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THE EPIDEMIOLOGY OF THE HIGHLY PATHOGENIC H5N1 AVIAN INFLUENZA IN MUTE SWAN (*CYGNUS OLOR*) AND OTHER *ANATIDAE* IN THE DOMBES REGION (FRANCE), 2006

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ABSTRACT: In February 2006, a highly pathogenic avian influenza (HPAI) H5N1 virus was isolated from Common Pochards (*Aythia ferina*) in the Dombes region of France, an important migrating and wintering waterfowl area. Thereafter, HPAI H5N1 virus was isolated from 39 swab pools collected from dead waterfowl found in the Dombes, but only from three pooled samples collected outside of this area but located on the same migration flyway. A single turkey farm was infected in the Dombes. The epizootic lasted 2 mo and was restricted to the Dombes area. Virus-positive pools were detected in 20 of 1,200 ponds and infected Mute Swans (*Cygnus olor*) represented 82% of the virus-positive pools. Other infected species included Common Pochard ($n=4$), Grey Heron (*Ardea cinerea*, $n=1$), Eurasian Buzzard (*Buteo buteo*, $n=1$), and Greylag Goose (*Anser anser*, $n=1$). Despite intensive monitoring during and after the outbreak, HPAI H5N1 virus was not isolated from healthy wild birds. Our results are consistent with an HPAI H5N1–virus introduction into the Dombes via migrating ducks. These birds could have been pushed west by a severe cold spell in central Europe where the virus had already been detected. The Mute Swan served as an excellent epidemiologic sentinel during this outbreak; swans appear to be highly sensitive to infection with these viruses and swan mortality was easy to detect. During the outbreak, the mortality rates for wild birds remained moderate and the virus affected a limited number of species.

Key words: *Cygnus olor*, epidemiology, HPAI H5N1, influenza A, Mute Swan, wild birds.

INTRODUCTION

Since 1996, when it was first identified in southern China (Xu et al., 1999), highly pathogenic avian influenza (HPAI) H5N1 has spread throughout much of Asia, primarily affecting poultry (Li et al., 2004; Chen et al., 2006). These HPAI H5N1 viruses are particularly pathogenic, and can induce clinical signs and mortality in many avian species, including wild birds (Ellis et al. 2004; Sturm-Ramirez et al., 2004), and in mammals (Keawcharoen et al., 2004; Thanawongnuweh, 2005). The virulence of these viruses in some species of water birds, especially the *Anatidae*, is unique; wild birds are generally asymptomatic carriers of low-pathogenic influenza viruses (Olsen et al., 2006; Fouchier et al., 2007; Stallknecht and Brown, 2007). In April 2005, HPAI H5N1 mortality in wild birds was observed in Qinghai, China (Chen et al., 2005; Liu et al., 2005). During the summer of 2005, the range of HPAI H5N1 expanded westward, affecting birds in Russia and Kazakhstan. By the fall of 2005 birds were infected in Turkey, Romania, Ukraine, and Croatia (FAO, 2006; Gilbert, 2006; Webster and Govorkova, 2006). The epizootic continued to spread westward throughout the European Union, affecting wild birds in 13 countries from February to May 2006 (OIE, 2006; Terregino et al., 2006; Nagy, 2007). However, infected poultry farms were detected in only five countries (France, Germany, Sweden, Denmark, and Hungary) where HPAI H5N1 virus was reported from wild birds.

Currently, it is thought that both human activities, including poultry trade through

Asia and Africa, and the movements of migratory birds contributed to the spread of this virus (Gauthier-Clerc et al., 2007). The latter is considered to be primarily responsible for the introduction of the virus into Europe (European Food Safety Authority, 2006; Kilpatrick et al., 2006; Olsen, 2006; Gauthier-Clerc et al., 2007; Pfeiffer, 2007).

In France, wild birds have been tested for highly pathogenic influenza viruses (H5 and H7) since September 2005. The monitoring programs relies on sampling dead or moribund birds through the Wildlife Diseases National Surveillance Network (SAGIR), which was organized in 1986 by the National Game and Wildlife Agency in partnership with the hunters' associations, the French Food Safety Agency (AFSSA) in Nancy, and the local laboratories. Diagnostic virology is done through the National Reference Laboratory (NRL) of the AFSSA Ploufragan and six local veterinary laboratories distributed throughout the French territory; all are authorized for the influenza screening tests. In addition to the passive monitoring, there is an active monitoring program of "healthy" birds that are captured, shot, or bred as sentinel birds in several wetlands.

