ANSES Opinion
Request No 2015-SA-0241

Maisons-Alfort, 14 December 2015

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

concerning the risk of avian influenza

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES's public health mission involves ensuring environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with the necessary information concerning these risks as well as the requisite expertise and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its opinions are made public.
This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 14 December 2015 shall prevail.

On 27 November 2015, ANSES received a formal request from the Directorate General for Food (DGAL) and the Directorate General for Health (DGS) to conduct a scientific expert appraisal relating to the risk of avian influenza.

BACKGROUND AND PURPOSE OF THE REQUEST

On 24 November, the National Reference Laboratory (NRL) for Influenza (ANSES - Ploufragan) identified a strain of highly pathogenic (HP) avian influenza (AI) H5N1 in a backyard flock of 32 birds of the species *Gallus gallus* (hens and chickens) located in Dordogne.

Since this first case, several outbreaks have been confirmed by the NRL. They involve different bird species (ducks, chickens, guinea fowl, geese), different *départements* (Dordogne, Gers, Haute-Vienne, Landes, Pyrénées-Atlantiques) and different virus types: H5N1, H5N2 and H5N9 (see the National Epidemiological Surveillance Platform for Animal Health[1]).

On 27 November 2015, in view of the first confirmed outbreaks in Dordogne, the DGAL and the DGS called on ANSES to answer the following questions:

- ***Animal health component***

1. What is the zoonotic potential of the HPAI H5N1 virus identified in Dordogne? Are the measures for restricting movements of domestic carnivores stipulated by the Ministerial Decree of 18 January 2008 relevant for this virus?
2. What are the most likely assumptions about the source of the infection?

[1] http://www.plateforme-esa.fr/?q=node/35869

3. Which surveillance measures, going beyond those stipulated by the regulations, would help assess the spread of the virus and ensure, if no other cases were identified, that the highly pathogenic strain is no longer circulating?

➢ ***Human health component***

4. In view of the information presented in advance, is it necessary to update ANSES's previous work and recommendations for the population, in light of the pathogenicity of the virus?
   4.1. Risk of exposure by ingestion, mainly by consumption:
   - of raw and cooked foods, such as meat (poultry), eggs, processed products;
   - of water potentially contaminated with the avian influenza virus;
   - of food contaminated with water, potentially contaminated with the avian influenza virus;

   4.2 Risk of exposure by the respiratory route, especially when handling poultry and preparing products from infected poultry.

ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with French Standard NF X 50-110 "Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)".

It was carried out by the HPAI H5 2015 Emergency Collective Expert Appraisal Group (GECU) which met on 30 November, 3 and 14 December 2015. An intermediate memorandum was drafted by the scientific coordination, then validated electronically on 4 December 2015.

This intermediate memorandum was sent to the requestors and appears in full in Annex 1. The answers to the questions raised by this intermediate memorandum are given below.

Following the complete sequencing of the H5N1 virus genome, the GECU was able to supplement the questions relating to the zoonotic risk. On 14 December 2015, it validated these additional answers, which are listed below.

ANALYSIS AND CONCLUSIONS OF THE HPAI H5 2015 GECU

1 - Reminder of the answers to the questions raised in the intermediate memorandum

- *Answer to question 1.b: Are the measures for restricting movements of domestic carnivores stipulated by the Ministerial Decree of 18 January 2008 relevant for this virus?*
  The GECU believes that in the current situation there is no evidence that restrictions on movements of dogs and cats are necessary and should be applied in the present case, apart from in the infected holdings, in order to avoid any dispersion of the virus outside the holding, as cats or dogs can roam freely and transport the virus mechanically.

- *Answer to question 2: What are the most likely assumptions about the source of the infection?*
  In the current state of knowledge, the GECU believes that the two most likely assumptions about the origin of the infection are (1) the circulation in poultry of low pathogenic (LP) AI that mutated into HP in these poultry, and (2) less likely, the circulation in wild birds of LPAI that mutated into HP in poultry.

- *Answer to question 3: Which surveillance measures, going beyond those stipulated by the regulations, would help assess the spread of the virus and ensure, if no other cases were identified, that the highly pathogenic strain is no longer circulating?*

In view of the detection of two other outbreaks after receipt of the formal request, it was agreed with the DGAL that this question would be reformulated and addressed at a later time.

2 - Answer to questions relating to the zoonotic risk

2.1. Question 1: a) concerning the zoonotic potential of the HPAI H5 viruses.

The experts recall that these HPAI viruses, H5N1, H5N2 and H5N9, are clearly different to the highly pathogenic H5N1 viruses of the A/goose/Guangdong/1/96 Asian lineage. It should be reiterated that the latter are the only HPAI H5 viruses described as being responsible for severe forms in humans[2].

In addition, the information from the complete sequencing of the H5N1 virus genome, carried out by the ANSES Ploufragan Laboratory and analysed by the National Reference Laboratory for avian influenza and Newcastle disease, and the National Reference Centre for influenza viruses (see annex 2), led the experts to the following conclusions:

- a comparison of the nucleotide sequence of the 150169a sample with databases or recent literature reviews identifying the determinants of adaptation of avian influenza viruses to humans, revealed that the studied virus does not present all of the determinants known to favour the transmission of avian influenza viruses to humans;
- however, and like most contemporary avian viruses with low pathogenicity for birds, circulating in Europe, the virus has a number of mutations previously identified as likely to promote replication and/or interfere with antiviral responses in mammals, which means that the occurrence of a respiratory infection in specific circumstances of high exposure to infected birds, cannot be ruled out;
- nevertheless, all of the segments analysed are avian-type, which means that the risk of transmission to humans can be regarded as nearly nil;
- the risk of human-to-human transmission is considered even lower than the above.

2.2. Question 4: In view of the information presented in advance, is it necessary to update ANSES's previous work and recommendations for the population, in light of the pathogenicity of the virus?

2.2.1. Risk of exposure by ingestion, mainly by consumption (1) of raw and cooked foods, such as meat (poultry), eggs, processed products, (2) of water potentially contaminated with the avian influenza virus, (3) of food contaminated with water potentially contaminated with the avian influenza virus

The experts recall that, apart from a few rare suspicions associated with the ingestion of blood and raw viscera from poultry in Asia (Gambotto *et al.*, 2008), no human cases have been confirmed for the Asian HPAI H5N1 virus *via* consumption of food or water, despite its proven zoonotic potential. In its Opinion No. 2005-SA-0258, which focused on the assessment of the risk to humans *via* the consumption of foodstuffs derived from poultry infected with the Asian HP H5N1 virus, AFSSA had thus estimated the risk for the consumer as nil to negligible (the negligible level resulting from these rare suspicions associated with highly unusual consumption patterns).

More specifically, the biomolecular conclusions relating to the HP H5N1 virus identified in Dordogne mean that it can be confirmed that the risk for the consumer is even lower than the above.

[2] http://www.who.int/influenza/human_animal_interface/HAI_Risk_Assessment/en

2.2.2. Risk of exposure by the respiratory route, especially when handling poultry and preparing products from infected poultry

In view of the answer presented in Section 2.1, the risk of infection by the respiratory route under these conditions of exposure cannot be totally ruled out.

AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses these initial conclusions of the HPAI H5 2015 GECU on the risk of avian influenza. They may be supplemented as new data, both epidemiological and genetic, become available.

Marc Mortureux

KEYWORDS

Avian influenza, HPAI, H5N1, H5N2, hens, geese, ducks, guinea fowl, birds, sequencing

ANNEX 1

INTERMEDIATE MEMORANDUM of 4 December 2015
of the French Agency for Food, Environmental and Occupational Health & Safety

concerning the risk of avian influenza

BACKGROUND AND PURPOSE OF THE REQUEST

On 24 November, the National Reference Laboratory (NRL) for Influenza (ANSES - Ploufragan) identified a strain of highly pathogenic (HP) avian influenza (AI) H5N1 in a backyard flock of 32 birds of the species *Gallus gallus* (hens and chickens) located in Dordogne.

In the framework of the European annual survey on the circulation of avian influenza, confirmed positive serological results regarding AI subtype H5 were reported, like every year, in holdings of domestic water fowl (Cherbonnel *et al.*, 2007; Briand *et al.*, 2010; Sadonès *et al.*, 2011; Sadonès *et al.*, 2012; Sadonès *et al.*, 2013; Guerry *et al.*, 2014; Guerry *et al.*, 2015). In 2015, the seropositive results involved farms in Dordogne (2), Landes (7), Aveyron (2), Vendée (3) and Deux-Sèvres (1), corresponding to 7 Peking breeder duck holdings, 5 breeder geese holdings and 3 ready-for-gavage duck holdings. Additional results are pending.

