

DEJELIA RAMONA GÓMEZ GONZÁLEZ

**Use of highly pathogenic Avian Influenza propagation models  
in the Dominican Republic**

São Paulo

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DEJELIA RAMONA GÓMEZ GONZÁLEZ

**Uso de Modelos de Propagación de Influenza aviar de alta patogenicidad en la República Dominicana**

Tese apresentada ao Programa de Pós-Graduação em Epidemiologia Experimental aplicada às zoonoses da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo para obtenção do Título de Doutor em Ciências.

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## CERTIFIED

We certify that the Research "", protocol number CEUax 5385310114, under the responsibility Fernando Ferreira, agree with Ethical Principles in Animal Research adopted by Ethic Committee in the Use of Animals of School of Veterinary Medicine and Animal Science (University of São Paulo), and was approved in the meeting of day February 19, 2014.

Certificamos que o protocolo do Projeto de Pesquisa intitulado "Uso de modelos para simulación de brotes de Influenza aviar de alta patogenicidad en la República Dominicana", protocolado sob o CEUax nº 5385310114, sob a responsabilidade de Fernando Ferreira, está de acordo com os princípios éticos de experimentação animal da Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, e foi aprovado na reunião de 19 de fevereiro de 2014.

Profa. Dra. Denise Tabacchi Fantoni  
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de São Paulo

# FOLHA DE AVALIAÇÃO

Autor: GOMEZ GONZÁLEZ, Dejelia Ramona

Título: Uso de Modelos de Propagación de Influenza aviar de alta patogenicidad en la República Dominicana.

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Instituição: \_\_\_\_\_ Julgamento: \_\_\_\_\_

*Nothing like feeling guided by God, nothing like feeling the support of the family; my God, my mother, my children, to you I dedicate this work.*

*To Natasha, Valerie and Dany Emmanuel. With all my love.*

*Nada como sentirse guiado por Dios, nada como sentir el apoyo de la familia; mi Dios, mi madre, mis hijos, a ustedes dedico con todo mi amor este trabajo.*

*A Natasha, Valerie y Dany Emmanuel con todo mi amor.*

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*Knowing is not enough, we must apply. Willing is not enough, we must also do.*

*Não basta saber, também deve aplicar-se. Não é suficiente querer, devemos também fazer.*

*No basta saber, se debe también aplicar. No es suficiente querer, se debe también hacer.*

Johann Wolfgang Goethe

## RESUMO

GOMEZ GONZÁLEZ, D. R. **Utilização de Modelos de Propagação de Influenza Aviar de alta patogenicidade na República Dominicana.** [Use of Highly Pathogenic Avian Influenza Propagation Models in the Dominican Republic.] 2017, 70 f. Tese (Doutorado em Ciências) - Faculdade de Medicina Veterinária y Zootecnia, Universidade de São Paulo, São Paulo, 2017.

A utilização de modelos epidemiológicos como uma ferramenta para avaliar o comportamento de algumas doenças é cada vez mais comum. Modelos têm sido utilizados para representar a infecção pela influenza aviária com base no histórico de surtos causados pelo subtipo H5N1 altamente patogênico, além disso, tem-se modelado transmissão intra-rebanho por causa do subtipo H7N7 a partir de dados de mortalidade. Os primeiros surtos de gripe aviária em aves de curral nas Américas vieram do subtipo H5N2; desde então, e por mais de 30 anos, a linhagem H5N2 Norte americana tem sido detectada em outros países das Américas. Um vírus do mesmo subtipo e linhagem foi detectado em 2007 na República Dominicana; para estudar o possível impacto de um surto sobre a população desenvolvemos um modelo SIR com vários cenários de infecção a partir de parâmetros de H5N2 linhagem Norte americano. O estudo foi baseado em uma população real através da rede de contato de aves formada por 951 granjas; foi representada transmissão por alta e baixa patogenicidade ao longo de um período de 100 dias sem utilização de estratégias de controle. Seis cenários para alta patogenicidade e seis para baixa patogenicidade foram simulados seguido com sete repetições; todos os cenários levaram a surtos com progressão semelhante com curva epidêmica em declínio a partir do dia 34; no cenário de baixa a infecção é mantida ao longo do tempo.