In France, the first HPAI H5N1 cases were detected on 13 February 2006; three dead Common Pochards (*Aythya ferina*) were found in the Dombes region, a wetland area of 1,100 km^2 located in the "department" (administrative unit) of Ain (eastern center of France: 46°00′08″N, 05°01′42″E). A few days after this first case, HPAI H5N1 was detected on a turkey farm in the vicinity of these cases; this was the only domestic bird outbreak in France. No outbreaks were observed in domestic fowl in the neighboring Bresse region, even though this area is famous for open-air chicken farming area. In all of France, all but three HPAI H5N1 virus-infected dead wild birds were found in the Dombes region. In this article, we describe the epizootic in the wild bird populations of the Dombes and the results of waterfowl monitoring during the winter and spring of 2006, which gave us an opportunity to investigate the respective roles of different species of *Anatidae* in the local emergence and persistence of the virus.

MATERIALS AND METHODS

Wintering waterfowl monitoring (December–March)

The Dombes region, with its 1,200 fish ponds, is a wintering area of international importance (Ramsar, 2007) that is utilized by more than 20,000 waterfowl; this includes more than 1% of the biogeographic population of several species of *Anatidae* and Eurasian Coots (*Fulica atra*; Fouque et al., 2005) wintering. Moreover, the population of Mute Swan (*Cygnus olor)* has been exponentially growing in the Dombes area (Benmergui et al., 2005); this same trend has been reported in France and in Europe (Fouque et al., 2007).

In France, a national winter census of migratory birds has been organized by the French Waterfowl and Wetland Network since 1987. This network is a collaboration between the National Game and Wildlife Agency and the hunters' associations. Each winter, more than 600 sites are observed in December, January, and February to estimate trends in the number of *Anatidae* and coots. In the Dombes region, the survey is conducted on a sample of 178 ponds that are utilized by the majority *Anatidae* wintering in this area. During the outbreak, an additional census was conducted on these sites in March 2006.

Breeding waterfowl monitoring (April–July)

During the nuptial period, more than 130 bird species breed in the Dombes. Since 1994, the reproduction of *Anatidae*, including the Mute Swan, has been monitored each spring. In 2006, as in previous years, pairs and broods of waterbirds were counted every week, from 15 April to 15 July, on a sample of 73 ponds.

The summer population size of Mute Swan is estimated by a global "flash" census performed throughout all the ponds of the Dombes area, during mid-June. The nesting population is estimated by counting pairs, and the nonbreeding population is estimated by counting groups of more than two individuals (Benmergui et al., 2005).

Dead bird surveillance

In 2006, well-preserved waterfowl carcasses were collected, principally by National Game and Wildlife officers during surveillance rounds or in response to mortality or morbidity reports from the public. For nonwaterfowl species, carcasses were collected if more than five dead birds were found in the same time and place. From February to July, this passive monitoring was expanded in the Dombes by a weekly census of 16 infected (HPAI H5N1–positive bird[s] detected) ponds and 16 control ponds (no infected birds detected) that were adjacent to the infected ponds. All birds on these 32 ponds were counted. Searches for dead or sick birds were conducted and dead birds were collected for testing. In addition to these sites, five waterfowl areas in the Ain department were observed three times a week in April.

Carcasses were submitted to the local veterinary laboratory of Ain, one of the six officially authorized labs for influenza screening tests. Tracheal and cloacal swabs were collected in Virocult® swab transport device, stored at 4 C, and analyzed within 48 hr. A screening test using the Real Time M-based Reverse Transcriptase PCR method (Spackman et al., 2002) was used for pooled swabs (pooled by species with a maximum of five samples). Pools of tracheal or cloacal swabs were tested separately. After 15 March, all individual swabs from positive pooled samples were tested. All positive swabs were tested by the NRL of the AFSSA by the H5 RT-PCR method (Slomka et al., 2007). For all H5 positives, the amino acid sequence of the hemagglutinin cleavage site was determined, and a RT-PCR targeting neuraminidase subtype 1 (N1) was performed. Finally, a phylogenetic analysis was done to characterize the virus (Le Gall-Reculé et al., 2008).

Healthy bird surveillance

After June 2006, in order to check for the possible persistence of the HPAI H5N1 virus in the Dombes area, 300 wild birds belonging to 29 species of passerines ($n=233$), ducks ($n=45$), turtledoves ($n=14$), and shorebirds ($n=8$) were captured (using nets and traps); 102 Mute Swans and 298 corvids were shot. These 700 birds were tested under the same laboratory protocol as for passive monitoring. Sera from the 102 swans were tested for antibodies to H5 with the official hemagglutination inhibition (HI) test (reference IHA Diagnostic Manual for avian influenza Commission Decision 2006/437/EC 4, August 2006) with two antigens. The first was A/Common Pochard/France/06167/2006 (H5N1) obtained from an isolate coming from positive birds found dead in the Dombes (Le Gall-Reculé et al., 2008). The second was: A/duck/France/05057b/2005 (H5N2) obtained from an isolate from free-range domestic ducks in France in 2005 (Cherbonnel et al., 2007). Only the swans that were positive (HI titer>1/16) for the two antigens were considered antibody positive.

