>
<td>IN, CON</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>500</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3,000</td>
<td>IT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>50,000,000</td>
<td>IN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Sandhill crane</td>
<td>juv</td>
<td>m</td>
<td></td>
<td>3,500</td>
<td>IN</td>
<td>ND</td>
<td>yes</td>
<td>yes</td>
<td>ND</td>
<td>8</td>
</tr>
</tbody>
</table>

* juv = juvenile, ad = adult
* IM = intramuscular, IC = intracoecal, PO = oral, IN = intranasal, CON = conjunctival, IT = intratracheal.
* ND = not done
* Euthanized.
* Stock IBDCV concentrated to 10^7 by polyethylene glycol dialysis.

least once during the first 8 days PI. Every second day, starting at day 10 PI, serum was obtained for antibody determination and oropharyngeal and cloacal swabs were collected for virus isolation attempts. If neither virus nor antibody was detected by day 21 PI, the bird was assumed not to have been infected and was reinoculated with IBDCV. On reinoculation, the dose of virus was increased and in one case (crane 6) the route of inoculation was varied several times. At necropsy, tissues for virus isolation and microscopic examination were obtained. Trigeminal ganglia from crane 6 were explanted.

Within the ICF quarantine area cranes both with and without IBDCV antibody were sampled periodically for 27 mo to detect virus shedding. The species and antibody status of the cranes tested are presented in Table 3. Swabs generally were taken once per month. This was increased to once every week or 2 wk during the breeding season and reduced to once every 2 mo during the last 11 mo of the study. Cloacal swabs were always taken and oropharyngeal swabs were obtained intermittently.

Sentinel cranes were used as another means to determine if IBDCV was shed by cranes in the quarantine area. Two juvenile cranes without antibody to IBDCV were established in November 1981 and housed separately in pens within a barn in the quarantine area. In March 1982 they were placed together in an outside pen. Oropharyngeal and cloacal swabs were obtained once or twice a week and the cranes were bled at least once a month during this period. One sentinel, a Stanley crane, died of unrelated causes after 6 mo, and the other, a sandhill crane, was removed after 8 mo and challenged experimentally with IBDCV (crane 12).

The entire flock of captive cranes at the ICF was sampled once a year for determination of serum neutralizing antibody and virus isolation was attempted from cloacal swabs.

Eggs were collected from cranes housed in the quarantine area at ICF in order to test for vertically transmitted virus. The eggs were incubated for a minimum of 8 days (range 8 to 27 days) before tissues and fluids were collected. The species tested and the antibody status of the cranes involved are given in Table 5. Thirty eggs from four species and one hybrid were tested in 1981 and 40 eggs from five species were tested in 1982. Allantoic-amniotic fluid

<table>
<thead>
<tr>
<th>Species</th>
<th>Number tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antibody positive</td>
</tr>
<tr>
<td>Hooded crane</td>
<td>4</td>
</tr>
<tr>
<td>Sarus crane</td>
<td>2</td>
</tr>
<tr>
<td>White-naped crane</td>
<td>0</td>
</tr>
<tr>
<td>Common crane</td>
<td>2</td>
</tr>
<tr>
<td>Sandhill crane</td>
<td>1</td>
</tr>
<tr>
<td>Red-crowned crane</td>
<td>1</td>
</tr>
<tr>
<td>Brolga crane</td>
<td>0</td>
</tr>
<tr>
<td>Gray-crowned crane</td>
<td>1</td>
</tr>
<tr>
<td>Black-crowned crane</td>
<td>0</td>
</tr>
</tbody>
</table>

*Serum neutralizing antibody of inclusion body disease of cranes virus at a titer of 1:8 or greater.

(AAF) was collected from infertile eggs. AAF and tissue explants were obtained from fertile eggs. Trigeminal ganglia, liver and spleen samples were explanted as separate tissues from six embryos 15 days or older. In 1982, 10% whole embryo suspensions were also tested.