**Palavras-chave:** Gripe aviária. H5N2. Modelo espacial. Taxa de transmissão. Rede de contato de aves.

## ABSTRACT

GOMEZ GONZÁLEZ, D. R. **Use of Highly Pathogenic Avian Influenza Propagation Models in the Dominican Republic.** [Utilização de Modelos de Propagação de Influenza Aviar alta patogenicidade na República Dominicana.] 2017, 70 s. Thesis - (Doctorate in Science) Faculty of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, 2017.

The use of epidemiological models as a tool to evaluate the behavior of some diseases is increasingly common. Models have been used to represent infection with avian influenza, based on the history of outbreaks caused by the highly pathogenic H5N1 subtype, besides that, within-flock transmission due to H7N7 has been modeled from mortality data. The first outbreaks of influenza in poultry in the Americas arose from subtype H5N2; since then and for more than 30 years the subtype H5N2 North American lineage has been detected in other countries of the Americas. A virus of the same subtype and lineage was detected in 2007 in the Dominican Republic; to study the possible impact of an outbreak on the population we have developed a SIR model with several infection scenarios using parameters from the H5N2 North American lineage. The study was based on a real population through the poultry network contact of 951 farms; high and low pathogenic transmission was represented during a period of 100 days without the use of control strategies. Six scenarios for highly pathogenic and six scenarios for low pathogenic were simulated with seven repetitions each; all scenarios led to outbreaks with similar progression with epidemic curve declining from day 34; in low pathogenic the infection is maintained over time.

**Keywords:** Avian Influenza. H5N2. Spatial model. Transmission rate. Poultry contact network.

## RESUMEN

GOMEZ GONZÁLEZ, D. R. **Utilización de Modelos de Propagación de Influenza Aviar alta patogenicidad en la República Dominicana.** [Use of Highly Pathogenic Avian Influenza Propagation Models in the Dominican Republic.] 2017, 70 p. Tesis (Doutorado em Ciências) - Faculdade de Medicina Veterinária y Zootecnia, Universidade de São Paulo, São Paulo, 2017.

La utilización de modelos epidemiológicos como instrumento para evaluar el comportamiento de algunas enfermedades en humanos y animales es cada vez más frecuente. En aves se han utilizado algunos modelos para representar infección por virus de Influenza aviar, basados en los antecedentes de brotes originados por el subtipo H5N1 de alta patogenicidad, además se ha modelado transmisión intra-parvada por causa del subtipo H7N7 a partir de datos de mortalidad. Los primeros brotes de influenza en aves de corral en las Américas surgieron a partir subtipo H5N2; desde entonces y durante más de 30 años el subtipo H5N2 linaje Norteamericano ha sido detectado en otros países de Las Américas. Un virus del mismo subtipo y linaje fue detectado en 2007 en la República Dominicana; para estudiar el posible impacto de un brote en la población desarrollamos un modelo SIR con varios escenarios de infección a partir del virus subtipo H5N2 de linaje norteamericano. El estudio fue basado en una población real a través de la red de contacto de aves representando transmisión por virus de alta y de baja patogenicidad a partir de parámetros de H5N2 linaje Norteamericano; la epidemia se representó durante un período de 100 días sin utilización de estrategias de control. Seis escenarios para alta patogenicidad y seis para baja patogenicidad fueron simulados con siete repeticiones cada uno, todos los escenarios dieron lugar a brotes de progresión similar con curva epidémica que disminuye a partir del día 34; la infección por baja patogenicidad se mantiene a lo largo de los 100 días.

