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Research Article—

Inclusion Body Disease and Columbid Alphaherpesvirus 1 Infection in a Eurasian Eagle-Owl (*Bubo bubo*) of Central Italy

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SUMMARY. Hepatosplenitis or inclusion body disease is a fatal disease in owls caused by Columbid alphaherpesvirus 1 (CoHV-1). A few old case reports describe it worldwide. In Italy, knowledge regarding virus circulation and disease development is lacking. Four Eurasian eagle-owls (*Bubo bubo*), two adults and two juveniles, were submitted for postmortem examination showing aspecific clinical signs a few hours before death. Grossly disseminated petechial hemorrhages on serosal surfaces ($n = 4$), hepatic and splenic necrosis ($n = 3$), bilateral and symmetric necrosis of pharyngeal tonsils ($n = 2$), and diffuse and bilateral dark-red discoloration and firmness in lungs ($n = 2$) were seen. Tissues were sampled for histology, bacteriology, molecular testing, and transmission electron microscopy (TEM). On histology, disseminated petechial hemorrhages ($n = 4$) and necrosis of liver ($n = 3$) and spleen ($n = 3$) were seen, as well as lympho-histiocytic interstitial pneumonia and meningoencephalitis ($n = 2$). Intranuclear inclusion bodies (INIBs) were detected in one case. A panherpesviral PCR led to positive results in one case, identified in sequencing as CoHV-1. On TEM, intranuclear and intracytoplasmic virions with herpesviral morphology were seen in the same case. For the other three birds, the lack of PCR positivity, INIBs, and TEM detection could be linked to a possible reduction of the virus to undetectable levels. Death possibly occurred secondarily to bacterial infections, supposedly established during the acute phase of CoHV-1 infection. This paper reports the presence of CoHV-1in Italy and the development of a fatal form of the disease in a Eurasian eagle-owl.

RESUMEN. Enfermedad con cuerpos de inclusión e infección por Alfaherpesvirus de las columbiformes 1 en un búho real euroasiático (*Bubo bubo*) del centro de Italia.

La hepatoesplenitis o enfermedad con cuerpos de inclusión es una enfermedad mortal en los búhos causada por el Alfaherpesvirus de las columbiformes 1 (CoHV-1). Algunos informes de casos antiguos lo describen en todo el mundo. En Italia, falta conocimiento sobre la circulación del virus y el desarrollo de enfermedades. Cuatro búhos reales euroasiáticos (*Bubo bubo*), dos adultos y dos juveniles, fueron sometidos a examen post mortem mostrando signos clínicos específicos unas horas antes de la muerte. Se observaron hemorragias petequiales muy diseminadas en las superficies serosas ($n = 4$), necrosis hepática y esplénica ($n = 2$), necrosis bilateral y simétrica de las tonsilas faríngeas ($n = 2$) y decoloración difusa y bilateral de color rojo oscuro y firmeza en los pulmones ($n = 2$). Se recolectaron muestras de tejidos para histología, bacteriología, pruebas moleculares y microscopía electrónica de transmisión (TEM). En la histología se observaron hemorragias petequiales diseminadas ($n = 4$) y necrosis de hígado ($n = 3$) y bazo ($n = 3$), así como neumonía intersticial linfohistiocítica y meningoencefalitis ($n = 2$). En un caso se detectaron cuerpos de inclusión intranucleares (INIB). Un método de PCR panherpesviral arrojó resultados positivos en un caso, identificado en la secuenciación como CoHV-1. Mediante microscopía electrónica de transmisión, se observaron viriones intranucleares e intracitoplasmáticos con morfología herpesviral en el mismo caso. Para las otras tres aves, la falta de positividad de PCR, la ausencia de cuerpos de inclusión intranucleares y de detección por microscopía electrónica de transmisión podría estar relacionada con una posible reducción del virus a niveles no detectables. La muerte posiblemente ocurrió de forma secundaria a infecciones bacterianas, posiblemente establecidas durante la fase aguda de la infección por el CoHV-1. Este artículo reporta la presencia de CoHV-1 en Italia y el desarrollo de una forma mortal de la enfermedad en un búho real euroasiático.