Additionally, 100 6-wk-old hand-reared Mallards (*Anas platyrhynchos*) were released on 1 June (6 wk after the last detection of a positive bird) in enclosures built on four previously positive ponds. All birds tested were virus and antibody negative for H5 and H7 influenza virus prior to release. Sentinel birds were in direct contact with water and the avifauna. Cloacal and tracheal swabs were collected twice a month from June to December 2006.

RESULTS

Wintering waterfowl monitoring

Mean temperatures during the winter of 2005–2006 in the Dombes region and all of France were 1–3 C lower than normal. Mean temperatures in December 2005 and February 2006 ranged from 2–4 C; similar below-normal temperatures occurred in eastern and central Europe. These colder temperatures resulted in some unusual bird observations in France during January and February; these included reports of Bewick's Swan (*Cygnus columbianus*), Barnacle Goose (*Branta leucopsis*), and White-fronted Goose (*Anser albifrons flavirostris*), which is relatively unusual.

During the 2005–2006 winter, the numbers of *Anatidae* and Eurasian Coots staging on the 178 monitored ponds in the Dombes region reached a peak of 30,000 birds in February; this coincides with the month of peak migration for the Common Pochard (*Aythya ferina*). As in previous winters, waterfowl populations were lower in December (13,206 birds), January (23,196 birds), and March (24,577 birds). The most important wintering species are the Mallard and the Common Pochard, with combined population size exceeding 10,000 birds (Fig. 1B, C). As in previous winters, species-related variations were observed; peak populations were observed

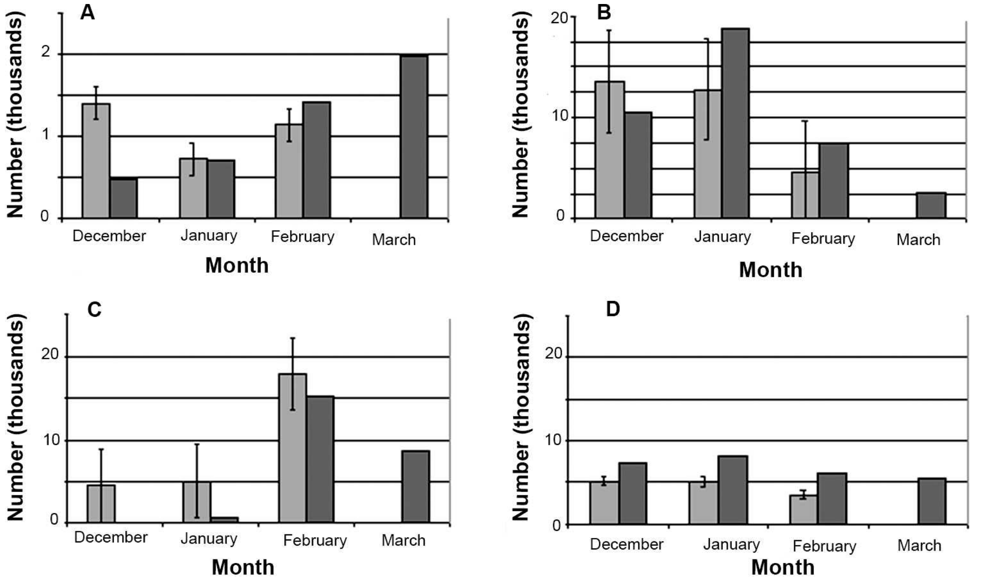

FIGURE 1. Monthly phenology of Green-winged Teal (A), Mallard (B), Common Pochard (C), and Mute Swan (D), counted in the Dombes area during the 2005–2006 winter (dark gray) and comparison with the mean (and standard error of the mean; light gray bars) of the five previous winters (2000/2001–2004/2005 period). Source: French waterfowl and wetland network ONCFS/Departmental Hunting Federation.

in January for Mallard (18,841; Fig. 1B), Mute Swan (n=809; Fig. 1D), and Eurasian Wigeon (*Anas penelope*, n=351); and in March for Eurasian Coot (n=4856), Green-winged Teal (*Anas crecca*, n=1983), Northern Pintail (*Anas acuta*, n=1943), Northern Shoveler (*Anas clypeata*, n=963), Gadwall (*Anas strepera,* n=932), Tufted Duck (*Aythya fuligula,* n=908), Red-crested Pochard (*Netta rufina*, n=733), and Garganey (*Anas querquedula*, n=113). Green-winged Teal, which, like the Eurasian Wigeon, is sensitive to cold weather conditions, was less numerous in December 2005 (Fig. 1A) than in the five previous winters. Common Pochards, which are normally absent in December and January, appeared in the Dombes early in February (15,332 counted birds, February 15), after a thaw of the ponds (Fig. 1C). A winter expansion of the Mute Swan population has been observed since 1995 in the Dombes, and since 1987 throughout France (Fig. 2). The population estimate was higher during the 2005–2006 winter than in the five previous winters (Fig. 1D). This growth is in partly due to increases in local breeding but also to the possible movements of swans into the Dombes region from outlying regions (Fouque et al., 2007). In the Dombes, a small part of the Mute Swan population is migratory, and in 2005 these migrants arrived in November and December. During winter, Mute Swans form groups of 10 to more than 100 individuals (including yearlings) that move locally between ponds. During summer, Mute Swans are territorial and their movements are limited.