Two further outbreaks of HPAI H5 were then identified in the same *département*, one 40 km to the north of the first one, the other 90 km to the south.

Regulated zones have been defined[3] around these outbreaks in Dordogne, as follows:

- a protection zone (PZ) of 3 km around each outbreak. A clinical examination of poultry is to be carried out in all holdings, whether professional or not, identified in the PZ and samples taken if necessary;
- a surveillance zone (SZ) of 10 km around each outbreak. Regular monitoring of commercial holdings is carried out here by the DDCSPP. Farmers must declare all morbidity, mortality or significant declines in production data;
- an additional low-risk zone, or Area B within the meaning of point (8) of Decision 2006/415/EC, encompassing the two outbreaks in the north of the *département*. This "low risk" zone separates the regulated zone affected by the disease from the disease-free zone. Its aim is to limit the risk of spread, mainly by restricting movements of poultry and their products and by-products.

In the *département*, gatherings of birds are prohibited[4].

[3] - Prefectoral Order no. DDCSPP/VESPA/20151125-0002 determining a restricted zone following a declaration of infection of highly pathogenic avian influenza (Biras)

- Prefectoral Order no. DDCSPP/VESPA/20151130-0001 determining a restricted zone following a declaration of infection of highly pathogenic avian influenza, as amended by the Order No DDCSPP/VESPA/20151201-0003 (Saint Paul la Roche)

- Prefectoral Order no. DDCSPP/VESPA/20151201-0002 determining a regulated zone following a declaration of infection of highly pathogenic avian influenza on the commune of Domme (Dordogne)

[4] Prefectural Order no. DDCSPP/VESPA/20151130-0002 on the ban on presenting ornamental birds, poultry and game birds at gatherings, markets, exhibitions or shows organised in the Dordogne *département* and on their participation in these events in other *départements*

Restrictions have also been implemented on bird hunting (PZ), on the use of dogs for hunting purposes and on game bird release (PZ and SZ).

At the national scale, the risk level has not changed.

Activation of wild bird surveillance through the monitoring of bird mortalities is under way, mainly by raising awareness in the SAGIR network and by conducting surveys in Dordogne.

In this context, the DGAL and the DGS called on ANSES to answer the following questions:

- ***Animal health component***

1 What is the zoonotic potential of the HPAI H5N1 virus identified in Dordogne? Are the measures for restricting movements of domestic carnivores stipulated by the Ministerial Decree of 18 January 2008 relevant for this virus?

2 What are the most likely assumptions about the source of the infection?

3 Which surveillance measures, going beyond those stipulated by the regulations, would help assess the spread of the virus and ensure, if no other cases were identified, that the highly pathogenic strain is no longer circulating.

In view of the detection of two other outbreaks after receipt of the formal request, it was agreed with the DGAL that this question would be reformulated and addressed at a later time.

- ***Human health component***

4 In view of the information presented in advance, is it necessary to update ANSES's previous work and recommendations for the population, in light of the pathogenicity of the virus?

4.1 Risk of exposure by ingestion, mainly by consumption:

- of raw and cooked foods, such as meat (poultry), eggs, processed products;
- of water potentially contaminated with the avian influenza virus;
- of food contaminated with water potentially contaminated with the avian influenza virus;

4.2 Risk of exposure by the respiratory route, especially when handling poultry and preparing products from infected poultry.

ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with French Standard NF X 50-110 "Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)".

It was carried out by the HPAI H5 2015 Emergency Collective Expert Appraisal Group (GECU) which met on 30 November and 3 December 2015. A draft intermediate memorandum from the GECU was written by the scientific coordination, then validated electronically on 4 December 2015.

ANALYSIS AND CONCLUSIONS OF THE HPAI H5 2015 GECU

As a preamble, it should be emphasised that the French health situation with regard to the highly pathogenic avian influenza (HPAI) viruses detected in Dordogne may continue to evolve rapidly. Accordingly, this opinion corresponds to the data available and to the situation on the date of its signature.

1. Data relating to the outbreaks of HPAI and the viruses identified in the Dordogne[5]

1.1. Health situation on 4 December 2015

As of 4 December 2015, **three outbreaks** of highly pathogenic (HP) avian influenza (AI) have been identified.

- *Outbreak 1*

The first outbreak was detected in a backyard flock of 32 chickens and laying hens (*Gallus gallus*) aged 9-10 months, located at Biras in Dordogne.

From 14 November 2015, sudden mortalities (22 cases), without any other symptoms, were recorded. In autopsy, the only macroscopic lesions found in certain subjects were a marked subcutaneous oedema in the head, extending to the neck, or even to the sternum.

On 20 November 2015, the Dordogne departmental testing laboratory (LDA24) identified the genome of an avian influenza virus subtype H5 from samples (oropharyngeal/tracheal and cloacal swabs) taken during the autopsy. The 10 remaining birds were culled and the premises disinfected.

On 24 November 2015, the NRL confirmed these results. Partial sequencing of the virus led to identification of a highly pathogenic strain of subtype **H5N1**.

- *Outbreak 2*

The second outbreak was detected in a holding with some 12,000 ready-for-gavage ducks aged 9 weeks with access to an outdoor run and 2,000 fattened ducks in a gavage facility, located in Saint-Paul-la-Roche in Dordogne, around forty kilometres to the north of the first outbreak.

On 10 November 2015, samples had been taken in the framework of the annual serological survey (programmed surveillance) in the absence of reported symptoms and received at the NRL for confirmatory analyses on 18 November 2015.

On 20 November 2015, the analysis of a batch of 20 sera, performed by the NRL, yielded positive results for AI subtype H5. Following these results, samples (oropharyngeal and cloacal swabs) were taken for virological testing.

These samples were received by the NRL on 26 November 2015. Partial sequencing of the virus resulted in identification of a highly pathogenic strain of subtype H5.

- *Outbreak 3*

The third outbreak was detected in an outdoor holding with 1,168 breeder geese (including 800 goslings) and 170 ducks located in Domme on the banks of the Dordogne River, in the south of the *département*, approximately 90 kilometres from the first outbreak.

On 3 November 2015, samples had been taken in the framework of the annual serological survey (programmed surveillance), which were received at the NRL for confirmatory analyses on 10 November 2015. On 13 November 2015, the analysis of the batch of 20 sera, performed by the NRL, yielded positive results for AI subtype H5. Following these results, samples (oropharyngeal and cloacal swabs) were taken on 25 November in order to conduct virological testing (targeting detection of the H5 gene), which proved negative on 27 November 2015.

Two days later, three goslings died in a batch from this holding. An autopsy was performed and samples taken from these goslings as well as from two other goslings presenting symptoms from the same batch, and from one gosling from a second batch. Lesions of the pericardium and hypertrophy of the liver, spleen and kidney were observed on the goslings from batch 1. Pancreatitis was observed in the gosling from batch 2.

[5] As of the date of signature of this memorandum, a total of 12 outbreaks have been detected: in Dordogne (7 outbreaks), Landes (3) Haute-Vienne (1) and Gers (1). Three HPAI viruses of subtype H5 have been identified: H5N1, H5N2 and H5N9.

On 30 November 2015, the NRL confirmed, from these latest samples, the presence of an HPAI virus subtype **H5N2**.

To date, no epidemiological link has been identified between these three outbreaks. The epidemiological investigations are in progress. All poultry present on these holdings have been slaughtered and the premises disinfected.

1.2. Characteristics of the viruses identified in the three outbreaks

1.1.1 HPAI virus subtype H5N1 (first outbreak)

The NRL obtained a partial sequence of the H5 gene (240 nucleotides). The sequence of the cleavage pattern, i.e. HQRRKRGLF, corresponds to the cleavage pattern of a highly pathogenic strain. The partial sequencing of the NA gene (549 nucleotides) enabled the subtype N1 to be identified.

A phylogenetic analysis of the H5 and N1 sequences showed that they are not directly related to the highly pathogenic H5N1 virus sequences from the A/goose/Guangdong/1/96 Asian lineage. The H5 sequences obtained are directly related to low pathogenic AI H5 sequences circulating in Europe and available in the data banks, and have at most 95% identity with the closest low pathogenic H5 virus sequences identified in France. Similarly, the N1 sequences obtained are directly related to the N1 sequences from AI circulating in Europe.

Consequently, the HPAI H5N1 virus identified in the Dordogne is different to the Asian HPAI H5N1 virus that appeared in 1996, which was responsible for the panzootic between 2004 and 2006 and which continues to circulate today, particularly in Asia, Egypt and West Africa. It is also different to the HPAI H5N1 virus that was circulating periodically in North America in early 2015. In Europe, apart from the outbreaks of Asian lineage HPAI H5N1, the most recent outbreaks of HPAI H5N1 were reported in turkeys in England in 1991. The virus detected in the Dordogne is not directly related to this A/turkey/England/50-92/91 H5N1 virus.