Key words: *Bubo bubo*, Columbid alphaherpesvirus 1, Eurasian eagle-owls, inclusion body disease

Abbreviations: CoHV-1 = Columbid alphaherpesvirus 1; IBD = inclusion body disease; INICB = intranuclear inclusion body; RT-PCR = reverse transcription polymerase chain reaction; TEM = transmission electron microscopy

INTRODUCTION

Hepatosplenitis, inclusion body disease (IBD), or hepatosplenitis infectiosa strigum is a long-known fatal disease in owls. Initially described in the United States (1) as a new viral disease of owls, further reports followed in Austria (2), Germany (3), South Africa (4), Canada (5), and Australia (6). In all cases, characteristic foci of pharyngeal, hepatic, and splenic necrosis were the main reported findings, in association with eosinophilic intranuclear inclusion bodies (INICBs) with and without optically empty halo and chromatin margination. Based on the histology and the morphologic features of the INICBs, the disease was thought to be caused by a species-specific owl herpesvirus (Strigid herpesvirus) (7). In addition, a similar disease has been described in diurnal raptors (1).

Historically, herpesviruses from owls, falcons, and pigeons have been described as distinct viruses. However, in recent years phylogenetic studies linked most or all cases of the herpesviral IBD in owl, falcons, and pigeons to the Columbid alphaherpesvirus 1 (CoHV-1) (8,9). CoHV-1, the agent of Smadel's disease (10), is known to cause foci of necrosis and inclusion body formation in

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parenchymatous organs as well as nonsuppurative meningoencephalomyelitis. The source of infection has not been fully elucidated, but, as most of the reported cases in raptors have been likely associated with the ingestion of infected pigeons (11), this has been suggested as the main source of infection.

In Italy, knowledge regarding CoHV-1 circulation and IBD development is lacking. The purpose of this case report is to describe the findings and the diagnostic procedures applied in a case of CoHV-1 infection in Eurasian eagle-owls (*Bubo bubo*) in central Italy.

MATERIALS AND METHODS

Bird species and categories. Between the end of April and beginning of May 2021, in a period of 3 wk, four (n = 4) Eurasian eagle-owls (*Bubo bubo*), two adults (one male and one female) and two juveniles (6 wk and 7 wk old; one male and one female) captive kept for falconry activities in the same enclosure of the same facility in Umbria (central Italy) were submitted to the Department of Veterinary Medicine at the University of Perugia (Italy). Adults were fed with pigeons, chickens, and other small birds. Juveniles were fed by the adult owls.

Pathology. For postmortem investigation, a complete recording of clinical signs was performed in association with subsequent full necropsy and tissue sampling. During necropsy, different organs (liver, spleen, brain, heart and pericardium, air sacs, lungs, thyroid and parathyroid, kidneys, adrenal glands, gonads, uterus, proventriculus and ventriculus, small and large intestine, bursa of Fabricius, and skin) were sampled. For histological examination, the samples were fixed in 10% neutral buffered formalin, and routine processing and paraffin embedding were carried out. Subsequently, 3–5 μm sections were stained with hematoxylin and eosin. Liver, lung, and spleen tissue sampled during necropsies from all cases were collected for bacteriological examination and molecular testing.

Bacteriology. From all the cases, lung, liver, and spleen were cultured for aerobic bacteria on blood agar, MacConkey agar, and mannitol salt agar, and incubated at 37° C in 5%–10% CO_2 for 24–48 h.

Virology, sequencing, and phylogenetic analysis. For molecular testing, liver, lung, brain, and spleen were collected at necropsy and stored at −80° C. For the PCR assay, DNA was extracted from 50 mg of ground tissue from liver, spleen, and brain samples using a commercial kit (Quick DNA Miniprep kit, Zymo Research) following the manufacturer's instructions. The DNA extracted was quantified with a NanoDrop2000® spectrophotometer, and its integrity was examined with electrophoresis (1% agarose gel) and subjected to nested PCR amplification using degenerate primers targeting the highly conserved herpesviral DNA polymerase gene, following the protocol of Vandevanter *et al.* (12). The PCR products were analyzed by electrophoresis on 1.5% agarose gel, stained with ethidium bromide (0.5 μg/ml), and visualized under UV light.

Reverse transcription polymerase chain reaction (RT-PCR) for West Nile virus and influenza virus was also performed on brain tissue samples of all cases at an external laboratory (Istituto Zooprofilattico Sperimentale "Togo Rosati," Umbria, Italy) following standard diagnostic protocols.

To investigate the possible transmission of a herpesviral agent through ingestion of infected pigeons, spinal cord and brain tissue of the domestic pigeons (*Columba livia* var. *domestica*) used for animal consumption and submitted from the owls' owner were tested using the PCR protocol described above (12).

