

# A Quantitative Assessment of the Risk for Highly Pathogenic Avian Influenza Introduction into Spain via Legal Trade of Live Poultry

Fernando Sánchez-Vizcaíno,<sup>1\*</sup> Andrés Perez,<sup>2,3</sup> Manuel Lainez,<sup>1</sup> and José Manuel Sánchez-Vizcaíno<sup>4</sup>

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Highly pathogenic avian influenza (HPAI) is considered one of the most important diseases of poultry. During the last 9 years, HPAI epidemics have been reported in Asia, the Americas, Africa, and in 18 countries of the European Union (EU). For that reason, it is possible that the risk for HPAI virus (HPAIV) introduction into Spain may have recently increased. Because of the EU free-trade policy and because legal trade of live poultry was considered an important route for HPAI spread in certain regions of the world, there are fears that Spain may become HPAIV-infected as a consequence of the legal introduction of live poultry. However, no quantitative assessment of the risk for HPAIV introduction into Spain or into any other EU member state via the trade of poultry has been published in the peer-reviewed literature. This article presents the results of the first quantitative assessment of the risk for HPAIV introduction into a free country via legal trade of live poultry, along with estimates of the geographical variation of the risk and of the relative contribution of exporting countries and susceptible poultry species to the risk. The annual mean risk for HPAI introduction into Spain was estimated to be as low as  $1.36 \times 10^{-3}$ , suggesting that under prevailing conditions, introduction of HPAIV into Spain through the trade of live poultry is unlikely to occur. Moreover, these results support the hypothesis that legal trade of live poultry does not impose a significant risk for the spread of HPAI into EU member states.

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**KEY WORDS:** Avian influenza; live poultry; risk assessment; Spain; trade

## 1. INTRODUCTION

Highly pathogenic avian influenza (HPAI) is a devastating disease of poultry caused by some H5 and H7 strains of the avian influenza (AI) A virus type (HPAIV). HPAI is characterized by high mortality rates, which may be as high as 100% under certain epidemiological conditions.<sup>(1)</sup> The first identification of the Asian lineage of the H5N1 HPAI virus in geese in China in 1996<sup>(2)</sup> was followed by worldwide reports of a large number of HPAI outbreaks. Some of the most recent HPAI epidemics, notably those reported in Italy in 1999–2000,<sup>(3)</sup> Chile in 2002,<sup>(4)</sup> the Netherlands in 2003,<sup>(5)</sup> Canada in

<sup>1</sup>Centro de Tecnología Animal, Instituto Valenciano de Investigaciones Agrarias, Segorbe, Castellón, Spain.

<sup>2</sup>Center for Animal Diseases Modeling and Surveillance, University of California, Davis, CA, USA.

<sup>3</sup>CONICET/Facultad de Ciencias Veterinarias UNR, Casilda, Argentina.

<sup>4</sup>Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, Madrid, Spain.

\*Address correspondence to Fernando Sánchez-Vizcaíno, Centro de Tecnología Animal, Instituto Valenciano de Investigaciones Agrarias, Polígono la Esperanza, N° 100, 12400, Segorbe Castellón, Spain; tel: +34 96 471 2115; fax: +34 96 471 0218; fernandosv@sanidadanimal.info.

2004,<sup>(6)</sup> and Southeast Asia from late 2003 to early 2005,<sup>(7)</sup> were reported to have a considerable impact on the economy and to lead to social disruption in affected countries and regions. For example, it has been estimated that the economic losses associated with the HPAI epidemic that affected the Netherlands in 2003 were approximately EUR 270 million,<sup>(8)</sup> and that the indirect impact of the disease on the country's economy, which includes losses of markets and restrictions to exports between 1996 and 2005, was in excess of \$10 billion.<sup>(9)</sup>

The HPAIV may be introduced into disease-free poultry populations by multiple transmission routes including, for example, migration or trade of wild birds, legal or illegal trade of live poultry and their products such as meat, and mechanical transmission associated with movement of people and contaminated objects.<sup>(8–13)</sup> The relative importance of specific transmission routes in the risk for HPAIV introduction into free regions is likely affected by local epidemiological and ecological conditions. Some believe that legal trade of live poultry imposes a high risk for the introduction of the Asian lineage of the H5N1 HPAIV into disease-free regions of Asia, the Americas, and Africa, but that this route is relatively less important in the European Union (EU).<sup>(9)</sup> The hypothesis that legal trade of poultry plays a minor role in the EU seems to be supported by the observation that 73.6% of the HPAI outbreaks reported in the EU from 2005 to 2008 occurred in wild bird species<sup>(14)</sup> and that at least some of the outbreaks reported in free-range bird farms were likely associated with transmission from wild bird species. However, at least 55% of the outbreaks reported in poultry farms of the EU were associated with direct or indirect effective contacts between infected and susceptible poultry flocks.<sup>(10)</sup> Moreover, for many outbreaks reported in poultry in the EU and in other regions of the world, the association with wildlife could not be demonstrated.<sup>(10,15)</sup> Certainly, the highest risk for HPAIV introduction into naïve regions of the EU is likely associated with migration of wild birds. However, one may hypothesize that the combination of free animal trade policy, relative short distances, and long latency periods for HPAI in certain poultry species may also result in some risk for the introduction of HPAIV into the EU via the legal trade of infected poultry during the silent phase of an epidemic. Because the model has been parameterized using data collected both before and after the emergence of the Asian strain in EU in 2005, the analysis refers to a generic HPAI strain; model