This genetic difference implies that this H5N1 virus may have different characteristics to the Asian and American viruses, particularly in terms of virulence and therefore pathogenicity to domestic or wild birds, and to humans. The GECU reiterates that viruses from the Asian lineage have very specific characteristics, notably a high pathogenicity to humans, that are not found in the other highly pathogenic H5N1 viruses.

1.1.2 HPAI virus subtype H5 (second outbreak)

Analyses by real-time RT-PCR targeting the M and H5 genes using RNA extracted from the swabs in a mixture of five, and then tested individually, produced late amplification signals (Ct>38 and Ct>35 respectively) due to an extremely low viral load, near the limit of detection. The partial H5 sequences obtained had between 98% and 99% identity with the H5 sequence from the first outbreak, on a common portion of gene of 143 nucleotides. Typing of neuraminidase (NA) by RT-PCR was unsuccessful, because the viral load was too low.

1.1.3 HPAI virus subtype H5N2 (third outbreak)

Analyses by real-time RT-PCR targeting the M and H5 genes gave negative results on the goslings from the first batch, and produced early amplification signals on the second batch. The partial sequences of the H5 gene obtained were closely related to the sequences identified in the first two outbreaks (97% to 98% identity). The partial sequencing of the NA gene enabled the subtype N2 to be identified. This virus is different to the HPAI H5N2 virus that was/is circulating in Asia, especially Taiwan, and North America in 2015. In Europe, the most recent outbreaks of HPAI H5N2 were reported in chickens in Italy in 1997. The H5N2 virus detected in the Dordogne is also different, presenting only 94% identity with the A/chicken/Italy/330/97 H5N2 virus.

In summary, as of 4 December 2015:

- three outbreaks of HPAI have been detected in three holdings: one in a backyard flock of hens/chickens and two in professional holdings (free-range geese and ducks) located in Dordogne;
- to date, no epidemiological link has been identified between these outbreaks. Additional investigations must still be conducted;
- at least two HPAI viruses have been identified in these three holdings:
  - an HPAI H5N1 virus in the hens/chickens (*Gallus gallus*) from the first outbreak. Within the limit of the partial sequences available, this virus is **different to the HPAI H5 viruses (contemporary or most recent reported in Europe) available in the sequence banks and in particular the HPAI H5N1 viruses from the A/goose/Guangdong/1/96 Asian lineage**. The French HP H5N1 virus is on the other hand related to the low pathogenic European viruses from the past ten years.
  - an HPAI H5N2 virus in geese corresponding to the third outbreak is also different (within the limit of the partial sequences obtained and the viral sequences available in the sequence banks) to the HPAI H5N2 viruses. The partial H5 gene from the French HP H5N2 virus is closely related to the H5 gene from the aforementioned French HP H5N1 virus as well as to the H5 gene from the virus with incomplete subtype from the second French outbreak.

2 Question 1: a) What is the zoonotic potential of the HPAI H5N1 virus identified in Dordogne? b) Are the measures for restricting movements of domestic carnivores stipulated by the Ministerial Decree of 18 January 2008 relevant for this virus?

Following receipt of the formal request, detection of the HPAI H5N2 virus led the GECU to take this virus into account in its replies to the questions raised.

2.1 Zoonotic potential of the HPAI H5N1 and HPAI H5N2 viruses identified in Dordogne

2.1.1 HPAI H5N1 virus

Because the complete sequencing of the HPAI H5N1 virus identified in Dordogne is still ongoing as of 4 December 2015, the GECU is unable to give a view on its zoonotic potential.

- However, the experts emphasise, as specified in point 1.2.1., that this HPAI H5N1 virus is clearly different to the highly pathogenic H5N1 viruses of the A/goose/Guangdong/1/96 Asian lineage. It should be reiterated that the latter viruses are the only HPAI H5 viruses described as being responsible for severe forms in humans (http://www.who.int/influenza/human_animal_interface/HAI_Risk_Assessment/en).

Consequently, the proven zoonotic nature of the Asian lineage, responsible for the human cases reported since 1997, particularly in Asia and Egypt, cannot be extrapolated to this virus identified in Dordogne.

The data provided by the sequencing of the complete genome will help predict whether or not this viral strain has zoonotic potential.

2.1.2 HPAI H5N2 virus

Because the complete sequencing of the HPAI H5N2 virus identified in Dordogne has not been determined as of 4 December 2015, the GECU is unable to give a view on the zoonotic potential of the virus.
It should be noted that to date, no human cases of infection due to HPAI H5N2 have been reported (Freidl *et al.*, 2014; Munoz *et al.*, 2015) despite a large population being exposed to different HPAI viruses belonging to this subtype.

Answer to question 1.a)
As of 4 December 2015, the GECU does not have the complete sequences of the HPAI H5N1 and H5N2 viruses, identified in Dordogne, which would enable it to give a view on their zoonotic potential.
The experts recall that no cases of human infection have ever been reported for H5N1 viruses other than those of the Asian lineage, nor for H5N2 viruses.

2.2 Are the measures for restricting movements of domestic carnivores stipulated by the Ministerial Order of 18 January 2008 relevant for this virus?

2.2.1 Context of the Ministerial Order of 18 January 2008

The Ministerial Order of 18 January 2008 laying down the technical and administrative measures for the control of avian influenza, stipulates, when the suspicion or confirmation of an outbreak of HPAI is due to a virus of subtype H5N1 (Article 6 Point 4 and Article 10 Point 9), that the following additional measures must be implemented in the regulated zones:

- "o*bligation to keep dogs tied up or confined. They may however use a public thoroughfare if they are kept on a leash or are under the direct control of their master. They can also be transported in a cage, a closed basket or inside a vehicle;*
- *obligation to keep cats confined. They can however be transported in a cage, a closed basket or inside a vehicle.*"

It should be recalled that the measures taken in this Order concerned the specific case related to the Asian lineage HPAI H5N1 virus, detected in 2006 and 2007 in France, for which:

- the zoonotic nature is proven;
- wild birds were contaminated, with mortalities observed;
- the role of cats in the epidemiology of infection by Asian HPAI H5N1 had been demonstrated, as the virus can multiply in these animals. Clinical cases had been reported in cats and infected wild felines.
  The confinement of cats was thus related to the risk that they become contaminated through ingestion of infected wild birds, found dead or hunted, and that they could then multiply and retransmit the virus;
- the possibility that dogs may disturb wild birds and cause them to disperse (thus increasing the dispersion of the infection on the territory by contaminated birds) or transport the virus mechanically after becoming contaminated, particularly during hunting, which had led to the restrictions on movement stipulated in the Order.

The susceptibility and sensitivity of cats to this Asian H5N1 virus is not a usual characteristic of influenza viruses, including highly pathogenic ones. Thus, in this 2005-2008 context of (1) proven susceptibility and sensitivity of cats to the Asian HPAI H5N1 virus and (2) mortalities of wild birds due to this virus and proven infection of birds, these measures were designed to limit the zoonotic risk and the spread of the virus. The risk associated with cats nonetheless remained nil to negligible (AFSSA 2006). It can thus be noted that, since the emergence of this zoonotic Asian lineage, no cases of human infection linked to carnivores have been reported in the world.

2.2.2 Relevance of the restrictions on movements of carnivores in the current context

In the current context of the Dordogne, several points should be emphasised:

- no abnormal mortality has been identified in wild birds, despite the fact that hunting associations and departmental services of the ONCFS in the framework of the SAGIR network were quickly made aware and mobilised to monitor wild birds by means of field surveys;
- the outbreaks are not located in areas where wild birds gather. In particular, the first two outbreaks were in the north of the Dordogne *département*, which has no wetland at risk of gatherings of Anatidae. The site of the third outbreak was on the banks of the Dordogne

River, but does not constitute a major wintering area. Thus, from some 30,000 rings returned or observations made of mallards and teals ringed in France by the ONCFS since 2002, only one was in Dordogne (Guillemain, personal communication), showing that this *département* does not constitute a major stopover site for wild birds;

- nothing indicates that cats are susceptible and sensitive to this new HPAI H5N1 virus, which is unrelated to the Asian lineage.

Answer to question 1.b)

The GECU believes that in the current situation there is no evidence that restrictions on movements of dogs and cats are necessary and should be applied in the present case, apart from in the infected holdings, in order to avoid any dispersion of the virus outside the holding, as cats or dogs can roam freely and transport the virus mechanically.

3 Question 2: What are the most likely assumptions about the source of the infection?

The experts stress the little data available to answer this question. In particular, the GECU has no epidemiological evidence for the three outbreaks, especially since the second outbreak was only discovered through annual surveillance.