PCR products were purified using Wizard SV Gel and PCR Clean-up System (Promega Corporation, Madison, WI) in accordance with the manufacturer's recommended protocol and subjected to direct sequencing. The final sequences were submitted to BLAST analysis to verify specific amplification. Nucleotide alignments were constructed with MUSCLE implemented in MEGA 11 software. Phylogenetic analysis of the 145 nucleotide-long sequences was performed by the neighbor-joining method with the Kimura two-parameter model and 1000 bootstrap replicates by MEGA 11 software (13).

Electron microscopy. For three cases (Cases 1, 2, 3), formalin-fixed liver samples (1 mm^3) were immersed in 2.5 % glutaraldehyde at room temperature for 8 h. For one case (Case 4), pieces of 1 mm^3 of liver tissue collected during necropsy were directly fixed in 2.5% glutaraldehyde at room temperature for 2 h. All samples were subsequently postfixed in 2% osmium tetroxide, dehydrated in graded ethanol (up to absolute ethanol), and embedded in epoxy resin (Epon 812). Twenty-nanometer-thick sections were mounted on 200-mesh copper grids, stained with uranyl acetate and lead citrate, and examined in a Philips EM 208 transmission electron microscope connected to a digital camera for picture acquisition (Centro di Microscopia Elettronica, University of Perugia, Italy).

RESULTS

Bird species and categories. The first owl, Case 1, an adult female, was submitted on April 20, just after death. The clinical history reported yearly seasonal recurrent conjunctivitis with final severe apathy and continuous eyes blinking. Based on postmortem findings, the remaining birds ($n = 3$) of the same enclosure were tentatively treated with acyclovir (333 mg/kg orally; twice a day) for 7 days. Case 2, a 6-wk-old owl, was submitted to necropsy 1 wk after the first case (April 27). Before death, it showed apathy, increased respiratory rate, open mouth breathing with head on the ground, and widespread wings. The bird died suddenly 2 h after the first acyclovir administration. On May 7, Case 3, a 7-wk-old owl, from the same brood was presented for postmortem investigation after being found suddenly dead (7 days after treatment suspension). A few hours later, Case 4, an adult male was found dead in the same enclosure. Summary of bird data is available in Table 1.

Pathology. For Case 1, the postmortem investigation led to the identification of small multifocal foci affecting 60% of the liver and 90% of the spleen (Fig. 1A; spleen not shown) and bilateral and symmetrical yellow and irregular areas in correspondence with the palatine tonsils (coagulative necrosis) (Fig. 1B). On the serosal surfaces, air sacs, and peri- and epicardium, petechial hemorrhages and diffuse opacity were found (Fig. 1C–D). In the gastrointestinal tract, oesophageal, proventricular, and intestinal ulcers were evident along with hemorrhages. Multifocal petechiae were also visible on the intestinal serosa. In the ovary, yellow nodules (0.2 cm in diameter) with sharply defined margins were present. No additional macroscopic findings were seen in other organs. For Case 2, the macroscopic findings consisted of disseminated petechial hemorrhages on the abdomen wall, air sacs, epicardium, intestinal serosa, diffusely pale liver, and diffusely edematous lungs with firm consistency and wet cut surface. The spleen showed multifocal pinpoint white foci (follicular hyperplasia), while the bursa of Fabricius was diffusely atrophic. For Case 3, macroscopic findings were consistent with petechial hemorrhages in the oral cavity and skin, as well as in subcutis, pectoral muscles, and serous surfaces (hemorrhagic purpura). Additionally, necrosis and hemorrhages of the proventriculus were noticed as well as multifocal foci of hepatic and splenic necrosis. Also, in this case, the bursa of Fabricius was diffusely reduced in size. Similarly to Case 3, Case 4 showed petechial hemorrhages in the oral cavity, skin and subcutis, pectoral muscles and serous surfaces, and multifocal foci of hepatic and splenic necrosis. A summary of macroscopic pathology data is available in Table 2.

For microscopic lesions, in Case 1 multifocal foci of coagulative necrosis were observed in the palatine tonsils, liver, spleen, thyroid,

Table 1. Summary data for the examined Eurasian eagle-owls (*Bubo bubo*).

