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## Deficient Reporting in Avian Influenza Surveillance, Mali

**To the Editor:** In response to influenza outbreaks caused by highly pathogenic avian influenza virus (HPAIV) throughout western Africa as of 2006, the National Veterinary Epidemiologic Surveillance Network of Mali (EPIVET-Mali) started conducting domestic and wild bird surveillance. No HPAI outbreaks were reported to the World Organisation for Animal Health. An evaluation survey conducted in 2009 enabled identification and correction of some weaknesses in the organization and functioning of the network (1). However, no attempt was made to assess how much information on bird health in backyard poultry farms (which account for ≈95% of the total

poultry population in Mali) actually reached EPIVET-Mali veterinarians and technicians. Therefore, we quantified reporting of clinical signs of avian diseases, especially those suggesting HPAI, by poultry owners in Mali.

We used a pilot-tested standardized quantitative and qualitative questionnaire to conduct face-to-face interviews in 32 randomly selected villages in the southern half of Mali (which accounts for 98% of the poultry population). In each village, we conducted interviews in 4 randomly chosen households. No eligibility criteria were used for household selection because all village households had poultry. Interviews were repeated 6 times (approximately every 3 months) during November 2009–February 2011 in the same villages and whenever possible in the same households. If it was not possible to repeat an interview in a previously interviewed household (absence of the household chief), the neighboring household was interviewed.

For each household, data were collected on number of sick and dead birds in the previous 3 months, clinical signs observed, and their notification or lack thereof to veterinary authorities. Households in which birds showed ≥3 of the following clinical signs (diarrhea, respiratory disorder, nervous signs, cyanosis of the combs or wattles, and mortality rate >50%) were considered as having clinical signs suggesting HPAI. The study was approved by the Direction Nationale des Services Vétérinaires and traditional authorities in all 32 villages, and oral consent was obtained from the poultry owners before interviews.

A total of 110–128 households were investigated at each study interval, depending on village accessibility and presence or absence of household chiefs (Table). We conducted 738 household investigations in 152 households (80 households were

Table. Observations and reporting of sick poultry and signs of influenza suggesting HPAI virus infection, Mali, 2009–2011\*

Date of investigation	Total no. households investigated	No. with sick poultry/ no. with information on sick poultry (%)	No. with HPAI signs/ no. with information on signs (%)	No. with reported sick poultry/ no. with sick poultry and information on reporting (%)	No. with reported HPAI signs/no. with signs and information on reporting (%)
2009 Nov	128	67/128 (52.3)	25/124 (20.2)	7/66 (10.6)	5/25 (20.0)
2010 Feb	128	68/128 (53.1)	21/123 (17.1)	4/68 (5.9)	0/21 (0.0)
2010 May	127	47/127 (37.0)	6/118 (5.1)	9/45 (20.0)	2/6 (33.3)
2010 Sep	110	37/110 (33.6)	17/107 (15.9)	3/34 (8.8)	2/17 (11.8)
2010 Nov	124	53/124 (42.7)	7/119 (5.9)	12/51 (23.5)	4/7 (57.1)
2011 Feb	121	57/121 (47.1)	10/115 (8.7)	8/55 (14.5)	2/10 (20.0)
Total	738	329/738 (44.6)	86/706 (12.2)	43/319 (13.5)	15/86 (17.4)

\*Information was obtained for 3 months before the interview. HPAI, highly pathogenic avian influenza.

interviewed 6 times, 26 five times, 11 four times, 21 three times, 7 two times, and 7 one time). Observation of sick poultry in the 3 months before the interview was reported in 44.6% of household investigations, and observation of signs suggesting HPAI was reported in 12.2% (Table). Notification of veterinary authorities was reported in 13.5% of household investigations with sick poultry and in 17.4% of household investigations with signs suggesting HPAI (Table).

When we considered the 80 households interviewed 6 times, observation of sick poultry and signs suggesting HPAI varied over time ( $p = 0.043$  and  $p = 0.018$ , respectively, by Cochran Q test), whereas variation over time could not be tested for notification because of an insufficient number of observations. When we considered all 738 household investigations as independent investigations, observation of sick poultry and signs suggesting HPAI varied over time ( $p = 0.008$  and  $p < 0.001$ , respectively, by  $\chi^2$  test), but reporting of sick poultry did not vary over time ( $p = 0.06$ , by  $\chi^2$  test). Reporting of signs of HPAI could not be tested.