scenarios, however, have been formulated considering conditions that currently prevail in the EU. Assessments aimed at estimating the risk for HPAI introduction that have recently been conducted in Europe<sup>(16,17)</sup> were performed at a qualitative, rather than quantitative, scale. Moreover, those early studies did not assess the risk associated with legal trade of live poultry. Thus, no quantitative assessment of the risk for HPAIV introduction into EU member states via legal trade of live poultry has been published in the peer-reviewed literature. For that reason, the hypothesis that legal trade of poultry imposes a risk for the introduction of HPAI into EU member states such as Spain has yet to be assessed.

Quantitative risk assessment (QRA) was used here to quantify the risk and to identify factors associated with the risk for HPAIV introduction into Spain through the legal importation of live poultry. QRA is the methodology recommended by the World Trade Organization (WTO) to solve international trade disputes. Quantitative knowledge of the risk and of the factors associated with the risk for HPAIV introduction is prerequisite for a country to allocate financial and human resources to the development of policy for prevention and eventual control of HPAI epidemics.

The aim of this article was to estimate the probability of HPAIV introduction into Spain as a consequence of the legal trade of live poultry and to quantify the geographical variation of the risk for HPAIV introduction into the country and the relative contribution of exporting countries and susceptible poultry species to the risk of HPAIV introduction. This information will be useful to assess whether there is a need to apply additional preventive measures or to modify existing preventive measures in order to reduce the risk for HPAIV introduction into the country. The analytical approach used here may be easily extended to assess the risk for HPAI introduction via importation of live poultry during the silent phase of an epidemic in other countries and regions of the world.

## 2. MATERIALS AND METHODS

### 2.1. Modeling Approach

A QRA model<sup>(18)</sup> was developed to quantify the annual probability of HPAI introduction into Spain via the legal trade of live poultry ( $P_{\text{HPAI}}$ ). According to Spanish national regulations, the detection of an HPAI case will immediately trigger a prohibition to

import poultry from the infected country into Spain. Therefore, the assessment was conducted assuming that risk is only associated with the silent phase of an HPAI epidemic in a third country, which was defined as the time period between the first infection and first detection of the disease in a country that exports live poultry into Spain. Risk was assessed separately for each HPAI-susceptible species imported by Spain, namely, chickens, turkeys, ducks, geese, and guinea fowls. The assessment was conducted up to the entry of at least one infected fowl into an agricultural operation; spread from this initial point of entry was not considered here.

Spain is divided into 17 territorial entities referred to as autonomous communities, which are subsequently divided into 50 smaller administrative units referred to as provinces. Provinces were considered the unit of analysis for the assessment here because (i) they are the smallest units of aggregation for which data are collected, organized, and processed in Spain, (ii) the number and type of susceptible species imported by each province is clearly heterogeneous, and (iii) selective control and prevention measures are typically coordinated and implemented at this level.

The model was formulated and run using @ Risk version 4.5.5 (Palisade Corporation, 1996–2007). ArcMap 9.1 (ESRI<sup>®</sup>, 2005) was used to map the results, which were categorized as negligible ( $\sim 0$ ), very low (provinces included within the first quartile), medium (second quartile), high (third quartile), and very high (fourth quartile).

## 2.2. Model Formulation

The value of  $P_{\text{HPAI}}$  was estimated as the sum of the probabilities of introduction [ $P(I_{\text{cds}})$ ] of at least one HPAI-infected fowl into each Spanish province  $d$ , from each exporting country  $c$ , and via the legal importation of each susceptible species  $s$ . The value of  $P(I_{\text{cds}})$  was modeled as a multilevel binomial process of the form:<sup>(19)</sup>

$$P(I_{\text{cds}}) = 1 - [1 - p_{F_{\text{cds}}}(1 - (1 - p_{A_{\text{cds}}})^{n_{\text{cds}}})]^{f_{\text{cds}}},$$

where  $f_{\text{cds}}$  is the number of species  $s$  flocks in country  $c$  from which animals were exported into a Spanish province  $d$  during the silent phase of an HPAI epidemic;  $p_{F_{\text{cds}}}$  is the probability of exporting fowls from an HPAI-infected flock of species  $s$  from country  $c$  into a Spanish province  $d$ ;  $n_{\text{cds}}$  is the number of species  $s$  poultry exported per flock; and  $p_{A_{\text{cds}}}$  is

the species- and country-specific probability that an infected fowl is introduced into an agricultural operation of province  $d$ .

The value of  $p_{F_{\text{cds}}}$  was estimated as the conditional probability of two events, namely, the probability that species  $s$  from country  $c$  become HPAI-infected in the lapse of one year  $P(A_1)$ , and the probability of selecting an infected species  $s$  flock for exporting fowls into a Spanish province  $d$  during the silent phase of the epidemic  $P(A_2)$ . The value of  $p_{A_{\text{cds}}}$  was estimated as the product of the probability that an HPAI-infected fowl was exported  $P(B_1)$ , of the probability that the infected-and-exported fowls survive the infection  $P(B_2)$ , and of the probability that the fowl reaches an agricultural operation in Spanish province  $d$   $P(B_3)$ .<sup>(20)</sup> Fig. 1 depicts a graphical representation of the conditional probabilities computed to estimate  $p_{F_{\text{cds}}}$  and  $p_{A_{\text{cds}}}$ .