Case	Sex	Age	Clinical signs
1	Female	Adult	Seasonal recurrent conjunctivitis; apathy and eyes blinking
2	Female	6-wk-old	Apathy, increased respiratory rate, open mouth breathing, widespread wings
3	Male	7-wk-old	Sudden death
4	Male	Adult	Sudden death

salpinx, and ovary, in association with disseminated hemorrhages and occasional fibrin thrombi. In the ovary, multifocal granulomas with epithelioid and multinucleated giant cells (foreign body type) surrounded areas of necrosis associated with proteinaceous vitelline material and large bacterial colonies of coccoid basophilic (1–2 μm) bacteria. Associated with the necrotic foci were occasional eosinophilic to amphophilic intranuclear inclusion bodies (INICBs), characterized by margination of the chromatin with or without a peripheral optically empty halo, strongly suggestive of alphaherpesvirinae. For Case 2, findings were characterized as marked dissociation of the hepatic plates in the liver, nonsuppurative multifocal meningitis, and arteritis, the last affecting medium caliber arteries, in association with hemorrhages and multifocal thrombosis. In the lungs, diffuse and moderate thickening of the interstitium was associated with oedema and mononuclear-cell infiltrates. In Case 3, microscopic findings were hepatic and splenic necrosis, often associated with intralesional bacterial colonies. In the spleen, bacteria were localized inside reticular cells of the splenic ellipsoids. Petechiae and multiorgan disseminated thrombosis (heart, lung, liver, spleen) and a mild multifocal nonsuppurative meningitis with thrombosis were also seen. Finally, for Case 4, hepatic and splenic necrosis were, as for Case 3, associated with intralesional bacterial colonies, petechiae, and disseminated thrombosis (heart, lung, liver, spleen). No INICBs were seen in Case 2, 3, and 4. Figure 2 shows major histological lesions. A summary of the major histological findings is available in Table 3.

Bacteriology. Bacterial cultures gave negative results in Case 1 and Case 2. Numerous colonies of bacteria morphologically consistent *E. coli* and *Enterococcus* spp. were isolated from the liver and spleen of Case 3 and Case 4, respectively.

Virology, sequencing, and phylogenetic analysis. The PCR products of the expected amplicon size for the panherpesviral nested PCR were found from the liver and spleen of Case 1. No amplification has been detected in organs from Case 2, 3, and 4. RT-PCR for West Nile virus and influenza virus performed on all cases gave negative results. Tissues from the domestic pigeons tested did not give PCR products.

Sequences obtained from the liver and spleen of Case 1 were compared with publicly available sequences in the GenBank database (National Center for Biotechnology Information) and submitted to GenBank under Accession No. OK079093.

The phylogenetic tree (Fig. 3) showed distinct monophyletic clades between CoHV-1 and other avian herpesviruses. Gallid alphaherpesvirus-2 forms a separate clade, representing an outgroup.

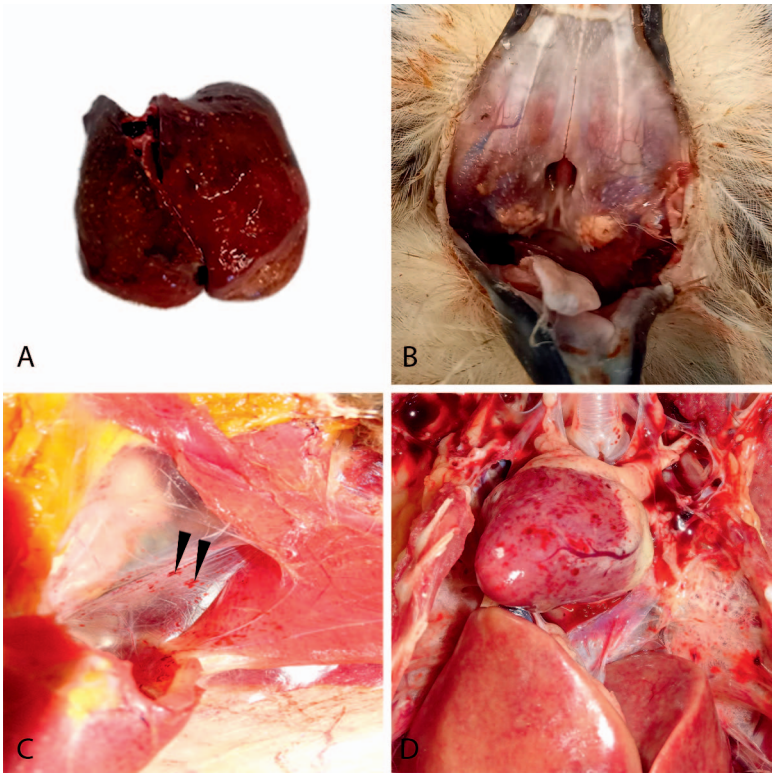

Fig. 1. Macroscopic findings of the examined Eurasian eagle-owls (*Bubo bubo*). (A) Liver. Disseminated small foci of hepatocellular necrosis. (B) Palate and pharynx. Bilateral and symmetric areas of lymphoid tissue necrosis. (C) Air sac. Multifocal petechial hemorrhages. (D) Heart and liver. Multifocal petechial hemorrhages in the epicardium and pinpoint foci of hepatic necrosis in the liver.