RESULTS

Reactivated IBDCV, confirmed by neutralization, was isolated from the trigeminal ganglion explant from crane 11 (Table 1) starting 25 days after explantation. Virus was isolated twice weekly until 56 days after explantation. The final isolation occurred on day 70 and the explant was terminated on day 89.

Of the cranes receiving CY, crane 2 was euthanized in extremis 10 days after inoculation and cranes 9 and 11 died 7 and 8 days after the last CY inoculation. Crane 10 was re inoculated with cyclophosphamide 2 wk after the first attempt at immunosuppression and died 8 days later. Crane 14 was euthanized 2 wk after the last DX inoculation. The liver and spleen of crane 10 were submitted to Dr. J. Price of the National Wildlife Health Laboratory, Madison, Wisconsin for bacteriologic examination. Escherichia coli was isolated and the histologic findings were consistent with a septicemia. Reactivation of IBDCV infection and virus shedding did not occur in cranes treated with CY or DX.

Of the cranes inoculated with IBDCV, only one (crane 6) developed specific antibody (Table 2). This crane produced a peak titer of 1:16 14 days after its fifth inoculation and the titer declined steadily to undetectable levels by day 40 (Fig. 1). No virus was isolated from tissue suspensions or from supernatants of any tissue explant cultures of this crane.

Five of six cranes inoculated with IBDCV died. The mortality pattern is given in Table 2. Virus was reisolated from the livers and spleens of three birds (cranes 3, 4, and 12). The titers were $10^{3.9}$ ml in

TABLE 4. Reciprocal serum neutralizing antibody titers to inclusion body disease of cranes virus in individual cranes in the International Crane Foundation (ICF) quarantine area over a 3-yr period.

<table>
<thead>
<tr>
<th>Species</th>
<th>ICF ident number</th>
<th>1980</th>
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<th>1982</th>
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<tbody>
<tr>
<td>Gray-crowned</td>
<td>10</td>
<td>8</td>
<td></td>
<td></td>
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<tr>
<td>Demoiselle</td>
<td>3</td>
<td>8</td>
<td>8</td>
<td>ND</td>
</tr>
<tr>
<td>Sandhill</td>
<td>12</td>
<td>8</td>
<td>8</td>
<td>ND</td>
</tr>
<tr>
<td>Sarus</td>
<td>10</td>
<td>16</td>
<td>32</td>
<td>ND</td>
</tr>
<tr>
<td>Common crane</td>
<td>1</td>
<td>64</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4b</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
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<td>16</td>
<td>8</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
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<td>32</td>
<td>16</td>
<td>8</td>
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<tr>
<td></td>
<td>13</td>
<td>32</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td>Hooded crane</td>
<td>4</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
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<td></td>
<td>ND</td>
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<td></td>
<td>16</td>
<td>32</td>
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<td></td>
</tr>
<tr>
<td>Red-crowned</td>
<td>10</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = negative serum neutralization titer (<1:8).

b Considered suspect.

ND = not done.
TABLE 5. Crane eggs from the International Crane Foundation (ICF) quarantine area sampled for inclusion body disease of cranes virus.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of eggs sampled per breeding season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICF female no (antibody status)</td>
</tr>
<tr>
<td>Demoiselle crane</td>
<td>3 (+)</td>
</tr>
<tr>
<td></td>
<td>8 (-)</td>
</tr>
<tr>
<td>Sandhill crane</td>
<td>1 (-)</td>
</tr>
<tr>
<td></td>
<td>2 (-)</td>
</tr>
<tr>
<td>Sarus crane</td>
<td>11 (+)*</td>
</tr>
<tr>
<td>Sarus crane x Brolga</td>
<td>11 (+)*</td>
</tr>
<tr>
<td>White-naped crane</td>
<td>13 (-)</td>
</tr>
<tr>
<td>Common crane</td>
<td>4 (+)*</td>
</tr>
<tr>
<td></td>
<td>7 (-)</td>
</tr>
<tr>
<td></td>
<td>9 (+)</td>
</tr>
<tr>
<td>Red-crowned crane</td>
<td>13 (-)</td>
</tr>
</tbody>
</table>

+ = titer >1:8, - = titer <1:8.