Breeding waterfowl monitoring

The 2006 monitoring of breeding waterfowl showed a decrease of the nesting populations (i.e., pairs and broods) of all species of Anatidae except the Mute Swan. The mean density of the juvenile Mute Swans was 0.05±0.03/10 ha (Table 1), which is clearly lower than had been observed in previous years (0.10±0.03/10 ha in average in the 4 previous years).

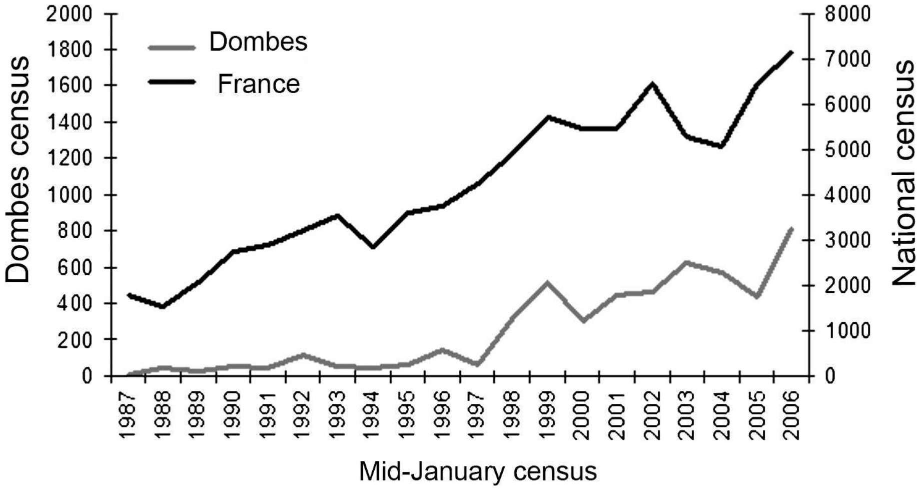

FIGURE 2. Trend in numbers of Mute Swan in the Dombes area and in France from January 1987 to January 2006, before the HPAI H5N1 outbreak. Source: French waterfowl and wetland network ONCFS/Departmental Hunting Federation.

The proportion of productive pairs dropped from 54.2% during the 2001–2005 period to 23.2% in 2006 (Table 1). The flash census revealed a lower density of adult and subadult swans (0.73 swans/10 ha), than that observed in 2005 (Table 1).

The epizootic of HPAI H5N1

From February 13 to August 31, 2006, 3,126 dead birds (2,011 swab pools) were collected and tested in France. In the Dombes, 244 carcasses (201 swab pools) were tested at the local laboratory of Ain.

In France, the HPAI H5N1 virus was isolated from 42 swab pools (2% of the tested pools); 39 (corresponding to 19% of the tested pools) of these were collected in the Dombes. Positive birds detected outside of the Dombes included one Tufted Duck and one Great-crested Grebe (*Podiceps cristatus*) from Leman Lake, located 100 km east of the Dombes, and one Mute Swan detected on the Mediterranean coast, 300 km south of the Dombes. These three cases occurred in late February, at the beginning of the outbreak.

The 39 positive pools from the Dombes included 32 pools of Mute Swan, four pools from Common Pochard, one pool from Grey Heron (*Ardea cinerea*), one

TABLE 1. Trends in densities of productive pairs and broods of Mute Swan (*Cygnus olor*) from 2002 to 2006 in the Dombes area (France).

	Year				
	2002	2003	2004	2005	2006
Breeding monitoring					
Number of observed ponds	58	90	87	79	73
Meaning density of young (birds/10 ha)	0.12	0.08	0.13	0.08	0.05
% of productive pairs	60.5	77.1	54.4	33.3	23.2
Flash census					
Number of observed ponds	918	948	1008	1030	982
Whole density (birds/10 ha)	0.80	0.53	0.57	0.90	0.73
Density of nonbreeding (birds/10 ha)	0.60	0.31	0.36	0.65	0.52

TABLE 2. Species distribution of collected carcasses in passive surveillance, number of tested pools, and number of highly pathogenic (HP) H5N1 virus–positive pools in the Dombes from mid-February to the end of August 2006.