**Palabras clave:** Influenza aviar. H5N2. Modelo espacial. Tasa de transmisión. Red de contacto avícola.

## SUMMARY

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## INTRODUÇÃO

O surgimento de novas cepas de vírus influenza tipo A em populações de animais representa um desafio permanente para os serviços de saúde e veterinários dos países, pelo seu potencial zoonótico e de pandemia. É de grande importância fortalecer a preparação dos serviços de saúde para responder a estas ameaças. A República Dominicana faz parte dos países da região das Américas que têm implementado novas estratégias em programas de saúde, desde o final de 2007, quando foi detectado o vírus da gripe H5N2 de baixa patogenicidade.

O objetivo deste trabalho é dirigido para tratar infecção de gripe aviária em vários aspectos, características virológicas, relação evolutiva e epidemiologia molecular do vírus; transmissibilidade; população suscetível; utilização de modelos estatísticos em saúde animal, registro e análise epidemiológica dos dados; e aplicação de ferramentas matemáticas e computacionais modernas, todas relacionadas com a dinâmica do hospedeiro, as características do agente e retenção no ambiente. A estrutura particular do vírus da gripe, a sua capacidade de mutação e a presença imprevisível em aves selvagens e aves de capoeira levam a desenhar novas estratégias de controle e prevenção.

Realizar um estudo nesta importante área da saúde animal permite que, do ponto de vista epidemiológico, redirecionar e concentrar-se várias questões que estão ligadas à dinâmica da transmissão da doença.

## **INTRODUCTION**

The emergence of new strains of the Influenza A virus in animal populations poses a permanent challenge for the health and the veterinary services of the countries for their zoonotic and pandemic potential. It is of great importance to strengthen the level of preparedness of health services to address these threats. The Dominican Republic is part of the countries of the Americas that have implemented new strategies in the health programs, since at the end of 2007 an influenza virus subtype H5N2 of low pathogenic was detected.

The objective of this work is to approach the avian influenza infection in several aspects, virological characteristics, evolutionary relationship and molecular epidemiology of the virus; transmissibility; susceptible population; use of statistical models in animal health, registration and epidemiological data analysis; and application of modern mathematical and computational tools, all related to the dynamics of the host, the characteristics of the agent and their permanence in the environment. The particular structure of influenza viruses, their ability to mutate and their unpredictable presence in wild and poultry lead to the development of new control and prevention strategies.

Conducting a study in this important area of animal health allows, from the epidemiological point of view, to redirect and concentrate several themes that are united in the dynamics of disease transmission.

## INTRODUCCIÓN

El surgimiento de nuevas cepas del virus Influenza Tipo A en poblaciones animales plantea un permanente desafío para los servicios los servicios veterinarios por su potencial zoonótico y pandémico. Resulta de gran relevancia fortalecer el nivel de preparación de los servicios sanitarios para hacer frente a estas amenazas. La República Dominicana es parte de los países de la región de Las Américas que han implementado nuevas estrategias en los programas sanitarios, desde que a finales del año 2007 fuera detectado un virus de influenza subtipo H5N2 de baja patogenicidad.

El objetivo de este trabajo es dirigido al abordaje de la infección por influenza aviar en varios aspectos, características virológicas, relación evolutiva y epidemiología molecular del virus; transmisibilidad; población susceptible; utilización de modelos estadísticos en salud animal, registro y análisis epidemiológico de datos; y aplicación de modernas herramientas matemáticas y computacionales, todo esto relacionado con la dinámica del hospedero, las características del agente y su permanencia en el medio ambiente. La particular estructura de los virus de influenza, su capacidad de mutar y su impredecible presencia en aves silvestres y de corral conducen a trazar nuevas estrategias de control y prevención.

Realizar una estudio esta importante área de la salud animal permite, desde el punto de vista epidemiológico, redirigir y concentrar varios temas que van unidos en la dinámica de transmisión de las enfermedades.