* Known mating with at least one antibody-positive male.

The spleen suspensions and $10^{2.5}$/ml in the liver suspensions. No virus was isolated from any other tissues tested. No virus was detected in the daily blood samples collected from cranes 1, 3, or 6.

The livers and spleens of all experimentally inoculated cranes that died were enlarged and turgid. Cream-colored foci, 1–2 mm in diameter, were visible over the entire surface and extended throughout the parenchyma of the liver and spleen of all cranes except for crane 5. All other organs were grossly normal. Histologically, foci of coagulative necrosis were observed in the liver, spleen and thymus of all experimentally inoculated cranes that died. Intraneurular inclusion bodies were infrequently observed around the periphery of the necrotic foci in the liver and spleen.

Virus was isolated from oropharyngeal and cloacal swab samples of only one of the five cranes that died after inoculation with IBDCV. The amount of virus recovered per ml of swab media from crane 12 is shown in Figure 2.

No virus isolations were obtained from oropharyngeal and cloacal swabs taken over a 27-mo period from cranes in the quarantine area. No virus was isolated from the swab samples of the sentinels. The susceptibility of one of these sentinels (crane 12) to IBDCV was established by experimental challenge with resulting mortality (Table 2).

The antibody titers of positive cranes monitored at ICF from 1980 to 1982 are given in Table 4. Neutralizing antibody to IBDCV was found in 23 of 121 cranes (seven species) in 1980, in 14 of 131 cranes (four species) in 1981, and eight of 137 cranes (two species) in 1982. A four-fold increase in antibody titer occurred in common crane 13 but all other cranes maintained or had a decline in antibody titer. The sentinel cranes did not develop neutralizing antibody to IBDCV.

No virus was isolated from AAF, embryo tissue explants or embryo tissue suspensions of 70 crane eggs.

DISCUSSION

Inclusion body disease of cranes virus was isolated from the trigeminal ganglion...
explant culture of a common crane with antibody to IBDCV. The 25-day incubation period required prior to detection of virus in this explant suggested that this was a true latent infection. The site of IBDCV latency is similar to herpesviruses such as bovine herpesvirus 1 (Krogman and McAdaragh, 1982), herpes simplex virus 1 (Montplaisir, 1979), pseudorabies virus (Beran et al., 1980), and duck plague virus (Burgess et al., 1979).

Cyclophosphamide and DX treatment of cranes with antibody to IBDCV did not result in detectable reactivation of IBDCV excretion. Reactivation of an avian herpesvirus with CY has been reported only for the pigeon herpesvirus (Vindevogel et al., 1980). Although DX is used to recrudesce herpesvirus infections in mammals (Montplaisir, 1979; Krogman and McAdaragh, 1982), no information is available on its effectiveness in reactivating herpesvirus infections of birds.

A white-naped crane produced a transient antibody titer after surviving five exposures to the virus, but no virus was isolated from the trigeminal ganglia explants. Sequential inoculation of four low doses of IBDCV probably immunized this bird, but neutralizing antibody remained undetectable. The high dose of virus given as a terminal challenge produced a transient antibody titer that appeared to be an anamnestic response based on the rapid appearance and early disappearance. This response may also represent resistance to IBDCV in white-naped cranes. Further studies will be necessary to determine the susceptibility of this and the other crane species to IBDCV infection and mortality. The failure to identify a latent infection in the trigeminal ganglia of this crane may have been due to the inefficiency of tissue explants (Hoorn and Tyrell, 1969; Nesburn et al., 1980) or to the immune response restricting the number of latently infected cells (Walz et al., 1976).