Species	Collected carcasses (number tested)	Number tested pools (% tested carcasses)	HP H5N1 virus–positive pools (% tested pools)	Number of birds in the H5N1 HP virus–positive pools (% min–max HP H5N1–positive birds)
Mute Swan (*Cygnus olor*)	130 (89)	65 (68%)	32[a] (49%)	54[b] (36–61%)
Common Pochard (*Aythya farina*)	13 (13)	11 (100%)	4 (36%)	6 (31–46%)
Great-crested Grebe (*Podiceps cristatus*)	2 (2)	2 (100%)	0	0
Grey Heron (*Ardea cinerea*)	19 (12)	12 (63%)	1 (9%)	1 (9%)
Greylag Goose (*Anser anser*)	3 (3)	3 (100%)	1	1
Eurasian Buzzard (*Buteo buteo*)	40 (16)	16 (40%)	1 (6%)	1 (6%)
Mallard (*Anas platyrhynchos*)	25 (23)	22 (92%)	0	0
Common Black-headed Gull (*Larus ridibundus*)	10 (9)	7 (90%)	0	0
Other Anatidae[c]	19 (12)	8 (63%)	0	0
Other wild birds out of the Dombes	210 (66)	55 (83%)	0	0
Tufted Duck (*Aythya fuligula*)	1	1	1	1
Great Crested Grebe	3 (3)	3 (100%)	1	1

[a] Corresponds to the minimal number of positive birds among the analyzed birds.

[b] Corresponds to the maximal number of positive birds among the analyzed birds.

[c] Northern Pintail (*Anas acuta*; *n*=1), Ruddy Duck (*Oxyura jamaicensis*; *n*=1), Red-crested Pochard (*Netta rufina*, *n*=8), teal (*n*=1), duck unspecified (8).

pool from Eurasian Buzzard, and one from a Greylag Goose (Table 2). Among these 39 positive pools, 62% were positive both on cloacal and tracheal swabs, 28% only on tracheal swabs (nine pools from Mute Swan and two pools from Common Pochard) and 10% only on cloacal swabs (two pools from Mute Swan, one pool from Greylag Goose, and one pool from Common Pochard). Virus was detected from oral swabs from most positive birds, but some birds shed the HPAI H5N1 only by the fecal route.

The first positive case involved three Common Pochards found dead on 13 February 2006. Positive swans, which represented 82% of the positive pools, were detected from 18 February to 15 March. The proportions of positive Mute Swans and Common Pochards were similar (respectively, 36–61% and 31–46%), but the sample size for pochards was inadequate for statistical comparison. No HAPI H5N1 positive Mallards or Common Black-headed Gulls (*Larus ridibundus*) were detected, and only one dead raptor was infected.

Positive dead birds were found in very restricted areas (Fig. 4). Infected birds were detected on 20 of the 67 ponds in the Dombes region where dead birds were observed (Fig. 4). Based on a 1-km radius around the positive cases, nine foci of infection were evident within a 25-km radius. Based on a weekly census on the 32 monitored ponds (Fig. 3), it was observed that most of the infected ponds were large (>10 ha), and in February, the densities of Mute Swans were six times higher on the infected ponds (2.5 birds/ha) than on the surrounding noninfected ponds (0.4 swans/ha). The same pattern was observed for the other species of *Anatidae* and coots; densities three times higher on infected rather than noninfected ponds (16.6 versus 5.7 birds/ha).

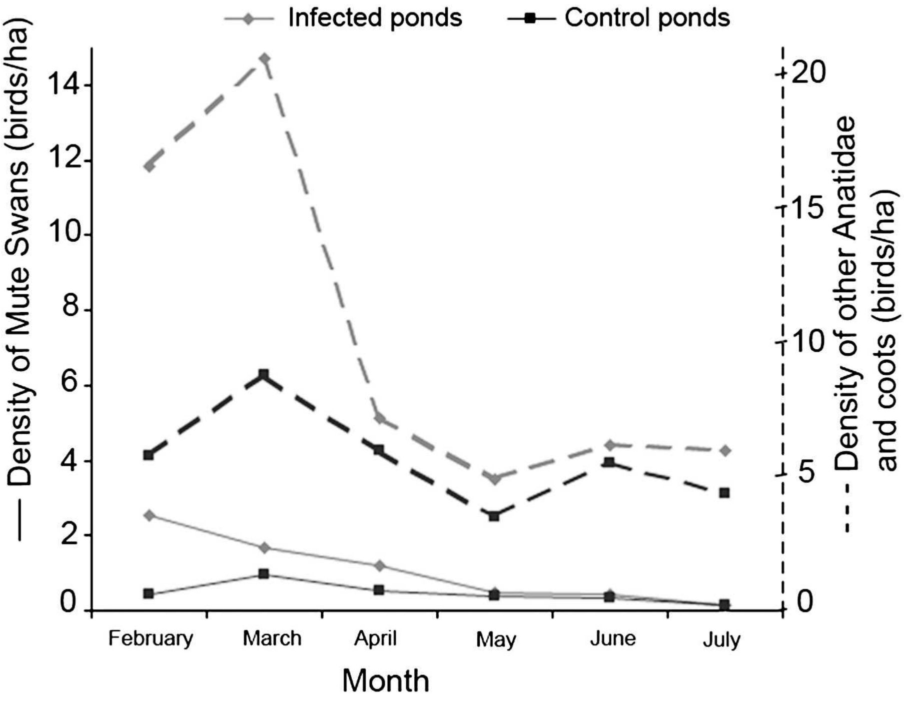

FIGURE 3. Densities of Mute Swans, other *Anatidae*, and Eurasian Coots in 16 infected ponds and 16 surrounding control ponds from February to July 2006.