## 2.3. Definition of Distributions of Input Variables

### 2.3.1. Number of Flocks Exported During the Silent Phase of the Epidemic [ $f_{\text{cds}}$ ]

The value of  $f_{\text{cds}}$  was estimated as the product of the parameters  $j_{\text{cds}}$  and  $e_{\text{cds}}$ , where  $j_{\text{cds}}$  denotes the number of shipments of species  $s$  sent from country  $c$  into Spanish province  $d$  per year, and  $e_{\text{cds}}$  indicates the number of flocks per shipment. In the model here, the parameter  $j_{\text{cds}}$  was assumed to follow a normal distribution with mean  $\mu j_{\text{cds}}$  and standard deviation  $\sigma j_{\text{cds}}$ , which were computed from data collected by the Spanish government between 2002 and 2007 (Table I).<sup>(21)</sup> Based on data collected by the autonomous region of Valencia in 2007 and 2008,<sup>(22)</sup> it was assumed that each shipment originated from an individual flock. Thus, because  $e_{\text{cds}} = 1$ , the term  $e_{\text{cds}}$  was eliminated from the equation and it was assumed that  $f_{\text{cds}} = j_{\text{cds}}$ . Consequently,  $f_{\text{cds}}$  was estimated as:

$$f_{\text{cds}} = (f_{\gamma_{\text{cds}}}/365) \times b_s,$$

where  $(f_{\gamma_{\text{cds}}}/365)$  approximates the number of flocks from which fowls of species  $s$  are exported from country  $c$  into the Spanish province  $d$  per day and  $b_s$  is the species-specific duration of the silent phase of the epidemic. Spain has banned introduction of poultry from areas of exporting countries where vaccination was practiced, which reduces the risk for introduction of subclinically infected poultry.<sup>(23)</sup> The duration of the silent phase of the epidemic was assumed to follow a Pert distribution with