Four assumptions about the source of the infection are possible, and are presented from the most likely to the least likely, in the opinion of the GECU members:

1) Circulation in domestic birds of a low pathogenic (LP) AI virus that mutated into an HPAI virus in these domestic birds

   LPAI H5 viruses typically circulate silently in domestic and wild birds. If they mutate into an HP virus, silent circulation remains possible, especially in ducks, a species which is usually less sensitive. In addition, few holdings are subject to screening in the framework of annual programmed surveillance (sampling is designed to detect a prevalence of at least 5% with a confidence level of 95 or 99% depending on the type of holding). During this surveillance, duck farms are nevertheless regularly found seropositive for H5, but the viral screening that follows most often remains negative, as it is not carried out within a time period conducive to virus detection (Jestin *et al.,* 2009; Guerry *et al.,* 2015). It should be noted that the longer the delay, the lower the likelihood of detecting the virus and identifying the pathotype (LP or HP).

   Therefore, mutation into an HP virus and then circulation of this mutant HP, without clinical signs and escaping surveillance for a period of time, is possible.
   In addition, the HP H5 sequences of the viruses identified in the three outbreaks have a very high percentage of nucleotide identity (97-99%), which raises the issue of possible viral circulation and possible reassortment of a highly pathogenic H5 virus. The data currently available do not enable the GECU to give a view on these questions.

   The GECU believes that this assumption about the source of the infection is the most likely.

2) Circulation in wild birds of an LPAI H5 virus which may have mutated, either after circulating in domestic poultry, or when passing into these poultry

   The dates of the signs of mortality in the backyard flock coincide with the end of the migration peak. This is not completely over and birds are still migrating. During such a period, the introduction by wild birds (mainly Anatidae) of influenza viruses in holdings is theoretically possible. However, this assumption seems less likely in the present case and on the basis of the information available as of 3 December 2015, since the outbreak zones (in particular the north of the *département*) are neither migratory stopover zones nor resting areas, nor wintering grounds for wild birds, nor major gathering sites for resident water

birds. The information from the epidemiological investigation does not at this stage indicate whether, in the outdoor poultry runs, the presence of relay birds other than Anatidae was noted (such as passerines or Laridae - gulls), likely to play a role in the emergence of these outbreaks.

The GECU believes that this second assumption, without being entirely excluded, is less likely than the previous one.

3) Circulation in wild birds of an HPAI virus transmitted to poultry

There are not, at the present time, any reports of HPAI viruses in wild birds in Europe, notably in countries that have established large-scale surveillance for avian influenza in birds and forming part of the same migration path as the west of France (the Netherlands, Sweden, Belgium, for example). This programmed surveillance focuses on birds killed by hunting or found dead, but also captured for diagnostic purposes.

In addition, the countries of Northern Europe, where birds gather before and during migration (for example Sweden or the Netherlands), have not reported any abnormal mortality in birds. The GECU considers it unlikely that an HPAI virus could circulate in birds without having been detected in the framework of these various surveillance schemes.

4) Introduction via importation of the virus by trade, markets, etc.

This assumption is unlikely, because the circulation of these viral strains has not been reported at a global level.

Answer to question 2

In the current state of knowledge, the GECU believes that the two most likely assumptions about the origin of the infection are (1) the circulation in poultry of LPAI that mutated into HP in these poultry, and (2) less likely, the circulation in wild birds of LPAI that mutated into HP in poultry.

4 Question 3: Which surveillance measures, going beyond those stipulated by the regulations, would help assess the spread of the virus and ensure, if no other cases were identified, that the highly pathogenic strain is no longer circulating?

In view of the detection of two other outbreaks after receipt of the formal request, it was agreed with the DGAL that this question would be reformulated and addressed at a later time.

For the time being, the GECU notes the decision taken by the authorities to take samples for serological and virological testing in all commercial holdings located in the regulated zones, including in smallholdings in the protection zone, this time by means of a survey. The test results obtained from these samples will provide evidence to enable the experts to decide on subsequent additional surveillance measures.

Regarding the surveillance of birds, the experts stress that there is a need to strengthen the detection of abnormal mortalities, even if the lethality of the viruses in question in wild birds is not currently known. The GECU notes that active surveillance cannot be implemented in a very effective and relevant manner on hunted birds or decoys in Dordogne. Indeed, hunting of waterfowl is rare, and decoy ducks are virtually absent from the *département*. Moreover, the hunting of migratory game in Dordogne mainly concerns pigeons (wood pigeons), which in principle are not highly susceptible or sensitive to influenza viruses (they are on the other hand sensitive to Newcastle disease caused by paramyxoviruses). Similarly, there is no Anatidae capture site in the Dordogne in the ringing campaigns of the ONCFS or the National Museum of Natural History, the closest being located in Indre (la Brenne), Gironde and the Hautes-Pyrénées. Taking samples from birds captured at these sites is, in any event, not being considered as long as circulation of the HP H5 virus is not proven in wild birds.

At this stage, the only surveillance of birds in Dordogne is therefore based on the strengthening of the detection of dead birds and, where appropriate, conducting *ad hoc* virological screening.

5 Question 4: In view of the information presented in advance, is it necessary to update ANSES's previous work and recommendations for the population, in light of the pathogenicity of the virus?

5.1 Risk of exposure by ingestion, mainly by consumption (1) of raw and cooked foods, such as meat (poultry), eggs, processed products, (2) of water potentially contaminated with the avian influenza virus, (3) of food contaminated with water potentially contaminated with the avian influenza virus

Pending the results of complete sequencing of the HPAI H5N1 and H5N2 viruses, the GECU can currently only provide a preliminary, limited response to this question.

The experts reiterate that, apart from a few rare suspicions associated with the ingestion of blood and raw viscera from poultry in Asia (Gambotto *et al.*, 2008), no human cases have been confirmed with the Asian HPAI H5N1 virus *via* consumption of food or water, despite its proven zoonotic potential. In its Opinion No. 2005-SA-0258, which focused on the assessment of the risk to humans *via* the consumption of foodstuffs derived from poultry infected with the Asian HP H5N1 virus, AFSSA had thus estimated the risk for the consumer as nil to negligible (the negligible level resulting from these rare suspicions associated with highly unusual consumption patterns).

5.2 Risk of exposure by the respiratory route, especially when handling poultry and preparing products from infected poultry

Pending the results of complete sequencing of the HPAI H5N1 and H5N2 viruses, the GECU cannot currently reply to this question. However, within the limits of the available data on the French HPAI viruses detected since the end of November in the first three outbreaks, the experts do not note any significantly more pronounced tropism for the respiratory tract of infected poultry, as these viruses were detected from cloacal as well as tracheal swabs, with a similar viral load. The same is not true for the Asian lineage HP H5N1 virus preferentially excreted by the respiratory route in birds and transmitted to humans via the respiratory route. Moreover, to this day, no human cases in connection with these three outbreaks have been reported.

CONCLUSIONS AND RECOMMENDATIONS OF THE HPAI H5 2015 GECU

Taking into account the data available on the date of signature of the Opinion, the GECU can only provide a very preliminary answer on the zoonotic potential of the HPAI H5N1 and H5N2 viruses detected in Dordogne. Nevertheless, the experts:

- recall that the H5N1 virus detected in Dordogne is different to the HPAI H5N1 viruses of the A/goose/Guangdong/1/96 Asian lineage and that cases of human infection have never been reported for H5N1 viruses other than those of this Asian lineage, nor for H5N2 viruses;
- believe that in the current situation there is no evidence that restrictions on movements of dogs and cats are necessary and should be applied in the present case, apart from in the infected holdings;
- emphasise that, apart from a few rare unconfirmed suspicions associated with the ingestion of blood and raw viscera from poultry in Asia, no human cases have been confirmed for the Asian HPAI H5N1 virus *via* consumption of food or water.

The GECU considers that:

- the two most likely assumptions about the origin of the infection are (1) the circulation in poultry of LPAI that mutated into HP in these poultry, and (2) less likely, the circulation in wild birds of LPAI that mutated into HP in poultry.
- at this stage, the only surveillance of wild birds in Dordogne consists in strengthening the detection of dead birds and, where appropriate, conducting *ad hoc* virological screening, given the *département's* specific characteristics regarding water birds.

In this regard, the experts recommend:

- within the framework of mandatory programmed surveillance within the European Union, taking samples for virological testing as soon as possible after a serological suspicion, to ensure they are compatible with a possible virus detection;
- in the framework of the surveillance implemented following these outbreaks, systematically conducting both serological and virological analyses;
- taking samples during visits to holdings, even in the absence of clinical signs, given the possibility of subclinical infections.