The avian influenza epizootic, defined as the period when HPAI H5N1 virus-infected wild birds were found, lasted 2 mo, with observed mortality decreasing during March (Fig. 5). From the end of April (week 17) on, bird mortalities markedly decreased and no additional positive birds were detected.

During the postepizootic period, surveillance was maintained by testing 102 Mute Swans that were shot during weeks 22–24. All birds were PCR negative, and during necropsy, no lesions suggestive of HPAI H5N1 (Baroux et al., 2007) were observed. However, more than 30% of these birds were H5 seropositive as determined by the hemagglutination inhibition test. Additionally, the 598 healthy birds (primarily corvids and passerines) were negative and HPAI H5N1 virus was not isolated from the 100 sentinel mallards that were tested twice a month. Low-pathogenic H5N2 viruses (close to the French LP strains previously described by Cherbonnel et al., 2007) were detected in August in three swab pools sampled on the sentinel Mallards of one enclosure.

DISCUSSION

Because of the abundance of waterfowl, the Dombes region was considered a high-risk area for avian influenza, and it was not surprising that the first case of HPAI H5N1 in France was detected at this site. Prior to 2006, a low prevalence of low-pathogenic avian influenza viruses had been documented in waterfowl in France, and particularly at this site (Jestin et al., 2006; Hars, unpublished data). There were no prior isolations of any HPAI strains in the Dombes (Durand, 2006).

Before February 2006, HPAI H5N1 had been detected in several European countries, as it moved westward from infected area of the Black Sea region. Based on the winter census in the Dombes, the Mute Swan population was stable between December 2005 and February 2006 (Fig. 1D), with only a small