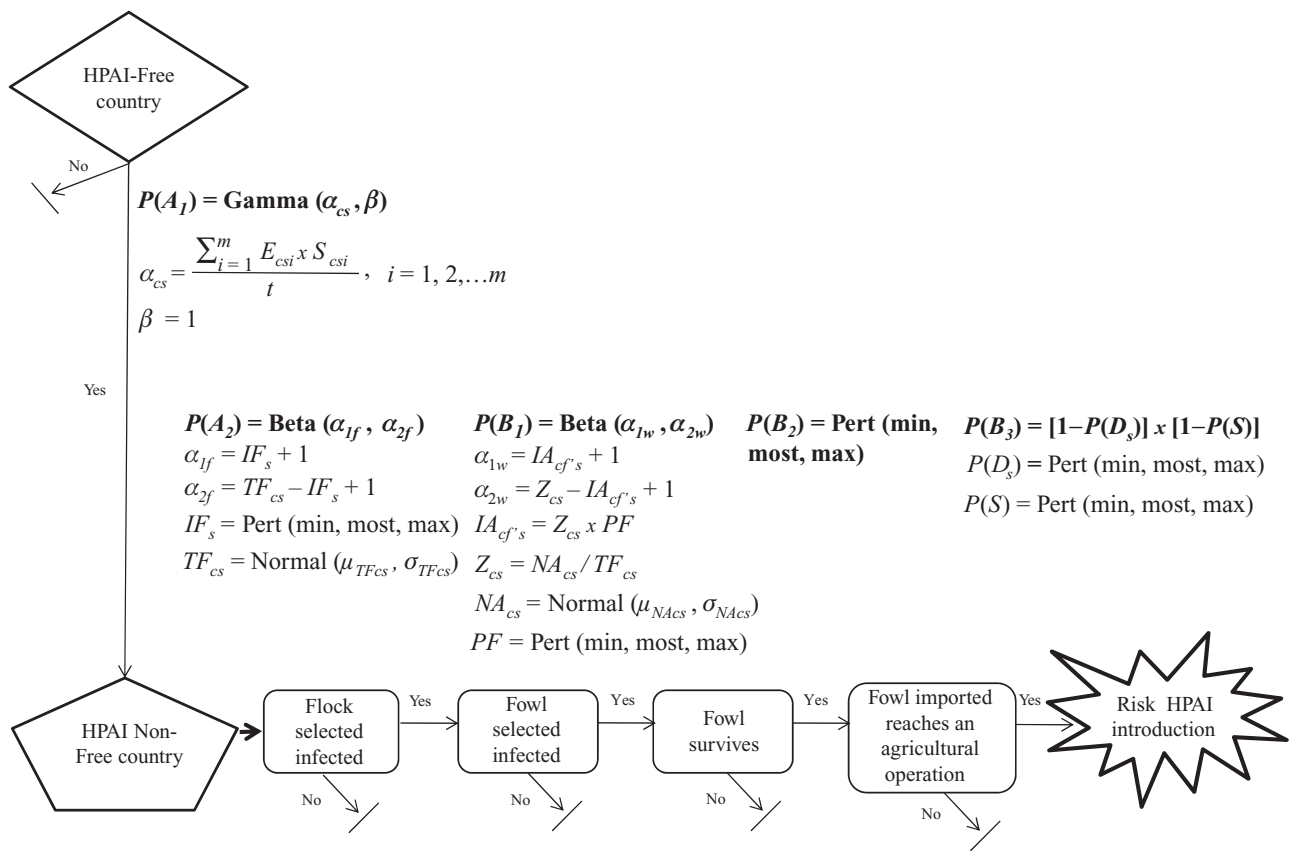


Fig. 1. Scenario-tree for the assessment of the risk for introduction of highly pathogenic avian influenza into Spain via the legal trade of live poultry.

minimum, most likely, and maximum values of, respectively, 11, 12, and 15 days for chickens, turkeys, and guinea fowls,<sup>(24)</sup> and 20, 40, and 60 days for ducks and geese.<sup>(25)</sup> The minimum value for duration of the silent phase of the epidemic represents the scenario in which diagnosis in the country of origin is facilitated by implementation of active surveillance programs for AI virus strains, which are currently enforced by law in EU countries, and assuming conservative figures for the parameterization. Conversely, the maximum value of the parameter represents the scenario in which diagnosis is delayed by factors such as nondiagnosed circulation of low pathogenic strains that may mask HPAI signs.

2.3.2. Probability that Species *s* from Country *c* Becomes Infected [ $P(A_1)$ ]

The value of  $P(A_1)$  was modeled using a gamma distribution with parameters  $\alpha_{cs}$  and  $\beta$ ,<sup>(20)</sup> where  $\alpha_{cs}$  is the probability of having one HPAI epidemic

affecting species *s* in country *c* through period of time  $\beta$ , which is the period of time considered in the assessment ( $\beta = 1$  year).

For countries in which species *s* was affected by at least one HPAI epidemic since 1996, which is the year when the emerging strain of H5N1 HPAIV was isolated for the first time in China,<sup>(2)</sup> the parameter  $\alpha_{cs}$  was estimated as:

$$\alpha_{cs} = \frac{\sum_{i=1}^m E_{csi} \times S_{csi}}{t}, \quad i = 1, 2, \dots, m,$$

where  $E_{csi}$  is the number of HPAI epidemics *i* that affected species *s* in country *c* and that were reported to the World Organization for Animal Health (OIE) from 1996 to 2008 ( $t = 13$ ); and  $S_{csi}$  is the probability that species *s* was HPAI-infected during the silent phase of the epidemic *i* that affected country *c*.  $S_{csi}$  was estimated for each country *c* using data from each specific epidemic *i*.<sup>(1,5,6,14,26-28)</sup> If species *s* was infected during the silent phase of the epidemic *i*

reported by country  $c$ , then a value of  $S_{csi} = 1$  was assumed; otherwise, a value of  $S_{csi} = 0$  was computed. Regarding the epidemics that affected Italy in 1997–1998 and 1999–2000, Belgium in 2003, and Germany in 2008, because more than one species was affected and the information on the species  $s$  that were affected during the silent phase of the epidemic was not available to us, the value of  $S_{csi}$  was computed as the proportion of outbreaks reported in species  $s$  through the duration of each specific epidemic  $i$ .

For countries that were affected by HPAI epidemics since 1996, but in which species  $s$  was not infected, the value of  $\alpha_{cs}$  was assumed to follow a beta distribution with parameters computed using Beta-Buster<sup>(29)</sup> and considering a most likely value of zero and 95% confidence that the probability was lower than the lowest value of  $\alpha_{cs}$  estimated for all the species that were HPAI-infected in the country. This procedure is equivalent to assuming that the probability of infection for noninfected species in countries that were affected by HPAI epidemics since 1996 is likely nil and that, at worst, one is 95% confident that the probability is not higher than the probability of infection in species in which outbreaks were reported.

For countries that were not affected by HPAI epidemics since 1996, the value of  $\alpha_{cs}$  was assumed to follow a beta distribution computed with Beta-Buster,<sup>(29)</sup> with most likely value of zero and 95% confidence that the probability was lower than the lowest value of  $\alpha_{cs}$  estimated in all countries in which species  $s$  was HPAI-infected. This procedure is equivalent to assuming that in countries in which no HPAI outbreak was reported since 1996, the species-specific probability of infection is likely nil and, at worst, one is 95% confident that it cannot be higher than the lowest probability estimated for countries and species that were actually HPAI-infected at least once since 1996.

**2.3.3. Probability of Exporting Poultry from an Infected Flock During the Silent Phase of an HPAI Epidemic [ $P(A_2)$ ]**

The value of  $P(A_2)$  was modeled using a beta distribution with parameters  $\alpha_{1f}$  and  $\alpha_{2f}$ , where  $\alpha_{1f} = IF_s + 1$ , and  $\alpha_{2f} = TF_{cs} - IF_s + 1$ .<sup>(20,30,31)</sup>  $IF_s$  is the number of species  $s$  flocks in country  $c$  infected before the detection of the epidemic, and  $TF_{cs}$  is the total number of species  $s$  flocks in country  $c$ .