As the French health situation with regard to the HPAI detected in Dordogne is evolving rapidly, this Opinion corresponds to the data available and the situation on the date of its signature. The GECU's answers and recommendations will be reconsidered as new data become available.

	Amino acid position	Virus H5N1 : A/chicken/France/150169a/2015	Comments	References
PB2	I63T	I63	Decrease pathogenicity in mice	PubMed : 21367983
	D256G	D256	Enhanced polymerase activity, mammalian host adaptation	PubMed : 19052090
	T271A	T271	"PB2-271A confers higher replication in mammalian cells and mice than does PB2-271T (typically found in avian influenza viruses)"	PubMed : 25812763
	Q591K	Q591	Enhanced replication efficiency and increased virulence in mice - A basic residue at PB2 position 591 (in 3 dimensional structure close to position 627) was shown to compensate for the lack of PB2-627K in the 2009 pandemic H1N1.	PubMed : 20700447;
	E627K	E627	A/Vietnam/1203/2004 isolate possessing 627Lys compared to A/Vietnam/1204/2004 with 627Glu increased replicated systemically in mice. Introduction of the Glu627Lys substitution in the A/chicken/Yamaguchi/7/2004 backbone conferred increased polymerase activity of RNP expressed. Introduction of the Glu627Lys subst	PubMed:20016035, PubMed:17922570, PubMed:17521765, PubMed:11546875, PubMed:15016548, PubMed:17098982
	D701N	D701	Introduction of Asp701Asn substitution in the A/duck/Guangxi/22/2001 backbone conferred efficient replication in the nose, trachea and lung of guinea pigs at titer levels comparable to A/duck/Guangxi/35/2001.	PubMed: 20041223, PubMed:19264775
	M28I ; A274T; K526R ; I553V; L607V	M28,A274,K526,I553,L607	A/duck/Guangxi/53/2002 differed from A/duck/Fujian/01/2002 by Met28Ile, Ala274Thr, Lys526Arg, Ile553Val, Leu607Val mutations. A/duck/Guangxi/53/2002 showed reduced polymerase activity.	PubMed:20211480
	L89V; G309D ; T339K; R477G; I495V; K627E; A676T	V89,D309,K339,G477,V495,E627,T6 76	Introduction of Leu89Val, Gly309Asp, Thr339Lys, Arg477Gly, Ile495Val, Lys627Glu, Ala676Thr naturally occurring substitutions in the A/wild duck/Hunan/021/2005 backbone conferred increased polymerase activity in mouse cells.	PubMed:19393699
	R368Q; Q391E; Q447H; K627E	R368,E391,Q447,E627	Introduction of the substitutions Arg368Gln, Gln391Glu, Gln447His, Lys627Glu in the A/Vietnam/1203/2004 backbone conferred reduced virulence as indicated by lethality in mice and conferred histologic alteration in the lungs, liver and brain of ferrets.	PubMed:16533883
	T271E	T271	adapatation hote mammifère H1N1	
PB1	K207R	K207	Introduction of Lys207Arg substitution in the A/Vietnam/1203/2004 backbone conferred increased virulence as indicated by mortality in mallards. Clinical signs of disease observed in mallards: cloudy eyes, appeared depressed, neurological signs. Introduction of Lys207Arg substitution in the A/Vietnam/1203/2004 backbone conferred decreased polymerase activity as indicated by the luciferase activity.	PubMed:17553873
	Y436H	Y436	Introduction of Tyr436His substitution in the A/Vietnam/1203/2004 backbone conferred decreased virulence as indicated by the survival rate of mice.	PubMed:17553873
	T677M	T677	Decrease virulence in mice	Pubmed : 21367983
	V3A; N328K; N375S	V3,N328,T375	Introduction of Val3Ala, Asn328Lys, Asn375Ser substitutions in the A/Vietnam/1203/2004 backbone conferred increased virulence as indicated by lethality in mice.	PubMed:16533883
	H99Y; I368V	H99,I368	Introduction of His99Tyr and Ile368val naturally occurring substitutions in the A/Indonesia/5/2005 backbone conferred increased airborne transmission in mammals	PubMed:22723413
	V473L; P598L	V473,L598	Introduction of Val473Leu and Pro598Leu substitutions in the recombinant virus A/Cambodia/P0322095/2005 (PB1, PB2, PA, NP) x WSN conferred decreased polymerase activity in 293 Tcells.	PubMed:22090209
PB1-F2	N66S	S66	Introduction of Asn66Ser substitution in the A/Hong Kong/156/1997 backbone conferred increased replication efficiency as indicated by growth kinetics in MDCK and lungs of mice. Mice inoculated with the mutant virus showed significant weight loss. Introduction of Asn66Ser substitution in the A/Vietnam/1203/2004 backbone conferred increased replication in CNS 8 days post infection using plaque assay.	PubMed:21852950
PA	T515A	T515	Introduction of Thr515Ala substitutions in the A/Vietnam/1203/2004 backbone conferred decreased polymerase activity as indicated by the luciferase activity, caused no mortality in ducks.	PubMed:17553873
	P149S; R266H; K357I; T515S	S149,R266,T357,T515	A/duck/Guangxi/53/2002 differed from duck/Fujian/01/2002 by Pro149Ser, Arg266His, Lys357Ile, Thr515Ser mutations. A/duck/Guangxi/53/2002 had limited lethality in mice.	PubMed:20211480
HA	D110N	N110	Introduction of Asp110Asn substitution in the A/chicken/Fujian/1042/05 backbone conferred increased binding to alpha 2-6 receptor as indicated by the hemadsorption assay with horse and guinea pig erythrocytes.	PubMed:19020946
	H119Y	H119	Increases HA heat stability as detected in ferret transmissible virus	
	S137N	S137	Introduction of Ser137Asn naturally occurring substitution in the A/Vietnam/1203/2004 backbone conferred increased binding to alpha 2-6 by measuring hemagglutination activities using enzymatically modified chicken RBCs.	PubMed:20427525
	S149A	S149	Introduction of Ser149Ala substitution in the A/Thailand/KAN 1/2004 backbone conferred alpha 2-6 linked receptor binding using resialylated HA assay.	PubMed:17690300
	A150V	A150	Introduction of Ala150Val substitution in the A/Cambodia/408008/2005 backbone conferred alpha 2-6 linked receptor binding using HA assay with human, horse and guinea pig RBCs.	PubMed:21343450, PubMed:18632950
	G155R	G155	Increased virus binding to alpha 2-6	PubMed : 17108965
	S171N; T172A	N171,A172	Introduction of Ser171Asn, Thr172Ala naturally occurring substitutions in the A/Vietnam/1203/2004 backbone conferred increased binding to alpha 2-6SAL without loss of binding to alpha 2-3SAL by comparing the hemagglutinin activity using enzymatically chicken RBCs.	PubMed:20427525
	N198K ou N198D	N198	Mutations at residue 198 (186 in the H3 HA) have been linked to changes in receptor specificity from viruses known to recognize avian receptor to ones that recognize the human receptor.	PubMed: 17108965
	D199G	D199	Introduction of Asp199Gly substitution in the A/Vietnam/1203/2004 backbone conferred increased binding to alpha 2-6 relative to WT using sialoglycan ELISA.	PubMed:22056389
	E202G	E202	Introduction of Glu202Gly substitution in the A/Vietnam/1203/2004 backbone conferred increased binding to alpha2-6 relative to WT using sialoglycan ELISA.	PubMed:22056389