Table 2. Summary of major macroscopic findings in the examined Eurasian eagle-owls (*Bubo bubo*).

Organ	Lesion	No. cases/4
Skin	Petechial hemorrhages	3/4
Palatine/pharyngeal tonsils	Coagulative necrosis	1/4
Serosal surfaces	Petechial hemorrhages	4/4
Liver	Pinpoint foci necrosis	3/4
Spleen	Pinpoint foci necrosis	3/4
Lung	Interstitial pneumonia	1/4
Gastrointestinal tract	Hemorrhages and necrosis	2/4
Bursa of Fabricius	Atrophy	2/4

Our sequence showed a high degree of nucleotide identity within the group of CoHV-1 strains, independently from the host species and the geographical origin.

Electron microscopy. On transmission electron microscopy (TEM), Case 1 showed the presence of intranuclear and intracytoplasmic spherical virions with morphologic features compatible with the herpesvirus. The structure consisted in an inner electron-dense core of condensed DNA and a peripheral icosahedral more electrodense capsid (Fig. 4). No virions were visible in the other examined birds (Case 2, 3, and 4).

DISCUSSION

The inclusion body disease (IBD) caused by the CoHV-1 has been reported as a sporadic cause of necrosis and inclusion bodies formation in parenchymatous organs in owls in Europe (2). Little is known about the morbidity and mortality cause by this virus, with susceptibility and fatal outcomes in various species of birds of prey (5,7). In Italy, knowledge regarding CoHV-1 circulation and IBD development is lacking.

To the authors' knowledge, the case reported here is the first signal of CoHV-1 infection in captive raptors in Italy. Birds submitted for postmortem examination consisted of four Eurasian eagle-owls (*Bubo bubo*), a species reported in the literature as susceptible to CoHV-1 infection (7).

Regarding the clinical presentation, a status of general and aspecific illness was reported in these cases, quickly progressing to sudden death. From the pathology point of view, classic reported lesions of CoHV-1, including pharyngeal, hepatic, and splenic necrosis, as well as petechial hemorrhages in various organs, were seen (5). As for other herpesviruses of mammalian species (e.g., elephant endotheliotropic herpesviruses) (17), these lesions suggest a wide tropism for different parenchymatous organs and a marked tropism for the endothelium. Additionally, nonsuppurative meningitis was seen in one case, as reported in CoHV-1 infection of pigeons, as a hallmark lesion of Smadel's disease (10) and in diurnal raptors (18) as a rare finding.

Regarding the identification of the virus, the classic appearance of the DNA-loaded capsids budding out of the nucleus seen on TEM in the liver, as well as the positive PCR and subsequent CoHV-1 in sequencing, made possible a straightforward diagnosis of IBD for Case 1. Additionally, phylogenetic analysis revealed that the sequence from Case 1 is highly related to CoHV-1 isolated worldwide.