## **Origins of the A/Chicken/Domin.Rep/2397-7/08 H5N2, a low pathogenic avian influenza virus from the Dominican Republic**

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### **Abstract**

Subtypes of avian Influenza viruses (AIV) H5N2 have caused important outbreaks in poultry populations in North America countries in its pathogenic form emerging from low pathogenic virus. Over the years low pathogenicity strains of the same subtype and lineage have been reported in other countries of the Americas; the control strategies used in the affected countries were different. During the 1983-84 Pennsylvania highly pathogenic AIV outbreaks, birds were depopulated without vaccination. During the 1994-95 Mexico outbreaks in presence of both, high and low pathogenicity viruses, strategies were based on the use of vaccines which to date are still used. In 2014-2015 a novel eurasian H5 reassorted with North American LPAI first reported in wild birds in British Columbia, Canada caused H5N8, H5N1 and H5N2 large outbreaks in United States in wild birds and poultry. Reassorted Eurasian H5N2 was North-America's wild bird H5N2 LPAI origin. The study of the origins of the Dominican strain involve the evolutionary relationship and molecular epidemiology of HPAI and LPAI H5N2 North American lineage isolated in different countries of the Americas, the Caribbean and Taiwan and is focused on low pathogenic strains that have given rise to new strains circulating in poultry population over 30 years. This subject leads to demonstrate the transmissibility of low pathogenic North American lineage which gave rise to A/Chicken/Domin.Rep./2397-7/08 H5N2 LPAI isolated in 2007 in the Dominican Republic and their likelihood of introduction to other countries or regions.

**Keywords:** Avian Influenza, low pathogenic, H5N2, North American lineage, evolutionary relationship, molecular epidemiology.

### **Orígenes de A/Chicken/Dom.Rep/2397-7/08 H5N2, un virus de influenza aviar de baja patogenicidad de República Dominicana**

Subtipos de virus de Influenza aviar H5N2 patógenos han causado importantes brotes en poblaciones de aves de corral en países de Norte América emergiendo de virus de baja patogenicidad. A lo largo de los años cepas de baja patogenicidad del mismo subtipo y linaje se han reportado en otros países de las Américas; las estrategias de control utilizadas en los países afectados fueron diferentes. En los brotes de alta patogenicidad emergentes en 1983-84 en Pennsylvania las aves fueron despobladas sin hacer uso de vacunas. En los brotes de México de 1994-95 en presencia de virus

de alta y baja patogenicidad, la estrategia fue basada en el uso de vacunas que hasta la fecha son utilizadas. En 2014-2015 un nuevo virus H5 de linaje Euroasiático reorganizado genéticamente con virus de baja patogenicidad de origen en Norteamérica fue reportado por primera vez en Columbia Británica, Canadá, además causó brotes de H5N8, H5N1 y H5N2 en Estados Unidos en aves silvestres y aves de corral. El virus H5N2 Euroasiático se originó a partir de virus Norteamericano H5N2 de aves silvestres. El estudio de los orígenes de la cepa Dominicana envuelve la relación evolutiva y la epidemiología molecular del linaje Norteamericano H5N2 presente en diferentes países de Las Américas, El Caribe y Taiwán enfocados en los virus de baja patogenicidad que han dado lugar a cepas que circulan en las poblaciones de aves de corral por más de 30 años. Este tema conduce a demostrar la transmisibilidad del linaje norteamericano de baja patogenicidad que dio origen al virus A/Chicken/Domin.Rep./2397-7/08 H5N2 aislado en el año 2007 en la República Dominicana República y la probabilidad de introducirse a nuevos países o regiones.

**Palabras clave:** Influenza aviar, baja patogenicidad, H5N2, linaje norteamericano, relación evolutiva, epidemiología molecular.