These results illustrate gaps in reporting signs suggesting HPAI by backyard poultry owners. Although these signs could also be those of Newcastle disease, which is present in Mali (2), these signs should be reported because HPAI and Newcastle disease are officially targeted by EPIVET-Mali. One survey attempted to similarly quantify the level of HPAI

reporting in Africa. In Kwara State in Nigeria, 56.5% of respondents indicated that they would not notify officials if they suspected HPAI in their flocks (3). Reluctance of poultry owners to comply with notification and culling obligations has also been reported in Indonesia (4). Several studies that assessed knowledge and practices of poultry workers with regard to avian influenza have been conducted in different countries, including developing countries (5,6). These studies were useful for better defining content of risk mitigation advice messages and the audience they should primarily target.

In our survey, occurrence of disease in Mali varied over time, which was expected because of the seasonal pattern of many avian diseases, especially Newcastle disease, in western Africa (7). However, reporting of sick poultry did not vary over time despite seasonality of activities in rural areas. Lack of awareness of who to report to, fatalistic attitudes toward animal diseases, and mistrust toward the government and its compensation schemes are among the major constraints affecting the likelihood of HPAI signs being reported (3,6,8). However, approaches associating socioanthropology and epidemiology have recently been developed to help solve the problem posed by deficient reporting (9).

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### Letters

Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 Figure or Table and should not be divided into sections. All letters should contain material not previously published and include a word count.

## Myxozoan Parasite in Brain of Critically Endangered Frog

**To the Editor:** More than three quarters of critically endangered species of amphibians are threatened by infectious disease; several are already extinct (1). In 2010, the yellow-spotted bell frog (*Litoria castanea*), which was presumed to be extinct, was rediscovered in the Southern Tablelands of New South Wales, Australia. This species of frog had not been seen for 30 years, and a chytrid fungus, *Batrachochytrium dendrobatidis*, was thought to be the reason (1,2). The number of frogs in the rediscovered population is estimated to be 100; if numbers are that low, the yellow-spotted bell frog is the most critically endangered frog in Australia.

Several yellow-spotted bell frogs were collected for a captive breeding program at Taronga Zoo in Sydney, New South Wales, Australia. Generalized edema developed in a subadult male frog after 8 months of captivity in strict quarantine conditions. The frog subsequently died, and later an adult male frog was also found dead. Results of necropsy on both frogs at the Australian Registry of Wildlife Health revealed subcutaneous edema, intracoelomic fluid, and swollen kidneys with pale foci. Histopathologic examination demonstrated chronic severe tubulonephropathy and acute severe encephalomalacia. Coalescing foci of hemorrhage and malacia were observed in the caudal brainstem and were associated with small multinucleated ( $1 \times 1 \mu\text{m}$ ) parasites forming plasmodia-like structures 10–20  $\mu\text{m}$  in diameter (Figure). Plasmodia were present in large numbers (1–5/40 $\times$  field) in the spinal cord. Organisms that were morphologically consistent with myxozoan parasites

detected in other frogs in Australia were found predominately within axons and were uncommonly present in vascular endothelial cells (3). Characteristic hepatic lesions, including lymphoplasmacytic hepatitis with biliary hyperplasia and loss of hepatocytes, were also present.

The cause of death was renal failure, a common problem in aged frogs; however, these frogs were young, and therefore the cause of the renal changes was perplexing. We considered whether toxins (e.g., improperly cured polyvinyl chloride glue) or an infectious process might be possible causes. Staff in the zoo's breeding program were questioned and indicated that the opportunity for introduction of a toxin was low. In addition, results for virus isolation and fungal and bacterial cultures were negative. We retrospectively reexamined histologic sections of an endangered booroolong frog (*Litoria booroolongensis*) that had similar brain lesions and intralesional myxozoan parasites (3). Tissue samples were submitted to the Faculty of Veterinary Science, The University of Sydney, for identification.

DNA was extracted from brain tissues (20 mg) by using the PureLink DNA Kit (Invitrogen, Mulgrave, Victoria, Australia). To test for myxozoans, we used a highly Myxozoa-specific PCR to amplify the complete internal transcribed spacer of the ribosomal DNA (3). Myxozoan-positive amplicons were directly sequenced at Macrogen Inc. (Seoul, South Korea), analyzed by using the CLC Main Workbench (CLC bio, Aarhus, Denmark), and deposited in GenBank (accession nos. JN977605–09).

PCR produced a 973-bp amplicon with DNA from brain and liver of the yellow-spotted bell frogs and the booroolong frog. DNA from the frogs showed 100% identity with each other, as did sequences from brain and liver. A BLASTN (4) search of