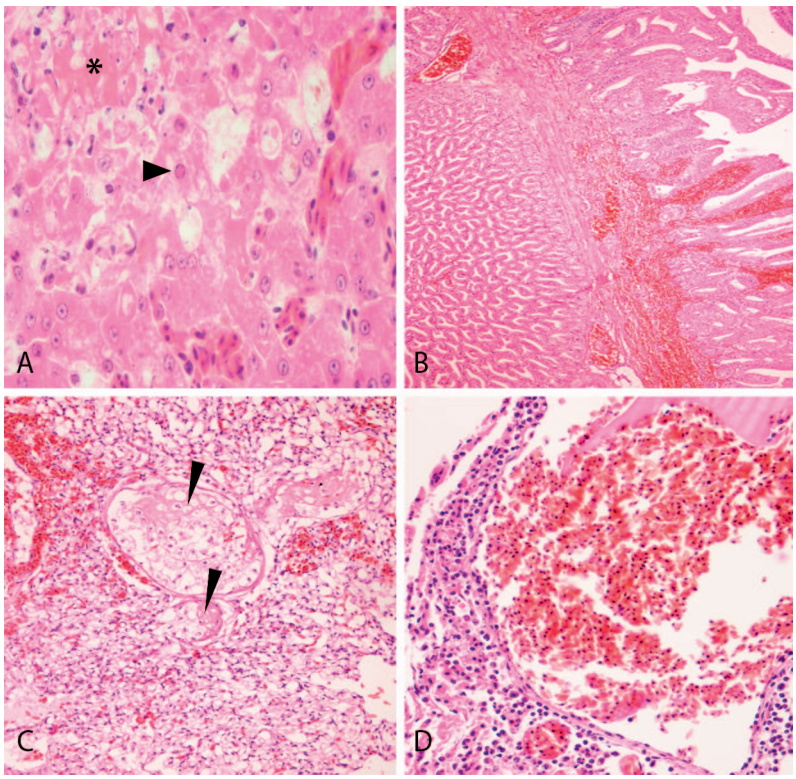

Fig. 2. Histologic lesion patterns (necrosis, hemorrhages, and thrombosis) of the examined Eurasian eagle-owls (*Bubo bubo*). (A) Case 1; liver. Focus of coagulative necrosis (*) and intranuclear intrahepatocellular herpetic inclusion body (arrowhead). (B) Case 1; proventriculus. Focal-extensive hemorrhage of the lamina propria. (C) Case 3; pulmonary vessel. Thrombus with fibrin and foamy activated platelets (arrowhead). (D) Case 2; brain. A perivascular cuff of mononuclear cells in the meninges.

Table 3. Summary of microscopic findings in the examined Eurasian eagle-owls (*Bubo bubo*).

Organ	Lesion	No.
Multiorgan	Thrombosis and hemorrhages	4/4
Serosal surfaces	Hemorrhages	4/4
Liver	Coagulative necrosis	3/4
Spleen	Coagulative necrosis	3/4
Heart	Hemorrhages	4/4
Lung	Pneumonia	4/4
Cerebrum	Perivascular cuffs	2/4
	Vasculitis	1/4

Unexpectedly, on TEM no viral particles were visible in Case 2, 3, and 4, possibly due to a low viral load. Additionally, PCRs resulted negative in Case 2, 3, and 4. However, some authors experienced false-negative PCR results after acyclovir administration (19). A possible explanatory reason is due to direct inhibition of the residual molecules of acyclovir and its metabolites against the *Taq* polymerase used in PCR protocol. In an attempt to verify this hypothesis, multiple PCR tests were performed increasing the concentrations of the enzyme in the PCR reaction master mix up to 10 units/100 µl, as suggested by Yedidag *et al.* (19). Unfortunately, the tests still failed to produce a positive PCR result. To date, known concentrations both of *Taq* polymerase and of acyclovir in the reaction mix have been tested, demonstrating that acyclovir inhibits PCR amplification products in a concentration-dependent manner (19). In our case the exact concentration of acyclovir and its metabolites were not known, so the hypothesis that in Case 2, 3, and 4 PCR assay did not amplify any product since an inhibition due to the acyclovir activity on *Taq* polymerase, although rare, cannot be excluded.

A more likely possibility to explain herpes-PCR negative results in Case 2, 3, and 4 is that the treatment with acyclovir lowered viral load below detectable levels and that the death of the birds in Cases 3 and 4 was the result of secondary bacterial infection established during the acute phase of CoHV-1 infection.

In an attempt to establish the source of infection, we performed a PCR assay from tissues of domestic pigeons used for food consumption by the owls. Literature reported positive results on testing brain and ganglion from infected pigeons (20). Unfortunately, in our report, the negative results did not allow the confirmation of an oral route of transmission of the virus, but this remains the most probable route of transmission of CoHV-1.

CONCLUSIONS

This paper reports the circulation of Columbid alphaherpesvirus 1 with the development of disease in Eurasian eagle-owls (*Bubo bubo*) in central Italy. Novel and not yet reported pathologic findings are here described suggesting a wider tissue and cell tropism, like other alphaherpesviruses affecting animals. Lack of PCR viral detection in Case 2, 3, and 4 could be explained by the acyclovir treatment that had likely reduced the viral load to undetectable levels. Lack of identification of CoHV-1 in tissues of domestic pigeons (*Columba livia* var. *domestica*) used for animal food consumption did not allow