For chickens, turkeys, and guinea fowls,  $IF_s$  was parameterized using a Pert distribution with minimum, most likely, and maximum values of, respectively, 1—i.e., at least one undetected outbreak, corresponding to the index case, 8—which is the mean number of undetected outbreaks during the silent phase of the HPAI epidemics of Italy in 1999–2000, Chile in 2002, and the Netherlands in 2003,<sup>(3–5)</sup> and 16—which is the largest number of undetected outbreaks for HPAI epidemics that affected those species in the EU since 1996.<sup>(3)</sup> Those values are conservative, considering that they were obtained before active surveillance was in place in the EU. For ducks and geese,  $IF_s$  was modeled assuming a Pert distribution with minimum, most likely, and maximum values of 1—at least one undetected outbreak, corresponding to the index case, 16—which represents the assumption that the most likely scenario for ducks and geese is equivalent to the worst-case scenario of chickens, turkeys, and guinea fowls, and 50—which is the approximate number of outbreaks expected in ducks in the United Kingdom through an entire epidemic.<sup>(25)</sup>

$TF_{cs}$  was assumed to be normally distributed, with parameters  $\mu_{TF_{cs}}$  and  $\sigma_{TF_{cs}}$  estimated as, respectively, the average and the standard deviation of the number of species  $s$  flocks in country  $c$  between 1996 and 2007.<sup>(32–38)</sup>

**2.3.4. Number of Poultry Exported per Flock [ $n_{cds}$ ]**

The value of  $n_{cds}$  was modeled using a normal distribution with parameters  $\mu n_{cds}$  and  $\sigma n_{cds}$  computed, respectively, as the average and the standard deviation of the number of species  $s$  poultry per shipment exported from country  $c$  into the Spanish province  $d$  between 2002 and 2007 (Table I).<sup>(21)</sup>

**Table I.** Number of Shipments of Live Poultry and Number of Live Poultry Imported into Spain per Year

Number of Shipments of Live Poultry	Number of Live Poultry	Year
647	19,624,042	2002
661	21,150,316	2003
643	22,625,135	2004
750	25,799,665	2005
690	24,363,983	2006
692	32,364,941	2007

### 2.3.5. Probability of Exporting an Infected Fowl from an Infected Flock [ $P(B_1)$ ]

The value of  $P(B_1)$  was modeled using a beta distribution with parameters  $\alpha_{1w}$  and  $\alpha_{2w}$ , where  $\alpha_{1w} = IA_{cf's} + 1$ , and  $\alpha_{2w} = Z_{cs} - IA_{cf's} + 1$ ,<sup>(20,30,31)</sup> with  $IA_{cf's}$  denoting the number of infected poultry of species  $s$  in an infected flock ( $f'$ ) of country  $c$ , and  $Z_{cs}$  indicating the average size of a species  $s$  flock in country  $c$ .  $IA_{cf's}$  was estimated as the product of  $Z_{cs}$  and the expected intraflock HPAI prevalence ( $PF$ ).  $Z_{cs}$  was estimated as the ratio between the number of species  $s$  poultry in country  $c$  ( $NA_{cs}$ ) and  $TF_{cs}$ .  $NA_{cs}$  was assumed to be normally distributed, with parameters  $\mu_{NA_{cs}}$  and  $\sigma_{NA_{cs}}$  estimated, respectively, as the average and the standard deviation of the size of the poultry population of species  $s$  in country  $c$  between 1996 and 2007.<sup>(32–34,36–39)</sup>  $PF$  was assumed to be Pert distributed, with a minimum, most likely, and maximum values of 0.05, 0.95, and 1, which denote, respectively, the expected intraflock prevalence at, respectively, 7, 9 to 12, and 12 to 15 days postinfection.<sup>(24)</sup>

### 2.3.6. Probability of Survival to HPAI-Infection [ $P(B_2)$ ]

The parameter  $P(B_2)$  was modeled using a Pert distribution, which was fitted assuming minimum, most likely, and maximum values for the variable. The minimum value of  $P(B_2)$  was assumed to be 0, which is equivalent to considering a 100% fatality rate in the affected population. The most likely value was assumed to be 0.07 for chickens, turkey, and guinea fowls because that was the fatality rate of HPAI outbreaks reported in those species to the OIE between 2005 and 2008,<sup>(14)</sup> and it was assumed to be 0.6 for ducks<sup>(40)</sup> and 0.621 for geese.<sup>(41)</sup> The maximum value was assumed to be 0.841 for chickens, 0.962 for turkeys and guinea fowls,<sup>(14)</sup> and 1 for ducks and geese.<sup>(40,41)</sup>

### 2.3.7. Probability that the Fowl Reaches an Agricultural Operation [ $P(B_3)$ ]

The value of  $P(B_3)$  was estimated as:

$$P(B_3) = [1 - P(D_s)] \times [1 - P(S)],$$

where  $P(D_s)$  and  $P(S)$  are, respectively, the probability that the fowl dies during the shipment and the probability that the fowl is shipped into a slaughterhouse. The parameter  $P(D_s)$  was modeled using a Pert distribution with minimum, most likely,