Of the cranes experimentally exposed to IBDCV, only one crane, a sandhill, excreted detectable amounts of infectious IBDCV prior to death. Although this crane was inoculated with the virus by the nasal route, virus recovered from oropharyngeal swabs was minimal. The amount of virus shed from the cloaca increased rapidly, however. This implicates infected feces as the probable source of infection for cranes. Spontaneous reactivation and shedding of IBDCV was not detected in the cloacal and oropharyngeal swab samples from antibody-positive cranes or from cranes exposed to IBDCV in the quarantine area at ICF. However, the sampling frequency of the quarantined cranes was insufficient to detect a very intermittent shedding pattern. Detection of IBDCV is difficult in the available avian cell systems. Thus the virus may be present in the swab samples, but at a level below the sensitivity threshold for the CEF cells.

The general decline in antibody titers and prevalence of seropositive cranes indicated that the ICF cranes were not exposed continuously to endogenous or exogenous virus, and concurs with the lack of IBDCV isolations from swab samples. Although antibody titers to herpesviruses
may remain elevated for several months, more often the titers tend to wane (Openshaw et al., 1981). Elevation of antibody titer has been used to monitor reactivation of bovine herpesvirus 1 (Pastoret et al., 1979), pigeon herpesvirus (Vindevogel and Pastoret, 1980) and herpes simplex virus 1 (Openshaw et al., 1981). Serum neutralizing antibody to IBDCV started to decline in 1981, and seven birds that were previously antibody positive became antibody negative. The antibody titer of common crane 13 increased four-fold between 1981 and 1982 but this may have been due to variability inherent in the test system. Antibody titers in other cranes never increased more than two-fold and none of the antibody-negative cranes developed antibody. The sentinel cranes did not produce antibody nor shed detectable amounts of virus, further suggesting that reactivation of IBDCV did not occur in the flock at ICF.

Inclusion body disease of cranes virus does not appear to be vertically transmitted. Virus was not isolated from any of the eggs tested. Further studies will be necessary to determine if vertical transmission occurs in the species that were not tested or if vertical transmission can occur during acute infection.

Attempts to produce persistent infection in cranes by experimental inoculation with IBDCV were unsuccessful. However, the resulting mortality provided an opportunity to examine the pathogenic effects of this virus in experimentally infected cranes. The cell propagated passage 7 IBDCV reproduced a fatal inclusion body disease in a sandhill and two red-crowned cranes, the species from which the original virus isolations were made (Docherty and Henning, 1980). The virus was isolated from the livers and spleens of these cranes. A similar inclusion body disease was produced in another red-crowned crane and in a common crane, but virus was not isolated. This red-crowned crane did not have gross lesions, but necrosis and inclusion bodies were identified histologically in the liver and spleen. The inability to isolate IBDCV from tissues of known infected cranes shows that identification of IBDCV as a cause of mortality in cranes is difficult. The detection of inclusion bodies in the liver and spleen is necessary to presumptively differentiate this disease from bacterial infections that may also produce necrosis in these organs.

Although IBDCV is capable of establishing a persistent infection in the trigeminal ganglia of cranes, the low frequency of detectable virus shedding suggests that IBDCV may be only a sporadic problem. However, the apparent difficulty in isolating this virus from swab samples and tissues of infected cranes makes detection of this disease troublesome and suggests that it will be easy to underestimate the frequency of IBDCV shedding and to overlook IBDCV as a mortality factor in cranes. The prevalence of IBDCV in wild and captive crane populations is unknown. Careful management of cranes in captivity to minimize cross contamination between individuals and testing of existing cranes and new introductions for evidence of IBDCV is recommended. Wild populations of cranes should also be tested for IBDCV. Reintroduction of captive bred cranes into wild flocks should be carefully considered in view of the significant mortality that may occur when susceptible cranes are exposed to this virus.

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LITERATURE CITED


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