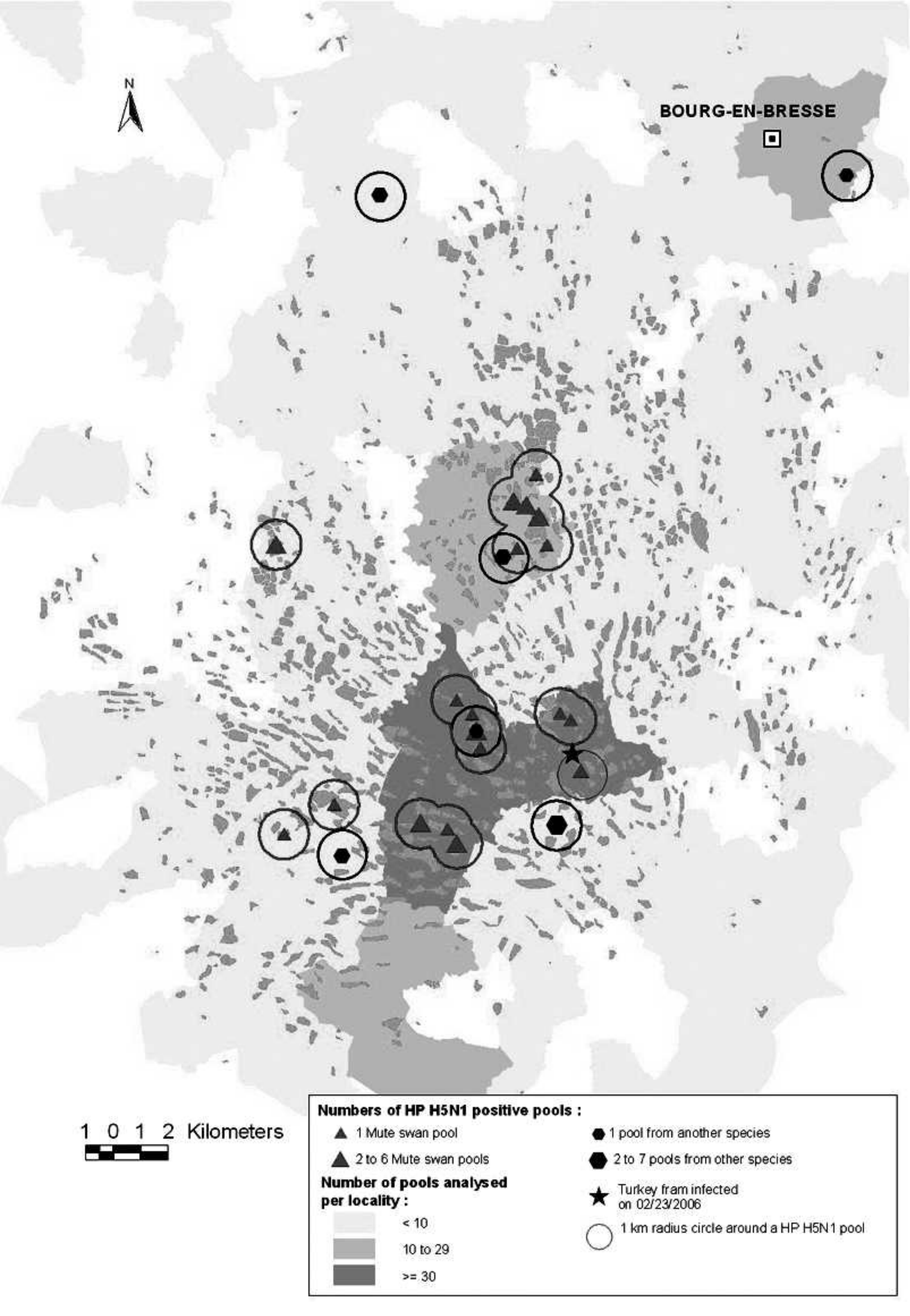

FIGURE 4. Distribution of HP H5N1–positive pools in the ponds of the Dombes in 2006.

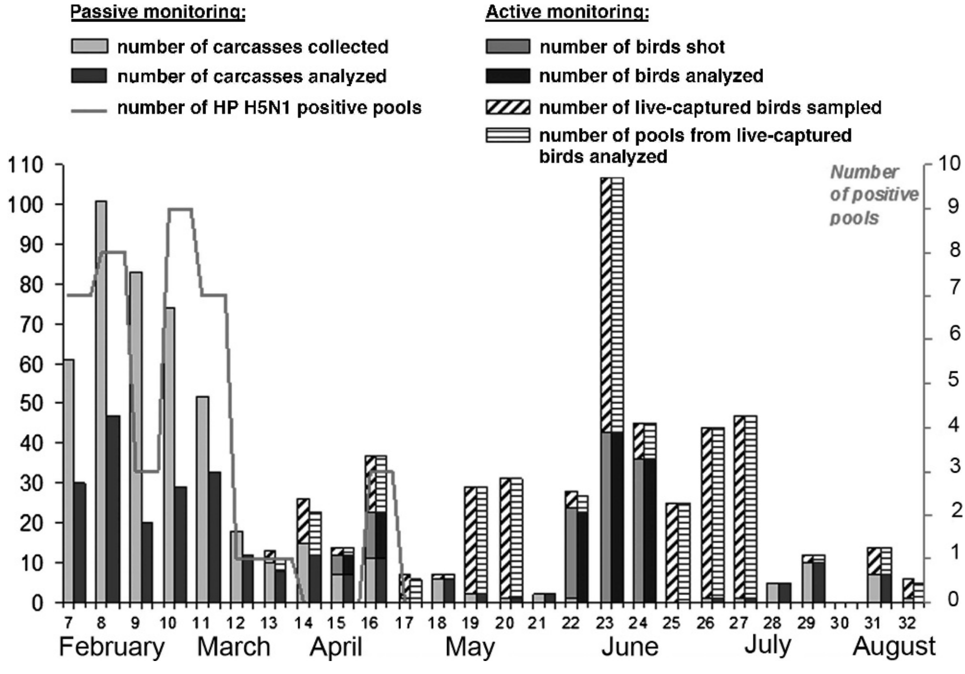

FIGURE 5. Temporal evolution (in weeks) of the number of collected and analyzed carcasses of sampled and analyzed healthy birds (except sentinel mallards) and of HP H5N1–positive pools.

number of migrants arriving in November and December. These swans arrived 2 mo before the first cases of HPAI H5N1. In contrast, large numbers of Common Pochards arrived between the end of January and the first days of February (Fig. 1C). The most likely hypothesis is that the virus was introduced by Common Pochards, or perhaps other migratory ducks, pushed from the East by the cold weather. Although HPAI H5N1 virus was not reported from Common Pochards elsewhere in Europe during the 2005/2006 outbreak, the virus was isolated in other diving ducks: 49 Tufted Ducks in Switzerland, Denmark, Germany, and France and seven Greater Scaup (*Aythya marila*) in Sweden (EUROPA, 2006).

Based on molecular data (Le Gall-Reculé et al., 2008) there seemed to be two HPAI H5N1 virus introductions into the Dombes region; two different subgroups, inside Qinghai clade 2.2.1, were identified. The first one was isolated from the first pochard case; the second was isolated 6 days later from the infected turkey farm and all of the subsequent wild bird cases. It is then likely that the two HPAI H5N1 strains were present when the outbreak began, which suggests that there were multiple introductions via migratory waterfowl.