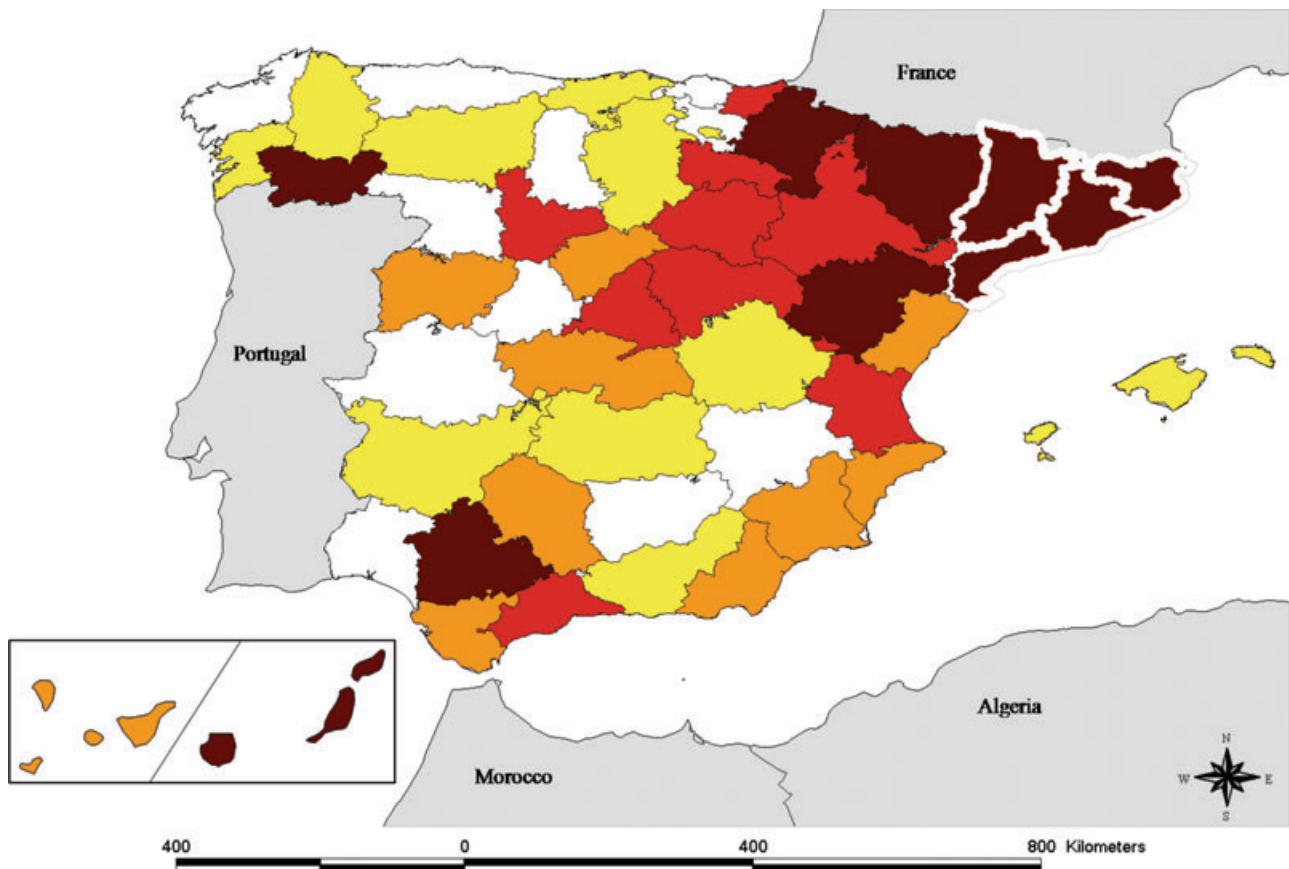
and maximum values of, respectively,  $0.151 \times 10^{-2}$ ,  $0.5725 \times 10^{-2}$ , and  $0.862 \times 10^{-2}$  for chickens,<sup>(42,43)</sup> and  $0.16 \times 10^{-2}$ ,  $0.32 \times 10^{-2}$ , and  $0.52 \times 10^{-2}$  for turkeys.<sup>(44)</sup> No references for the value of  $P(D_s)$  were found in the literature for ducks, geese, and guinea fowls. For those three species,  $P(D_s)$  was modeled using the values assumed for turkeys, to reflect the assumption that the value of  $P(D_s)$  in ducks, geese, and guinea fowls will likely be more similar to the value observed in turkeys than in chickens.  $P(S)$  was assumed to be Pert distributed, with maximum and minimum values of 1 and 0, which is equivalent to assuming that the shipment was, respectively, sent to a slaughterhouse and to an operation other than a slaughterhouse. The most likely value of the distribution was assumed to be 0.13, which was the proportion of imported shipments that were sent to slaughterhouse in 2007 and 2008 in the autonomous region of Valencia.<sup>(22)</sup>