	T204I	T204	Introduction of Thr204Ile substitution in the A/Thailand/KAN 1/2004 backbone conferred alpha 2-6 linked receptor binding suing glycan microarrays.	PubMed:17690300
	K205R	K205	Introduction of Lys205Arg naturally occurring substitution in the A/Vietnam/1203/2004 backbone conferred increased binding to alpha 2-6 without loss of binding to alpha 2-3 by comparing HA activities using enzymatically modified chicken RBCs.	PubMed:20427525
	Q208H	Q208	Introduction of Gln208His substitution in the A/duck/Egypt/D1Br12/2007 backbone conferred increased binding to alpha 2-6 using solid phase direct binding assay with sialyglycopolymer containing N-acetylneuraminic acid linked to galactose.	PubMed:21637809
	N209K	N209	Introduction of Asn204Lys substitution in the A/Vietnam/1194/2004xPR8 backbone conferred increased binding to alpha 2-6 using a solid phase binding assay with the sodium salts of sialylglycopoymers.	PubMed: 17108965
	V226I	V226	Introduction of Val226Ile substitution in the A/duck/Egypt/D1Br12/2007 backbone conferred increased binding to alpha 2-6 using solid phase direct binding assay with sialyglycopolymer containing N-acetylneuraminic acid linked to galactose.	PubMed:21637809
	K234E	K234	Introduction of Lys234Glu substitution in the A/Thailand/KAN 1/2004 backbone conferred increased replication efficiency since the virus replicated to high titers at each time point investigated in lung. The mutant also decreased virulence as indicated by lethal dose in mice.	PubMed:20519408, PubMed:18632950
	N236K	N236	Reduces heat stability of HA, results in change from avian-type to huma,-type receptor binding specificity	Pubmed : 25812763
	Q238L	Q238	Introduction of Gln238Leu substitution in the A/Indonesia/05/2005 backbone conferred agglutinated alpha 2-6 but not alpha 2-3 in turkey red blood cells (TRBC) using hemagglutination assay.	PubMed:20392847
	S239N	S239	Introduction of Ser239Asn substitution in the A/Vietnam/1203/2004 backbone conferred increased binding to 6' sialyl lactosamine relative to WT parental virus using ELISA based assay.	PubMed:16226289, PubMed:20130132, PubMed:20392847, PubMed:22056389, PubMed:18632950
	G240S	G240	Introduction of Gly240Ser naturally occurring substitution in the A/Vietnam/1203/2004 backbone conferred increased binding to alpha 2-6 but lost affinity to alpha 2-3 by comparing hemagglutination activities of enzymatically modified chicken red blood cells (cRBCs).	PubMed:16543414, PubMed:20392847, PubMed:20427525
	S251P	P251	Introduction of Ser251Pro substitution in the A/duck/Egypt/D1Br12/2007 backbone conferred slight increased binding to alpha 2-6 using solid phase binding assay.	PubMed: 21637809
	E91K	E91	Introduction of Glu86Lys, Ser134Pro, Arg508Lys substitutions in the A/Vietnam/1194/2004 (HA,NA) x PR8 backbone conferred increased binding to alpha 2-6 using a solid phase binding assay with the sodium salts of sialylglycopoymers.	PubMed:17108965
	S139P	S139	Introduction of Glu86Lys, Ser134Pro, Arg508Lys substitutions in the A/Vietnam/1194/2004 (HA,NA) x PR8 backbone conferred increased binding to alpha 2-6 using a solid phase binding assay with the sodium salts of sialylglycopoymers.	PubMed:17108965
	R509K	R509	Introduction of Glu86Lys, Ser134Pro, Arg508Lys substitutions in the A/Vietnam/1194/2004 (HA,NA) x PR8 backbone conferred increased binding to alpha 2-6 using a solid phase binding assay with the sodium salts of sialylglycopoymers.	PubMed:17108965
	H119Y; T172A; Q238L; G240S	H119,A172,Q238,G240	Introduction of the His119Tyr, Thr172Ala, Gln238Leu, Gly240Ser naturally occurring substitutions in the A/Indonesia/5/2005 backbone conferred increased airborne transmission in ferrets using paired transmission cages.	PubMed:22723413
	L145V; A150V	S145,A150	A/Thailand/676/2005 with Leu145val, Ala150Val mutations that conferred alpha 2-6 linked receptor binding using solid phase direct binding assay with sialylglycopolymer.	PubMed:17626098
	L145V; I167T	L145; T167	Increased virus binding to alpha 2-6	PubMed : 20427525; PubMed : 17626098
	S149; T204I	S149,T204	Introduction of Ser149Ala, Thr204Ile substitutions in the A/Thailand/KAN 1/2004 backbone conferred alpha 2-6 linked receptor binding using resialylated hemagglutination assay.	PubMed:17690300
	G155R; N198K	G155, N198	Mutations at residue 182 (186 in the H3 HA) have been linked to changes in receptor specificity from viruses known to recognize avian receptor to ones that recognize the human receptor.	PubMed: 17108965
	N170D; Q238L; N260D	N170,Q238,N260	Introduction of Asn170Ser, Gln238Leu, Asn260Asp naturally occurring substitutions in the A/Vietnam/1203/2004 backbone conferred increased affinity to sialoglycopolymers possessing SAalpha2-6Gal using solid phase assay.	PubMed:18404209
	S171N; T172A	N171,A172	Introduction of Ser171Asn, Thr172Ala naturally occurring substitutions in the A/Vietnam/1203/2004 backbone conferred increased binding to alpha 2-6SAL without loss of binding to alpha 2-3SAL by comparing the hemagglutinin activity using enzymatically chicken RBCs.	PubMed:20427525
	T172A; Q238L	A172,Q238	Introduction of Thr172Ala, Gln238Leu naturally occurring substitutions in the A/Vietnam/1203/2004 backbone conferred increased binding to alpha2-6SAL by comparing the hemagglutinin activity using enzymatically chicken RBCs.	PubMed:20427525
	S171N; T172A; S239N	N171,A172,S239	Introduction of Ser171Asn, Thr172Ala, Ser239Asn substitutions in the A/Vietnam/1203/2004 backbone conferred increased affinity for alpha2-6SAL using solid phase assay. The mutant virus showed 100 fold reduction in the lethality of WT.	PubMed:19116267
	N198K; Q208R; Q238L; S239N; G240S	N198,Q208,Q238,S239,G240	Introduction of Asn198Lys, Gln208Arg, Gln238Leu, Ser239Asn, Gly240Ser substitutions in the A/Indonesia/5/2005 backbone agglutinated alpha 2-6 and retained affinity for alpha 2-3 in shown using hemagglutination assay with modified turkey red blood cells (TRBC).	PubMed:20392847
	N198K; Q238L; S239N; G240S	N198,,Q238,S239,G240	Increased virus binding to alpha 2-6	PubMed : 20392847
	N198K; Q238L; G240S	N198,,Q238,G240	Increased virus binding to alpha 2-6	PubMed : 203992847
	E199G; E202D; K205S; Q238L; G240S	D199,E202,K205,Q238,G240	Introduction of Glu199Gly, Glu202Asp, Lys205Ser, Gln238Leu, Gly240Ser substitutions in the A/Hong Kong/486/1997 backbone conferred increased binding to alpha 2-6 compared to parent using hemagglutination assay with resialylated turkey red blood cells (TRBC).	PubMed:21397290
	E199G; Q238L; G240S	D199,Q238,G240	Increased virus binding to alpha 2-6	PubMed : 22056389