As 82% of the positive pools analyzed in the Dombes originated from Mute Swans, this species represented the best indicator species present on these wetlands. Similar results were seen throughout Europe; 63% of 746 infected dead birds found in Europe from February to May 2006 were Mute Swans (Eurosurveillance, 2006). Carcasses of Mute Swans are easy to detect and may have biased these results. However, the estimate of the Mute Swan population in the Dombes during the epizootic approximated 500–600 individuals in February–March 2006, and 54 swans were represented in the positive pools. This may represent an underestimate of the true mortality rate, as some virus-negative swans with characteristic lesions (hemorrhages, pancreatitis, encephalitis) and nervous signs also may have been infected (Gavier-Widen et al., 2006; Baroux et al., 2007; Teifke et al., 2007). Based on these numbers, we estimate that mortality in swans probably exceeded 10%. In February–March 2006, 25,000 to 30,000 ducks, of which half were Common Pochards, were present on the ponds. If ducks were affected by the same 10% mortality rate, we would have expected 2,000 or 3,000 dead ducks during the outbreak, which would not have been missed by surveillance efforts. Based on our field observations it appears that the Mute Swan is particularly sensitive to the HPAI H5N1 virus, and this has been subsequently confirmed by experimental infection of this species (Brown et al., 2008). This sensitivity and its visibility make for a very good epidemiologic sentinel for these viruses.

It is unknown if Mute Swans are reservoirs for these viruses or if asymptomatic carriers for these viruses exist in wild birds. The 102 swans that were tested 2 mo after the epizootic had no lesions compatible with HPAI H5N1 infection and no virus was detected. However, 30% of these birds had H5 antibodies, 6 wk after the outbreak. This may have been related to contacts with the HPAI H5N1 virus or to contacts with LPAI H5 strains. There was no indication of residual infection in the Mute Swan population.

Among the *Anatidae*, only swans and pochards demonstrated significant susceptibility to the HPAI H5N1 virus. This virus was not detected in either dead or sentinel Mallards, despite the large number that was tested in January (Fig. 1B), which raises the question of the sensitivity of this species. In the same way, neither mortality nor virus detections were observed in gulls and corvids, although these were very numerous in the Dombes region. As with raptors, these species could become infected by predation or scavenging of infected birds. One infected Eurasian Buzzard was found in the Dombes, and similar results have been reported from other European countries (EUROPA, 2006; Mörner et al., 2006).

The results of the flash census in June 2006 revealed a clear decrease in the Mute Swan population as compared to 2005. This may have resulted from HPAI H5N1 mortality and the surveillance-related removal of 100 birds in early June. There was also a decrease in reproductive success in 2006 compared to previous years. This reduction may have resulted from the illegal destruction of eggs, as Mute Swans were locally perceived as the carrier of HPAI H5N1. Because of these confounders, the impact of HPAI H5N1 on swan reproduction is uncertain. For the other *Anatidae* species, the impact of the disease is probably very low. Reproduction during in 2006 showed a decrease of the nesting populations that is consistent with a decline observed since 1994; this is probably related to changes in agricultural practices.

The outbreak of avian influenza, which principally involved wild birds, lasted only 2 mo, from mid-February to mid-April. Afterwards, no HPAI H5N1 virus was isolated, despite the passive and active surveys. The infection of a pond, defined as the period during which wild birds are found infected, did not generally last more than 5 wk. The same temporal evolution has been observed in the other outbreaks in Europe. This fact can be partially explained by the increase in water temperatures (Brown et al., 2007) and by the decrease in the density of waterfowl in April. Moreover, the rainy spring period, increasing the water surface of the ponds, likely had a diluting effect on the virus and its transmission.

This outbreak of HPAI also was spatially restricted. The Dombes was the only area in France where significant mortality was observed, and even within the Dombes, infected birds were detected on only 20 ponds. Only 39 swab pools, which included 63 dead wild birds, were found positive, although more than 30,000 aquatic birds were staging on the ponds during the outbreak.

The local movements of Mute Swans can explain the diffusion of the disease into the Dombes area, but cannot explain those cases that were detected in other areas of France. It is unlikely that the three positive cases observed in February, in the Leman Lake and the Mediterranean coast, came from migrant swans of the Dombes. It is also possible that the Leman Lake cases could be related to an outbreak in Switzerland.

The restricted spread of the disease in time and space, associated with a low rate of mortality in wild birds and the contamination of only one local poultry farm, are consistent with the pattern observed throughout Europe (EFSA, 2006; OIE, 2006; Terregino et al., 2006; Holko et al., 2006; Nagy, 2007). The epizootic observed in the Dombes area provides evidence that wild bird mortality associated with the HP H5N1 viral strain that circulated in Europe in 2006 was species dependent, and transmission among wild birds was limited.

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