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Highly Pathogenic Avian Influenza Virus (H5N1) Outbreak in Captive Wild Birds and Cats, Cambodia

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From December 2003 through January 2004, the Phnom Tamao Wildlife Rescue Centre, Cambodia, was affected by the highly pathogenic influenza virus (H5N1). Birds from 26 species died. Influenza virus subtype H5N1 was detected in 6 of 7 species tested. Cats from 5 of 7 species were probably infected; none died.

On January 24, 2004, the first confirmed outbreak of highly pathogenic avian influenza virus (HPAIV) subtype H5N1 in Cambodia was reported to the Office International des Epizooties (OIE). During the previous month, an unusually high mortality rate had been noted among captive wild birds at the Phnom Tamao Wildlife Rescue Centre (PTWRC) in Takeo Province, 45 km South from Phnom Penh. We report the results of a retrospective investigation of this outbreak.

The Study

During the outbreak period, PTWRC housed 600–1,000 wild animals (70 species of mammals, birds, and reptiles). The center is divided into 3 main sections that cover 37 ha. Birds were kept in sections S1–1, S1–2, and S2, and the cats were in all sections (Figure). The information on bird deaths at PTWRC was systematically recorded by WildAid staff members who were at the Centre at the time of the outbreak. In June 2004, a complete investigation was conducted at PTWRC, and semistructured interviews of key informants were used to identify deaths of domestic poultry in the surrounding villages. Every bird death between December 15, 2003, through January 15, 2004, was defined as a suspected case of HPAIV (H5N1). For S1, the cumulative mortality rate could not be estimated because the exact bird population was not known and the birds were difficult to observe in that section (the semicaptive waterfowl population is able to mix with the wild population and disperse to breed). For S2, information was complete (Table 1).

The first case, in a crested serpent eagle (Spilornis cheela), was reported on December 15, 2003, in S2 (Figure). On December 19, the outbreak had reached every section and continued until January 12; a total of 86 birds, representing 8 taxonomic orders and 12 families, died (Table 1). Of 7 cat species, cats from 5 species were reported sick (16/39 total cats) (Table 2). In S2, 80% of the reported bird deaths were observed from December 15 to 21. Of the 29 wild bird species kept in S2 at the beginning of the outbreak, no birds from 12 species showed signs of disease (Table 1). Mortality rates varied among the orders, 0–100%

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The only mammals present in the aviaries in S2, slow lorises (Nycticebus sp.), did not become ill. None of the 27 animal keepers, who were 20–50 years of age, were reported to have gotten sick. Most of the birds died within a few hours without showing any clinical signs of infection. A few birds died 1–2 days after onset of clinical signs (anorexia, extreme lethargy, occasional dark green diarrhea, respiratory dis-

Table 1. Cumulative deaths during an outbreak of highly pathogenic avian influenza virus (H5N1), Phnom Tamao Wildlife Rescue Centre, Cambodia, December 15, 2003–January 13, 2004*