	E199G; S239N	D199,S239	Introduction of Asp199Gly and Ser239Asn in the A/Vietnam/1203/2004 backbone conferred increased binding to alpha 2-6 while retaining strong preference for alpha 2-3 sialoglycans using glycan array analysis.	PubMed:22056389
	E202G; Q238E, G240S	E202,Q238,G240	Introduction of Glu185Gly, Gln221Leu ,Gly223Ser substitutions in the A/Egret/Egypt/1162/NAMRU 3/2006 backbone conferred increased binding to alpha 2-6 and decreased binding to alpha 2-3 sialoglycans using glycan array analysis.	PubMed: 22056389
	Q208R; Q238L; S239N; G240S	Q208,Q238,S239,G240	Introduction of Asn198Lys, Gln208Arg, Gln238Leu, Ser239Asn, Gly240Ser substitutions in the A/Indonesia/5/2005 backbone agglutinated alpha 2-6 and retained affinity for alpha 2-3 in shown using hemagglutination assay with modified turkey red blood cells (TRBC).	PubMed:20392847
	Q208R; Q238L; G240S	Q208,Q238,G240	Introduction of Gln191Arg, Gln221Leu, Gly223Ser substitutions in the A/Egret/Egypt/1162/NAMRU 3/2006 backbone conferred increased binding to alpha2-6 and decreased binding to alpha2-3 sialoglycans using glycan array analysis.	PubMed:20392847, PubMed:22056389
	Q208R; S239N;	Q208,S239	The Gln192Arg mutation in the HA of enhanced the capacity of the avian H5N1 HA to recognize human-type SAa2,6Gal receptors. Introduction of the Ser223Asn mutation further increased the binding capacity although the latter did not have an effect on its own.	PubMed: 17108965
	N209K; R513K	N209,R513	Introduction of Asn204Lys, Arg508Lys substitutions in the A/Vietnam/1194/2004 backbone conferred increased binding to alpha 2-6 using a solid phase binding assay with the sodium salts of sialylglycopoymers.	PubMed: 17108965
	Q238L; S239N; G240S	Q238,S239,G240	Increased virus binding to alpha 2-6	PubMed : 20392847
	Q238L; G240S	Q238,G240	Introduction of reversions of Gln233Leu and Gly235Ser substitutions in the A/Vietnam/1194/2004 backbone conferred decreased expression of proinflammatory response in human respiratory epithelial cells by measuring levels of TNF alpha, IL-6 mRNA after infection of virus.	PubMed:14671130, PubMed:16543414, PubMed:18672252, PubMed:18404209, PubMed:21397290, PubMed:20427525, PubMed:20392847, PubMed:21345953, PubMed:22056389, PubMed:20041223, PubMed:19924306
	T331I	T331	Restores the heat stability of HA, possibly compensating other HA mutations in some viruses with human-type receptor binding specificity	Pubmed : 25812763
	K400I	K400	Reduces the pH value at which fusion occurs in H5 HA	Pubmed : 25812763
	N319K	N319	The NP 319K, together with PB2 701N and 714R, PA 615N, PB1 13P and 678N causes increase in polymerase activity and confers adaptation of avian influenza virus to the mammalian host.	PubMed: 16339318
NP	Q357K (with PB2627K)	Q357	Enhanced virulence in mice	PubMed : 18248089
	R99K; S345N	R99, S345	Introduction of Arg99Lys and Ser345Asn naturally occurring substitutions in the A/Indonesia/5/2005 backbone conferred airborne transmission in mammals.	PubMed:22723413
	deletion	No		
	V116A	V116	Introduction of Val95Ala substitution in the A/Turkey/15/2006 backbone conferred decreased sensitivity to oseltamivir and zanamivir using NA inhibition assay and measuring NA enzyme kinetics.	PubMed: 20016036; PubMed : 20523902; PubMed : 17112602;
	I117V	I117	A/Chicken/Indonesia/Wates/77/2005 isolate with Ile97Val substitution conferred decreased sensitivity to oseltamivir using fluorescence based NA enzyme inhibition assay.	PubMed:17112602, PubMed:20523902, PubMed:18836532
	E119A/G/V	E119	Introduction of Glu119Gly substitution in the A/Quebec/144147/09 backbone conferred resistance to oseltamivir, zanamivir and peramivir using NA inhibition assay.	PubMed: 21148493
	Q136L/K/R	Q136	Introduction of Gln136Lys naturally occurring substitution in the A/Panama/1310/2008 backbone conferred reduced susceptibility to zanamivir and peramivir.	PubMed: 19917319; PubMed : 19641000
	V149A	V149	Introduction of Val129Ala substitution in the A/CAM/408008/2005 backbone conferred decreased sensitivity to zanamivir using NA inhibition assay.	PubMed:21343450
	R156K	R156	Introduction of Arg156Lys naturally occurring substitution in the A/Hong Kong/213/03 backbone conferred resistance to oseltamivir, peramivir and zanamivir using NA inhibition assay.	PubMed:22379077
	D199N	D199	A/New York/4438/2009 isolate contained the Asp199Asn substitution that conferred decreased sensitivity to oseltamivir using NA inhibition assay.	PubMed: 21288815
N1	I223M/V/L/R/K	I223	Compared to A/California/07/2009, A/Ontario/313762/2009(H1N1) isolate contained the Ile223Arg substitution that conferred decreased sensitivity to oseltamivir, zanamivir using NA inhibition assay.	PubMed: 21801626; PubMed : 20858074; PubMed : 20879894
	S247N	S247	A/chicken/Laos/13/08 isolate with Ser227Asn substitution conferred decreased oseltamivir sensitivity using NA inhibition assay	PubMed: 20016036
	H275Y	H275	Introduction of His255Tyr naturally occurring substitution in the A/Vietnam/1203/2004 backbone conferred decreased oseltamivir sensitivity as indicated by measuring inhibition of neuraminidase activity.	PubMed: 19651908; PubMed : 1170976; PubMed : 17296744; PubMed : 18368779; PubMed : 19022400; PubMed : 16228009; PubMed : 16371632;
	E278Q	E278	Introduction of Glu258Gln naturally occurring substitution in the A/Vietnam/1203/2004 backbone decreased oseltamivir sensitivity using plaque reduction assay in MDCK cells.	PubMed:17296744
	N295S	N295	Asn275Ser substitution found in A/Egypt/1425 NAMRU3/2006 isolate conferred decreased oseltamivir sensitivity from patients treated with oseltamivir, increased replication in ferrets.	PubMed: 20701864; PubMed : 21367898; PubMed : 19022400; PubMed : 21148493 ; PubMed : 17855542;
	N30D	D30	Increased virulence in mice	PubMed : 19117585
M1	T139A	T139	All five mutations (T139A (and silent mutation: T121C) of M1, D538G in PB1, K482R (silent mutation: G912A) in PB2, N369I in NA and W47G in HA2) control virulence and replicative capacity in mice.The PB1 and PB2 mutations are shown to be host restrictive in changing the virus to a mouse specific strain.	PubMed: 10426210
	T215A	A215	Increased virulence in mice	PubMed : 19117585
	L26F	L26	This residue is one of the critical amino acid occuring within the transmembrane domain of M2 protein. Substitution at this residue results in loss of sensitivity to M2 inhibitor drugs.	PubMed: 15673732
	V27A	V27	It is shown that single-amino-acid substitution at this position within the transmembrane domain of M2 produces amantadine resistance in Influenza viruses	PubMed: 15673732
M2	A30V/T/S	A30	It is shown that single-amino-acid substitution at this position within the transmembrane domain of M2 produces amantadine resistance in Influenza viruses	PubMed: 15673732
	S31N/S	S31	It is shown that single-amino-acid substitution at this position within the transmembrane domain of M2 produces amantadine resistance in Influenza viruses	PubMed: 15673732
	G34E	G34	It is shown that single-amino-acid substitution at this position within the transmembrane domain of M2 produces amantadine resistance in Influenza viruses	PubMed: 15673732
	P42S	S42	Introduction of Pro42Ser substitution from A/Duck/Guangxi/27/03 in the A/Duck/Guangxi/12/03 backbone conferred increased virulence as indicated by lethality in mice and the systemic spread of infection. This substitution also affects IFN pathway. Human epithelial lung A549 cells were infected with mutant A/Duck/Guangxi/12/03. Then supernatants from A549 cells were used to determine the levels of secreted IFN alpha/beta in bioassay. Infected cells did not inhibit viral replication.	PubMed:18032512
	deletion	No	increase virulence in Mice	PubMed : 18317917; PubMed : 12195436
	E92D	D92	Introduction of Glu92Asp in the A/HK/156/97 backbone conferred cytokine resistance using antiviral activity assay by comparing viral titers after pretreatment with IFN gamma, IFN alpha, TNF alpha. Introduction of Glu92Asp in the A/HK/156/97 backbone had viral titers similar to PR8 when inoculated pigs.	PubMed: 12195436
NS1	L103F; I106M	F103,M106	Introduction of Leu103Phe and Ile106Met substitutions in the A/Hong Kong/483/1997 backbone conferred increased virulence compared to WT by measuring lethality in mice. This dual substitution also spread systemically after measuring viral titers in lung, peripheral blood, spleen and brain. The histopathological assessment of lungs in mice show lung inflammation, accumulation of neutrophils and exudate in the alveolar spaces.	PubMed: 19052083; PubMed : 21593152
	N205S; G210R	S205,G210	Residues at positions 200 and 205 of NS1 contribute to enhanced type I interferon (IFN) antagonistic activity. Togehter, amino acid differences at residue 134 of HA, at 200 and 205 of NS1, and positions 47 and 51 of NS2 cause difference in virulence between high and low pathogenic H5N1 viruses.	PubMed: 20862325
	227-230 (presence of PDZ ligand domain)	ESEV	ESEV is consensus among contemporary HP H5N1	PubMed : 18334632
	227-230 (presence of PDZ ligand domain)	ESEV	Introduction of the PL motif at the C terminal in the virus A/WSN/33 conferred significant weight loss compared to WT. The virus variant showed severe alveolitis and hemorrhage in lung tissue of mice.	PubMed: 18334632
NS2	T47A	A47	decreased antiviral reponse in host	PubMed : 20862325
	M51I	M51	decreased antiviral reponse in host	PubMed : 2086225