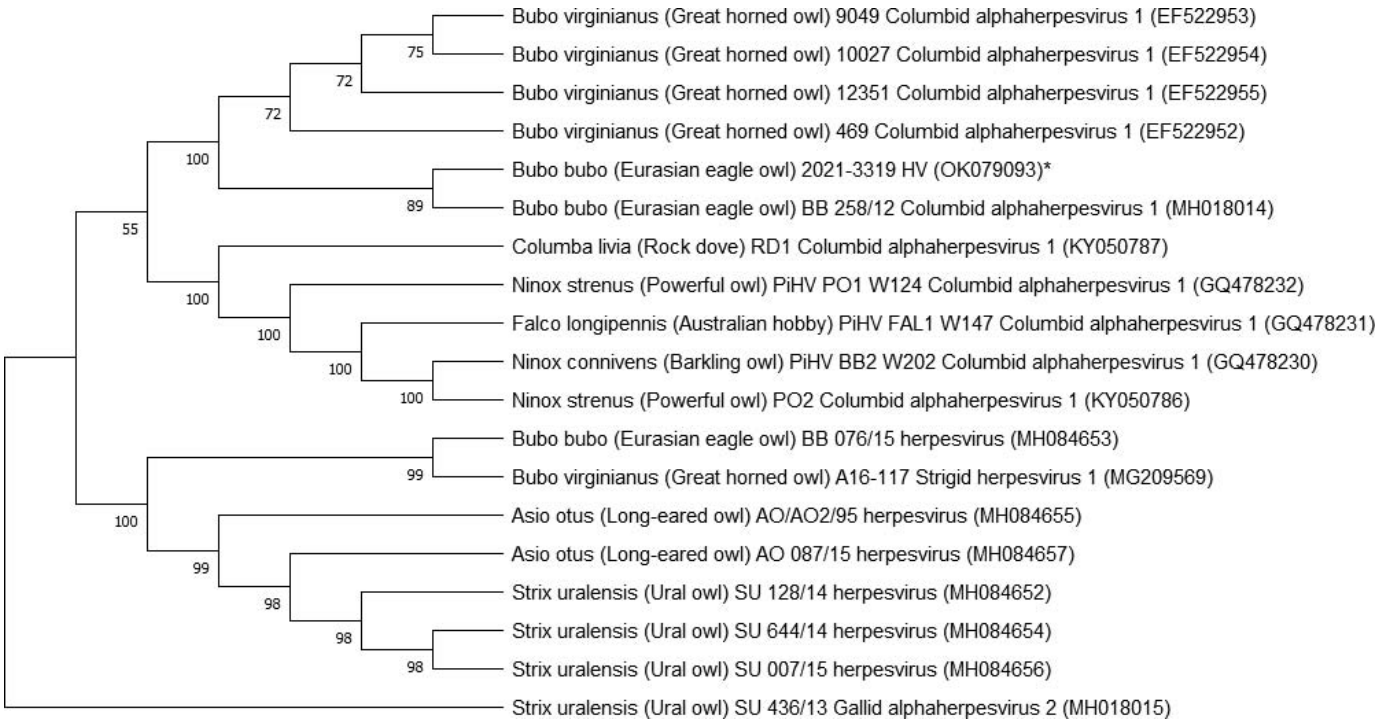

Fig. 3. Phylogenetic tree constructed based on the conducted alignment of the DNA-dependent DNA polymerase gene from our strains (OK079093) marked with an asterisk (*) and herpesviruses derived from the GenBank database. Sequences are reported in the following order: scientific name, common name, ID strain, viral name, and the GenBank Accession Number. The evolutionary history was inferred using the Neighbor-Joining method (14). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (15). The evolutionary distances were computed using the *p*-distance method (16) and are in the units of the number of base differences per site. This analysis involved 19 nucleotide sequences. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA11 (13).