| Order                  | Family     | Species (common name), no. sampled | No. dead birds in S1 | No. birds not dead in S1 | Cumulative deaths in S2, % (dead/total at risk) |
|------------------------|------------|------------------------------------|----------------------|--------------------------|------------------------------------------------|
|                        |            |                                     |                       |                          | Per species Per order                          |
| Anseriformes           | Anatidae   | Anas poecilorhyncha (Indian-spot-billed duck) | NP                    | NP                       | 0 (0/4) 0 (0/4)                                |
|                        |            | Ardea cinerea (grey heron), n = 2   | 4                    | 2                        | NP                                             |
|                        | Ardeidae   | Ardea speciosa (Javan-pond heron), n = 1 | 7                    | 0                        | 100 (7/7)                                     |
|                        |            | Butorides striatus (little heron)   | NP                    | NP                       | 0 (0/1)                                       |
|                        |            | Egretta garzetta (little egret)     | NP                    | NP                       | 18 (2/11)                                     |
| Ciconiiformes          |            | Ephippiorhynchus asiaticus (black-neck stork) | 1                    | 3                        | NP                                             |
|                        |            | Leptoptilos dubius (greater adjutant stork) | 2                    | 1                        | NP                                             |
|                        |            | Leptoptilos javanicus (lesser adjutant stork) | 3                    | 21                       | NP                                             |
|                        |            | Mycteria leucocephala (painted stork) | 6                    | 20                       | NP                                             |
|                        |            | Ciconia episcopus (wooly necked stork) | 0                    | 3                        | NP                                             |
|                        |            | Anastomus oscitans (Asian openbill stork) | 0                    | 5                        | NP                                             |
| Colombiformes          | Colombidae | Treron curvirostra (thick-billed green pigeon) | NP                    | NP                       | 0 (0/7) 0 (0/17)                               |
|                        |            | Streptopelia chinensis (spotted dove) | NP                    | NP                       | 0 (0/10)                                      |
| Coraciiformes          | Buceritidae| Buceros bicornis (great hornbill)    | NP                    | NP                       | 100 (1/1)                                     |
|                        |            | Gyps bengalensis (white-rumped vulture) | NP                    | NP                       | 100 (1/1)                                     |
|                        |            | Haliastur indus (Brahminy kite)     | NP                    | NP                       | 0 (0/1)                                       |
|                        |            | Ichthyophaga ichtyaetus (grey-headed fish eagle) | 3                    | 0                        | 100 (4/4)                                     |
|                        |            | Ictinaetus malayensis (black eagle) | NP                    | NP                       | 100 (1/1)                                     |
|                        |            | Milvus migrans (black kite)         | 1                    | 0                        | NP                                             |
|                        |            | Spilornis cheela (crested serpent eagle), n = 1 | 1                    | 0                        | 100 (5/5)                                     |
|                        |            | Spizaetus cirratus (changeable hawk eagle), n = 1 | 0                    | 0                        | 100 (2/2)                                     |
| Galliformes            | Numididae  | Agelastes sp (guineafowl)            | NP                    | NP                       | 33.3 (1/3) 36 (5/14)                           |
|                        | Phasianidae| Pavo muticus (green peafowl)         | 0                    | 3                        | 100 (3/3)                                     |
|                        |            | Gallus gallus (red jungle fowl)      | NP                    | NP                       | 12.5 (1/8)                                    |
| Gruidae                |            | Grus antigone (Sarus crane)          | 3                    | 0                        | NP                                             |
| Passeriformes          | Corvidae   | Corvus macrorhynchos (large-billed crow), n = 1 | 2                    | 0                        | 100 (3/3) 25 (3/12)                            |
|                        | Sturnidae  | Gracula religiosa (hill mynah)       | NP                    | NP                       | 0 (0/3)                                       |
|                        |            | Acridotheres tristis (common mynah)  | NP                    | NP                       | 0 (0/4)                                       |
|                        |            | Acridotheres javanicus (white-vented mynah) | NP                    | NP                       | 0 (0/1)                                       |
|                        |            | Sturnus contra (Asian pied starling) | NP                    | NP                       | 0 (0/1)                                       |
| Pelecaniformes         | Anhingidae | Anhinga melanogaster (oriental darter) | 0                    | 1                        | NP                                             |
|                        | Pelecanidae| Pelecanus philippinus (spot-billed pelican) | 3                    | 2                        | NP                                             |
| Psittaciformes         | Psittacidae| Psittacula eupatria (Alexandrine parakeet), n = 1 | 1                    | 0                        | 50 (1/2) 0 (1/146)                             |
|                        |            | Psittacula roseate (blossom-headed parakeet) | NP                    | NP                       | 0 (0/20)                                      |
|                        |            | Psittacula alexandri (red-breasted parakeet) | NP                    | NP                       | 0 (0/20)                                      |
|                        |            | Psittacula finschii (grey-headed parakeet) | NP                    | NP                       | 0 (0/104)                                     |
| Strigiformes           | Strigidae  | Bubo nipalensis (spot-billed eagle owl), n = 1 | 0                    | 0                        | 100 (1/1) 92 (12/13)                           |
|                        |            | Ketupa ketupu (buffy fish owl)       | NP                    | NP                       | 100 (3/3)                                     |
|                        |            | Ketupa zeylonensis (brown fish owl)  | NP                    | NP                       | 86 (6/7)                                      |
|                        |            | Strix selaputo (spotted wood owl)    | NP                    | NP                       | 100 (2/2)                                     |
|                        | Tytonidae  | Tyto alba (barn owl)                | 5                    | 0                        | NP                                             |
| Total                  |            |                                     | 42                   | 61                       | 18.3% (44/240)                                 |