## INTRODUCTION

Many countries in The Americas have been reported avian influenza (AI) virus in poultry. In North America in 1983 low pathogenic avian influenza (LPAI) H5N2 virus was detected in the state of Pennsylvania, United States, mutating into high pathogenic (HPAI) in 1984 (Swayne, 2008a; Alexander, Parsons, and Manvell, 1986; Alexander 2007; Alexander and Brown, 2009). In 2004, an H5N2 avian influenza virus that met the molecular criteria for classification as a HPAI virus was isolated from chickens in the state of Texas, United States (Lee et al., 2005). It was the first highly pathogenic strain reported in the United States in 20 years (Lee et al., 2005), was the only highly pathogenic virus reported to the OIE not pathogenic for chickens (Seene, 2006).

Ten years later in Mexico LPAI H5N2 was detected in March 1994; in December 1994 the virus mutated to HPAI (Villarreal, 2009). The last HPAI virus was isolated in June 1995, and since then only LPAI virus has been found (Villarreal-Chávez and Rivera-Cruz, 2002). Studies of some North American H5N2 LP lineage viruses resulted in variable morbidity and mortality rates in chickens. 0% morbidity and mortality in A/chicken/Mexico/26654-1374/94 (H5N2) and A/chicken/Jalisco/14589-660/94 (H5N2) inoculated by intranasal route (Swayne et al., 1997); 0% mortality in A/chicken/Hidalgo/26654-1368/94 (H5N2) by different routes (Horimoto et al., 1995); 6% morbidity and mortality by intranasal route in A/chicken/Hidalgo/26654-1368/94 (H5N2) and 31% by intravenous route (Swayne et al., 1997); A/chicken/Jalisco/14589-660/94 (H5N2) 19 % morbidity and mortality by intravenous route (Swayne et al., 1997); A/chicken/Pennsylvania/1/83 (H5N2) 20% morbidity and mortality by intranasal route; 10% morbidity and mortality by intramuscular route; 10% morbidity and mortality by direct contact (Alexander et al. (1986); 33% morbidity and 20% mortality in A/chicken/Pennsylvania/21525/83 (H5N2) (Mo et al., 1997).

HPAI A/chicken/Pennsylvania/1370/83 (H5N2) by intranasal route resulted in 100% morbidity and mortality (Bean et al, 1985; van der Goot et al, 2003); and 90% morbidity and mortality (Alexander et al, 1986).

Despite being low pathogenicity some H5N2 North American lineage viruses continued to spread; two Central America countries, Guatemala (2000) and El Salvador (2001) were also affected with LPAI virus (OIE). In the Caribbean, at the end of 2007 the Dominican Republic reported the first occurrence of H5N2 virus (OIE) and subsequently in mid-2008 Haiti reported first occurrence LPAI H5N2 sub clinical infection (OIE). A/chicken/Domin.Rep/2397-7/08 H5N2 LPAI was isolated and classified by the National Veterinary Diagnostic Laboratory in Iowa, NVSL, of United States of America. In early 2015 in Central America, Belize reported first occurrence of LPAI H5N2. The Belize report indicates that the virus was classified by NVSL as North American LPAI H5N2 98.8% similar to A/CK/Mexico/2012 H5N2 (OIE). All the H5N2 low pathogenic viruses reported in Central America and in the Caribbean were classified as North American lineage.

In late 2014 and early 2015 a new HPAI H5N2 virus was reported in North America. A novel Eurasian H5 reassorted with North American LPAI, first reported in wild birds in British Columbia, Canada, caused H5N8, H5N1 and H5N2 outbreaks in United States, was proposed as HPAI intercontinental group A (icA). Reassorted Eurasian H5N2 was North-America's wild bird A/Northern/Pintail/40964/2014 H5N2 LPAI origin. The novel H5N2 virus first reported in British Columbia, Canada, possessed gene segments related of HPAI H5N8 viruses and North American lineage waterfowl viruses (Pasick et al. 2015); next a similar virus was reported in wild birds in United States causing large outbreaks in some US states (OIE), (NWHC-USDA). In August of 2015 Mexico reported LPAI H5N2 subclinical infection in commercial layers. The event probably will be an important

piece in the phylogeny and transmissibility of the low pathogenic H5N2 viruses, after more than two decades of the emergence of low pathogenic virus in Mexico.