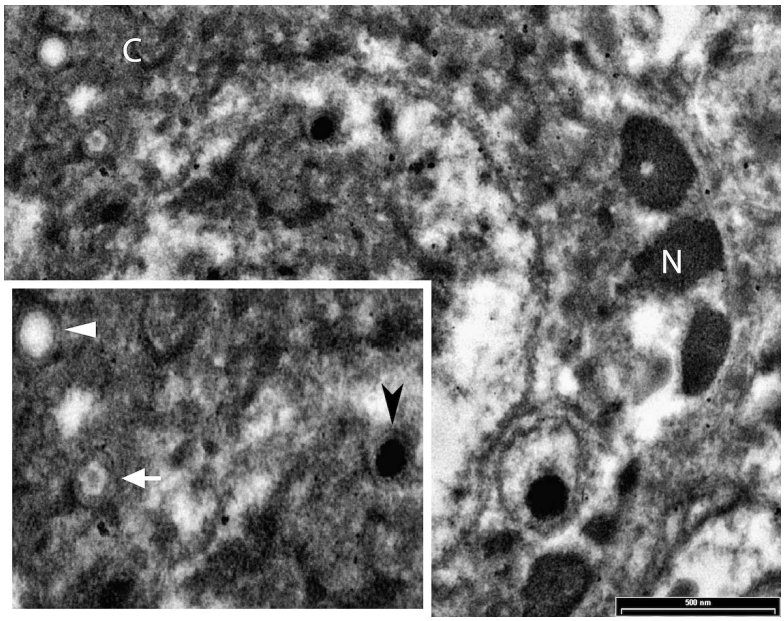

Fig. 4. Electron microscopy of the adult female Eurasian eagle-owl (*Bubo bubo*) (Case 1). Liver. Empty capsid in the cytoplasm (C) of a necrotic hepatocyte and DNA-loaded capsids budding out of its nucleus (N). Inset: Multiple stages of herpes virion development. Stages are represented by highly electron-lucent capsids with angular profile (white arrow and arrowhead), a viral particle with an electron-dense core (DNA), and an outer lighter electron-dense icosahedral capsid (black arrowhead).

the confirmation of a possible oral source of transmission of the virus that remains the most probable route of transmission.

REFERENCES

1. Green RG, Shillinger JE. A virus disease of owls. *Am J Pathol*. 12:405–410; 1936.

2. Burtscher H. Die virusbedingte Hepatosplenitis infectiosa strigorum: 1. Mitteilung: Morphologische Untersuchungen. *Vet Pathol*. 2:227–255; 1965.

3. Mare, J. Herpesviruses of birds of prey. *J Zoo Anim Med*. 6:6–11; 1975.

4. Williams MC, Nesbit JW. Fatal herpesvirus infection in a cape eagle owl *Bubo capensis* and a spotted eagle owl *Bubo africanus*. *Ostrich* 67:60–62; 1996.

5. Rose N, Warren AL, Whiteside D, Bidulka J, Robinson JH, Illanes O, Brookfield, C. Columbid herpesvirus-1 mortality in great horned owls (*Bubo virginianus*) from Calgary, Alberta. *Can Vet J. = Rev Vet Can*. 53:265–268; 2012.

6. Phalen DN, Holz P, Rasmussen L, Bayley C. Fatal columbid herpesvirus-1 infections in three species of Australian birds of prey. *Aust Vet J*. 89:193–196; 2011.

7. Burtscher H, Sibalin M. Herpersvirus strigis: host spectrum and distribution in infected owls. *J Wildl Dis*. 11:164–169; 1975.

8. Gailbreath KL, Oaks JL. Herpesviral inclusion body disease in owls and falcons is caused by the pigeon herpesvirus (Columbid herpesvirus 1). *J Wildl Dis*. 44:427–433; 2008.

9. Woźniakowski GJ, Samorek-Salamonowicz E, Szymański P, Wencel P, Houszka M. Phylogenetic analysis of Columbid herpesvirus-1 in rock pigeons, birds of prey and non-raptorial birds in Poland. *BMC Vet Res*. 9:1–9; 2013.

10. Smadel JE, Jackson EB, Harman JW. A new virus disease of pigeons: I. Recovery of the virus. *J Exp Med*. 81:385–398; 1945.

11. Kaleta EF. Herpesviruses of birds—a review. *Avian Pathol*. 19:193–211; 1990.

12. Vandevanter DR, Warrener P, Bennett L, Schultz ER, Coulter S, Garber RL, Rose TM. Detection and analysis of diverse herpesviral species by consensus primer PCR. *J Clin Microbiol*. 34:1666–1671; 1996.

13. Tamura K, Stecher G, Kumar S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol Biol Evol*. 38:3022–3027; 2021.

14. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol*. 4:406–425; 1987.

15. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791; 1985.

16. Nei M, Kumar S. Molecular evolution and phylogenetics. Oxford University Press, Oxford, England/New York, USA, 2000.

17. Perrin KL, Kristensen AT, Bertelsen MF, Denk D. Retrospective review of 27 European cases of fatal elephant endotheliotropic herpesvirus-haemorrhagic disease reveals evidence of disseminated intravascular coagulation. *Sci Rep*. 11:14173; 2021.

18. Pinkerton ME, Wellehan JFX, Johnson AJ, Childress Al, Fitzgerald SD, Kinsel MJ. Columbid herpesvirus-1 in two Cooper's hawks (*Accipiter cooperii*) with fatal inclusion body disease. *J Wildl Dis*. 44:622–628; 2008.

19. Yedidag EN, Koffron AJ, Mueller KH, Kaplan B, Kaufman DB, Fryer JP, Stuart FP, Asecassis M. Acyclovir triphosphate inhibits the diagnostic polymerase chain reaction for cytomegalovirus. *Transplantation* 62:238–242; 1996.

20. Kamionokowski M. Herpesvirus inflammation of the brain and spinal cord in pigeons. *Med Vet J*. 531, 532. 1980.

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