*S1, aviary section in which cumulative mortality rate could not be estimated because exact bird population was not known and birds were difficult to observe; S2, aviary section in which captive bird population was exactly known and number of dead birds was precisely recorded; NP, species not present in S1 or S2.

†Only sample that was negative for highly pathogenic avian influenza virus (H5N1); all other birds sampled were positive.
The clinical outcome of wild birds with suspected HPAIV (H5N1) infection at PTWRC ranged from severe illness and death to complete absence of clinical signs, as described (8). Several species from the orders Ciconiiformes, Galliformes, Passeriformes, Gruiformes, Coraciiformes, and Pelecaniformes were affected during the outbreak. This observation is consistent with data published earlier, except for Coraciiformes represented by 1 bird in our study (9). Only the carnivorous species (Corvus macrorhynchos) among the 5 species of Passeriformes in the aviaries showed clinical signs and later was confirmed by RT-PCR to be positive for HPAIV (H5N1). This outbreak confirms that Falconiformes and Strigiformes are sensitive to HPAIV (H5N1) infection and disease (10–12) and shows that numerous species of these orders can be affected by HPAIV (H5N1) (Table 1). Psitaciformes and Columbiformes were not visibly affected by the outbreak although they were kept in large numbers in S2, where large numbers of deaths occurred. As non–water-bird species, they do not belong to groups in which avian influenza is commonly reported (13). Anseriformes, represented in PTWRC by only 4 birds (Anas poecilorhyncha), did not show any clinical signs. Heterogeneity in the susceptibility of wild ducks to HPAIV (H5N1), including asymptomatic infection, has been demonstrated (14); this species also belongs to the group of wild ducks found asymptptomatically infected with HPAIV (H5N1) in the People’s Republic of Cambodia.

### Table 2. Morbidity rates for wild cats during outbreak of highly pathogenic avian influenza virus (H5N1), Phnom Tamao Wildlife Rescue Centre, Cambodia, December 15, 2003–January 13, 2004

| Order     | Family      | Species (common name)       | Cumulative morbidity rate, % (sick/at risk), no. sampled |
|-----------|-------------|-----------------------------|--------------------------------------------------------|
| Carnivora | Felidae     | Panthera leo (lion)         | 100 (2/2)                                               |
|           |             | Panthera tigris (tiger)     | 80 (8/10), n = 1*                                       |
|           |             | Catopuma temminckii (Asiatic golden cat) | 100 (2/2), n = 1*                                   |
|           |             | Panthera pardus (leopard)   | 100 (3/3), n = 1*                                       |
|           |             | Neofelis nebulosa (clouded leopard) | 100 (1/1), n = 1*                                   |
|           |             | Prionailurus bengalensis (leopard cat) | 0 (0/16)                                             |
|           |             | Prionailurus viverrinus (fishing cat) | 0 (0/5)                                              |
| Total     |             |                             | 41 (16/39)                                              |

*All serum samples were positive (date of collection: March 4, 2004).
China during the winter of 2005 (15).

The serologic evidence of influenza virus (H5N1) infection in 4 species of wild cats is in agreement with previous infection in Thailand (6,7). The report of illness in the Asiatic golden cat (Catopuma temminckii) and the clouded leopard (Neofelis nebulosa) broadens the host range of the virus among mammals.

This report confirms the great variability of wild bird and mammal responses to HPAIV (H5N1) infection. It also confirms the broadening range of susceptible species that may be specific to this clade 1 virus.

Dr Desvaux is a veterinary epidemiologist working at the Centre de Cooperation Internationale en Recherche Agronomique pour le Développement. Her current research interests focus on HPAIV epidemiology and surveillance in Vietnam.

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