## 2.4. Sensitivity Analysis

As in most risk assessments, the true distribution of many of the parameters necessary to formulate the model was uncertain. One possible strategy is to quantify the sensitivity of the model to variations in the value of the parameters, which provides evidence of how robust the model is to the uncertainty on the true value of the parameters. The sensitivity analysis was performed using a two-stage procedure. On a first stage, the standardized regression coefficient  $\beta_i$  of the association between the input parameter  $i$  and the probability of HPAI introduction into Spain was computed to identify input parameters most likely to be influential on the model output ( $\beta \geq 0.1$ ). The methodology used to estimate  $\beta_i$  was a multiple linear regression analysis. Therefore,  $\beta_i$  represents the change in the probability of HPAI introduction into Spain associated with a change of one standard deviation in the value of the input parameter  $i$ . In the second stage of the sensitivity analysis, the value of each input parameter with  $\beta \geq 0.1$  was varied through seven steps from minimum and maximum values equal to, respectively, a 30% reduction and 30% increase in the base value of the parameter, and the model was run for each step while keeping constant on their base values all other input parameters.

## 3. RESULTS

The mean  $P_{\text{HPAI}}$  was  $1.36 \times 10^{-3}$  ( $P_{\text{HPAI}}$  95% PI =  $7.87 \times 10^{-6}$  –  $8.99 \times 10^{-3}$ ), suggesting that



**Fig. 2.** Geographical variation of the risk for introduction of highly pathogenic avian influenza into Spain via the legal trade of live poultry estimated using a quantitative risk assessment. Risk has been categorized using quartiles as nil ( $\sim 0$ ), very low ( $4 \times 10^{-8}$  to  $9.7 \times 10^{-7}$ ), medium ( $9.8 \times 10^{-7}$  to  $8.46 \times 10^{-6}$ ), high ( $8.47 \times 10^{-6}$  to  $4.421 \times 10^{-5}$ ), and very high ( $4.422 \times 10^{-5}$  to  $1.89 \times 10^{-4}$ ). Provinces with nil risk and those included within the first, second, third, and fourth quartile are indicated in white, yellow, orange, red, and maroon (in the online version of this figure), respectively. Provinces of Catalonia, which is the region that concentrated most of the risk, are indicated with a white outline.

if the conditions and parameters assumed here persist, then it would be expected that HPAI epidemics caused by the legal introduction of live poultry occur once every 735 years in Spain.

Countries that most contributed to the  $P_{\text{HPAI}}$  were France (country-specific  $P_{\text{HPAI}} = 8.06 \times 10^{-4}$ ), the Netherlands (country-specific  $P_{\text{HPAI}} = 3.24 \times 10^{-4}$ ), and the United Kingdom (country-specific  $P_{\text{HPAI}} = 1.62 \times 10^{-4}$ ). Those three countries concentrated 94.6% of the  $P_{\text{HPAI}}$ . Risk from France, the Netherlands, and the United Kingdom was mostly associated with imports of, respectively, ducks (65.3% of the country-specific  $P_{\text{HPAI}}$ ), chickens (58.3% of the country-specific  $P_{\text{HPAI}}$ ), and turkeys (85.2%, of the country-specific  $P_{\text{HPAI}}$ ).

Most (50.8%) of the risk was associated with importation of ducks, whereas turkeys, chickens, guinea

fowls, and geese contributed with 30.2%, 18.7%, 0.2%, and 0.1% of the  $P_{\text{HPAI}}$ , respectively. France was the most likely origin of epidemics caused by the introduction of ducks (76.0% of the species-specific  $P_{\text{HPAI}}$ ), turkeys (61.5% of the species-specific  $P_{\text{HPAI}}$ ), guinea fowls (92.4% of the species-specific  $P_{\text{HPAI}}$ ), and geese (100% of the species-specific  $P_{\text{HPAI}}$ ), whereas the Netherlands was the most likely origin of epidemics associated with the introduction of chickens (74.4% of the species-specific  $P_{\text{HPAI}}$ ).

Much (45.7%) of the risk was clustered in the region of Catalonia, in northeastern Spain, which includes the provinces of Barcelona ( $P_{\text{HPAI}} = 1.89 \times 10^{-4}$ ), Gerona ( $P_{\text{HPAI}} = 1.72 \times 10^{-4}$ ), Tarragona ( $P_{\text{HPAI}} = 1.39 \times 10^{-4}$ ), and Lerida ( $P_{\text{HPAI}} = 1.22 \times 10^{-4}$ ) (Fig. 2). Chickens, turkeys, and ducks were estimated the most likely route of entry for 41.0%,

30.8%, and 28.2% of the 39 Spanish provinces that imported poultry from 2002 to 2007, respectively. France, the Netherlands, Portugal, and the United Kingdom were the most likely origin of introduction for 51.3%, 17.9%, 12.8%, and 7.7% of the provinces, respectively.

Results of the sensitivity analysis suggest that the  $P_{\text{HPAI}}$  was affected ( $\beta \geq 0.1$ ) by the probability of an HPAI epidemic affecting ducks ( $\beta = 0.80$ ) and turkeys ( $\beta = 0.23$ ) in France, and ducks ( $\beta = 0.16$ ) and chickens ( $\beta = 0.14$ ) in the Netherlands. A 30% increase in the base value of those four parameters did not result in a substantial increase of the  $P_{\text{HPAI}}$ , with HPAI epidemics being expected once every 662, 697, 714, and 706 years, respectively.