Conclusion : La comparaison de la séquence nucléotidique de l'échantillon 150169a avec les bases de données ou les synthèses bibliographiques récentes recensant les déterminants de l'adaptation des virus influenza A à l'homme révèle que le virus étudié ne présente pas l'ensemble des déterminants connus pour favoriser la transmission des virus aviaires à l'homme. Cependant, et comme une majorité de virus aviaires contemporains faiblement pathogènes pour les oiseaux, circulant en Europe, le virus présente un certain nombre de mutations préalablement identifiées comme susceptibles de favoriser la réplication et/ou d'interférer avec les réponses antivirales chez les mammifères, ce qui ne permet pas d'exclure la survenue d'une infection respiratoire dans des circonstances particulières de forte exposition aux oiseaux infectés.

A Ploufragan, le 13 décembre 2015

François-Xavier Briand, Audrey Schmitz, Nicolas Eterradossi et Eric Niqueux (LNR influenza Aviaire)
Sylvie van der Werf, (CNR virus influenzae, Institut Pasteur)

Conclusion : The comparison of the nucleotidic sequence of sample 150169a with databanks and recent litterature reviews compiling molecular determinants promoting adaptation of influenza A viruses to humans reveals that the studied virus does not exhibit all determinants previously reported as allowing the transmission of avian viruses to humans. However, as many contemporary avian viruses currently circulating in Europe, the virus exhibits several changes previously described as possibly increasing virus replication and/or interfering with antiviral responses in mammals, so that respiratory infections cannot be excluded under specific circumstances of intense human exposure to infected birds.

In Ploufragan, 13th December 2015

François-Xavier Briand, Audrey Schmitz, Nicolas Eterradossi et Eric Niqueux (LNR influenza Aviaire)
Sylvie van der Werf, (CNR virus influenzae, Institut Pasteur)

La séquence génomique complète de l'échantillon 150169a a été établie par la Plate-forme Anses de séquençage à haut débit (Unité Génétique Virale et Biosécurité, Anses laboratoire de ploufragan-Plouzané, France)

Légende

La numerotation est réalisée à partir de la première methionine (quel que soit le segment) / Numbering from the first methionin residue in all segments.
Les commentaires sont basés sur la publication "H5N1 genetic changes Inventory : A tool for Influenza Surveillance and preparedness" du CDC (http://www.cdc.gov/flu/avianflu/h5n1/inventory.htm) ainsi que sur l'annotation automatique de la séquence analysée, réalisée sur le site "Influenza Research database" (http://www.fludb.org/brc/search_landing.spg?decorator=influenza); enfin sur la récente revue Neumann & Kawaoka (2015), Transmission of influenza A viruses, Virology, 479-480: 234-246

Présence dans la séquence analysée d'un acide aminé décrit dans la littérature comme i) favorable à la réplication (*in vivo* ou *in vitro sur cellules) de mammifères, ou ii*) favorable au pouvoir pathogène ou à la transmission entre individus chez une espèce mammifère, ou iii) favorable à la résistance aux antiviraux
Présence dans la séquence analysée d'un acide aminé non associé dans la littérature à aucun des critères i), ii) ou iii) mentionnés plus haut
Au sein d'une série de positions aminopeptidiques qui ont été étudiées en association, présence à la fois de positions répondant aux deux critères précédents

Table caption

Numbering from the first methionin residue in all segments.

Comments are baesd on the publication "H5N1 genetic changes Inventory : A tool for Influenza Surveillance and preparedness" from CDC (http://www.cdc.gov/flu/avianflu/h5n1/inventory.htm) and on the automated annotation of the studied sequence using the "Influenza Research database" online ressource (http://www.fludb.org/brc/search_landing.spg?decorator=influenza), and on recent review Neumann & Kawaoka (2015), Transmission of influenza A viruses, Virology, 479-480: 234-246

The amino acid present at this position in the studied sequence has been reported in the scientific litterature as i) associated with replication efficeinecy (*in vivo* or *in vitro in cultured cells) in mammals, or ii*) associated with pathogenicity or transmission between individuals in mammals, or iii) associated with decreased sensitivity to antivirals
The amino acid present at this position in the studied sequence has not been associated with criteria i), ii) or iii).
In a series of aminoacid positions that have been studied jointly, some positions of the studied sequence have been associated with criteria i), ii) or iii), whereas other positions do not.

REFERENCES

AFSSA (2006) Avis de l'Agence française de sécurité sanitaire des aliments relatif à l'évaluation qualitative du risque sanitaire pour l'homme lié à la présence dans l'eau destinée à la consommation humaine et dans divers effluents aqueux de virus Influenza hautement pathogène, dans le cas d'une épizootie ou dans le cas d'une épidémie humaine [Opinion of the French Food Safety Agency on the qualitative assessment of the health risk to humans associated with the presence in water intended for human consumption and in various aqueous effluents of highly pathogenic influenza virus, in the event of an epizootic disease or in the event of a human epidemic]

AFSSA (2006) Avis de l'Agence française de sécurité sanitaire des aliments sur l'*Influenza* aviaire : évaluation du risque sanitaire représenté par les chats, en tant que vecteurs du virus *Influenza* aviaire H5N1 hautement pathogène d'origine asiatique, pour les autres espèces animales et pour les personnes en contact avec les chats [Opinion of the French Food Safety Agency on Avian Influenza: assessment of the health risk represented by cats, as vectors of the highly pathogenic avian Influenza H5N1 virus of Asian origin, for other animal species and for people in contact with the cats] (Request No 2006-SA-0074)

AFSSA (2005) Avis de l'Agence française de sécurité sanitaire des aliments relatif à l'évaluation du risque de transmission des virus *Influenza* aviaires de sous-types H5 et H7 hautement pathogènes, à l'homme, lors de l'ingestion de denrées alimentaires d'origine animale issues de volailles ou de gibier à plume [Opinion of the French Food Safety Agency on the assessment of the risk of transmission of highly pathogenic avian influenza viruses of subtypes H5 and H7 to humans, during ingestion of foodstuffs of animal origin from poultry or game birds]

Briand FX, Le Gall-Reculé G, Guillou-Cloarec C, Ogor K, Jestin V (2010) Phylogeny and genotyping of recent avian low-pathogenic H5 subtype influenza viruses from French ducks. J Gen Virol 91(4) 960-970

Cherbonnel M, Lamandé J, Allée C, Schmitz A, Ogor K, Le Gall-Reculé G (2007) Virologic Findings in Selected Free-Range Mule Duck Farms at High Risk for Avian Influenza Infection. Avian Diseases 51,408-413

Freidl GS, Meijer A, de Bruin E, de Nardi M, Munoz O, Capua I, Breed AC, Harris K, Hill A, Kosmider R, Banks J, von Dobschuetz S, Stark K, Wieland B, Stevens K, van der Werf S, Enouf V, van der Meulen K, Van Reeth K, Dauphin G, Koopmans M, Flurisk consortium (2014) Influenza at the animal-human interface : a review of the literature for virological evidence of human infection with swine or avian influenza viruses other than A(H5N1). *Euro Surveill*. 19(18):pii=20793. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20793

Gambotto A, Barratt-Boyes SM, de Jong MD, Neumann G, Kawaoka Y (2008) Human infection with highly pathogenic H5N1 influenza virus. *Lancet*; 371: 1464-1475

Guerry et al (2014) Bilan de la surveillance de l'Influenza aviaire et de la Maladie de Newcastle en France en 2013. *Bull épid santé anim et alim* n°64 / Spécial MRE - Bilan 2013, 54-59

Guerry I, Schmitz A, Rautureau S, Niqueux E, Briand FX, Jestin V (2015) Bilan de la surveillance de l'Influenza aviaire et de la maladie de Newcastle en France en 2014. *Bull épid santé anim et alim* n°59 / Spécial MRE - Bilan 2014, 59-65

Jestin V, Schmitz A, Niqueux E, Briand FX, Brochet AL, Picault JP, Hars J, Sadonès H (2010) Maintien des objectifs et modalités de la surveillance de l'influenza aviaire en 2009 : bilan stable par rapport à 2008. *Bull épid santé anim et alim* n°40 / Spécial MRC - Bilan 2009, 41-46

Munoz O, de Nardi M, van der Meulen K, Van Reeth K, Koopmans M, Harris K, von Dobschuetz S, Freidl G, Meijer A, Breed A, Hill A, Kosmider R, Banks J, Stark KDC, Wieland B, Stevens K, van der Werf S, Enouf V, Dauphin G, Dundon W, Cattoli G, Capua I, Flurisk consortium (2015) Genetic Adaptation of Influenza A Viruses in Domestic Animals and Their Potential Role in Interspecies Transmission: A Literature Review. Ecohealth, DOI: 10.1007/s10393-014-1004-1

Sadonès H, Schmitz A, Niqueux E, Briand FX, Jestin V (2011) Surveillance de l'influenza aviaire en France en 2010. *Bull épid santé anim et alim* n°46 / Spécial MRC - Bilan 2010, 44-46

Sadonès H, Hars J, Schmitz A, Briand FX, Niqueux E (2012) Surveillance de l'influenza aviaire et de la maladie de Newcastle en France en 2011. *Bull épid santé anim et alim* n°54 / Spécial MRE - Bilan 2011, 49-53

Sadonès H, Hendrikx P, Hars J, Niqueux E, Schmitz A, Briand FX (2013) Bilan de la surveillance de l'Influenza aviaire et de la Maladie de Newcastle en France en 2012. *Bull épid santé anim et alim* n°59 / Spécial MRE - Bilan 2012, 54-59