#### 4. DISCUSSION

The study here is the first quantitative assessment of the risk for HPAIV introduction into an HPAI-free country via the legal trade of live poultry, discriminated by susceptible species imported, country of origin of the export, and administrative unit of destination. The risk assessment performed here suggests that legal trade of poultry does not impose a substantial risk for HPAI introduction into Spain. Such low risk probably results from the combination of a number of factors influencing the path of risk, including adequate biosecurity conditions, high sensitivity of the surveillance system, and good diagnostic capacity in most of the countries that export poultry into Spain, along with the high mortality levels expected in susceptible species.

There are several routes or paths through which HPAIV may be introduced and subsequently disseminated into disease-free poultry populations. Identifying which of those potential transmission routes impose a significant risk of spread into naïve regions has critical implications for predicting and preventing the future spread of the virus. Moreover, identifying routes that do not impose a substantial risk for HPAIV introduction is also important because resources can be selectively allocated to prevent the spread of the disease through the risk pathways that are estimated to be most important. Some believe that legal trade of live poultry may impose a high risk for the emergence and spread of the Asian lineage of the H5N1 HPAIV around of world.<sup>(9)</sup> However, no quantitative assessment of the risk for HPAIV introduction into disease-free poultry populations via legal trade of live poultry has been published in the

peer-reviewed literature. The results presented here suggest that legal trade of live poultry does not impose a substantial risk for the introduction of HPAIV into Spain. Therefore, current national and EU legislations seem to be sufficient to prevent the risk for HPAIV spread through this pathway into EU member states. If the conditions presented here also apply to other EU member countries, then one may also assume that the likelihood of HPAIV transmission via legal trade of poultry in the EU is negligible. These observations and findings support conclusions from early qualitative assessments that assumed that legal trade of live poultry does not represent a risk for HPAI introduction into the EU.<sup>(16,17)</sup> Consequently, risk assessments and resources to minimize the risk would be most effective in preventing the introduction of HPAIV into Spain if they focus on routes other than the legal trade of poultry, such as, for example, migration of wild bird species or illegal trade of animals and products.

Although the risk for HPAI introduction into Spain estimated here was low, interestingly, risk was concentrated in terms of most likely species of introduction and spatial distribution. The highest risk was imposed by imports of ducks. In addition to the large number of duck shipments sent to Spain, this result could also be explained, at least in part, by the “carrier state” attributed to some individuals of this species.<sup>(25,45)</sup> Such epidemiological feature resulted in that ducks, compared to other species, have larger (i) probability of survival from HPAIV infection; (ii) number of infected flocks by the time the epidemic is detected in this species; and (iii) temporal extent of the silent phase of the epidemic. Only seven of the 50 Spanish provinces concentrated 66.21% of the risk for HPAI introduction into the country (Fig. 2). Much (45.7%) of the risk was estimated for the four provinces of Catalonia region. For that reason, even if the risk for HPAI introduction into Spain is nil, one may expect that in the event of an HPAIV epidemic in Spain caused by the legal introduction of poultry, then the index case would likely be located in this region of the country.

Model estimates were mainly affected ( $\beta \geq 0.1$ ) by the probability of an HPAI epidemic affecting ducks in France ( $\beta = 0.80$ ). France, which concentrates 35.3% of the total number of poultry imported by Spain, including 99.6% of the total number of imported ducks,<sup>(21)</sup> was also the country that most contributed to the  $P_{\text{HPAI}}$ . However, and because of the low value of  $P_{\text{HPAI}}$  estimated here, the second stage of the sensitivity analysis showed that model



outputs were not substantially affected by changes in the values of the parameters. Therefore, the model was found to be robust to changes in the input parameters.

The comparison between model outputs and real distribution of HPAIV introductions into Spain would be required in order to validate the model. However, validation of the model was not possible because Spain has never experienced an HPAI epidemic in poultry. Indeed, such observation is consistent with the results presented here that suggest that HPAI epidemics are extremely unlikely to occur in the Spanish poultry industry, at least through the pathway assessed here. Moreover, if the conditions presented here persist and if the conclusions of the assessment were accurate, one would expect that the model will never be able to be validated, or in other words, that the absence of HPAI epidemics caused by the introduction of live poultry into Spain would result, in itself, a validation of the model.

## 5. CONCLUSION

This is the first comprehensive risk assessment that quantified the risk for HPAI introduction into an HPAI-free country via legal trade of live poultry. Results presented here suggest that the risk for HPAI introduction into Spain via legal trade of live poultry is nil, and that therefore, current preventive measures are sufficient to prevent the occurrence of HPAI epidemics through this pathway of introduction.

## ACKNOWLEDGMENTS

F. Sánchez-Vizcaíno was supported by a research grant from Conselleria de Agricultura, Pesca y Alimentación (Generalitat Valenciana). The study was also supported in part by the Ministerio de Medio Ambiente y Medio Rural y Marino (MARM) and by the U.S. National Center for Medical Intelligence. The authors thank Marta Martínez (CISA) and Beatriz Martínez-López (UCM) for their assistance with the formulation of the model.

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