Some H5 and H7 subtypes AIVs can undergo mutational changes that have serious consequences when allowed to circulate in poultry for an extended period of time (Lee et al, 2005), as demonstrated close relationship in the phylogenetic analysis of the HA and several internal genes (NP, M, and NS) between two isolates from chicken in Texas (A/Chicken/Texas/298313/04 HPAI and A/Chicken/Texas/167280-4/02 LPAI), which indicates a reassortment event had occurred with unknown viruses. (Escorcia et al., 2010).

This study reveal the origins of the H5N2 subtype virus of low pathogenicity isolated in 2007 in the Dominican Republic, and its similarity with the available H5N2 strains of North America lineage demonstrating the persistence of these viruses in some countries in the Americas, making obvious the potential of transmissibility of LPAIV of this lineage over more than 30 years.

## MATERIALS AND METHODS

### Phylogenetic analysis of North American Lineage H5N2

GenBank information has been used to build the North American avian influenza HA (H5N2) tree. The nucleotide sequences were aligned using CLUSTAL W application running in BioEdit v. 7.2.5 with nucleotide the sequence of A/Chicken/Domin.Rep/23977/08 (kindly provided by Istituto Zooprofilattico Sperimentale delle Venezie) and sequences were obtained from the GenBank .

The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model. The tree with the highest log likelihood (-5668.3318) is shown [Fig. 1]. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the

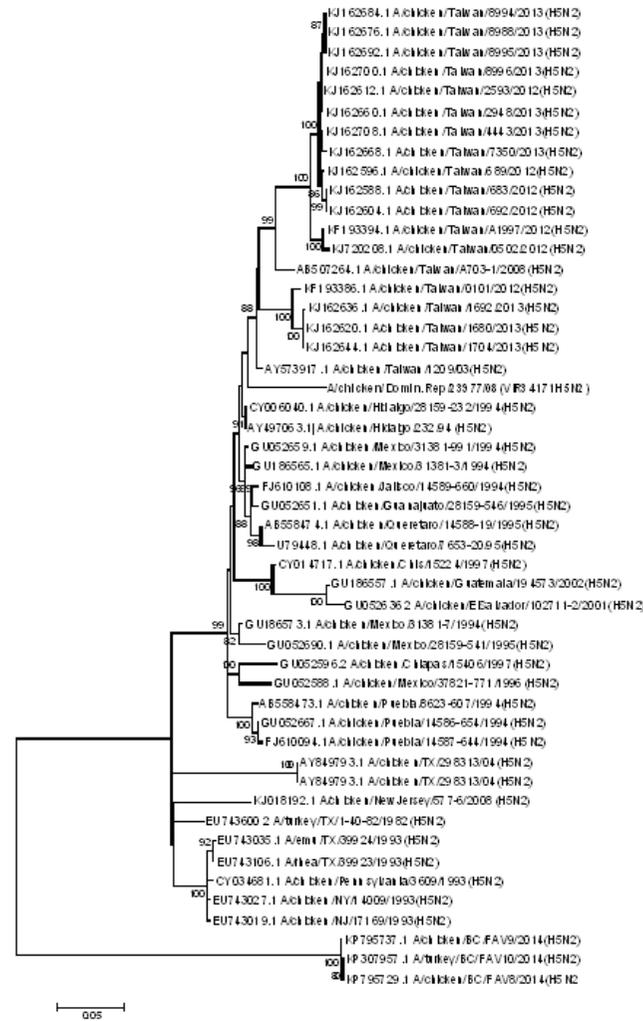
heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 48.0105% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 50 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 986 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar, Stecher AND, Tamura, 2016).

## RESULTS AND DISCUSSION

The phylogenetic tree shows the evolutionary relationship of the Dominican strain A/Chicken/Domin.Rep/2397-7/08 with the low pathogenic strain A/chicken/Hidalgo/28159-232/1994, their most recent common ancestor.

The low pathogenic strain A/Chicken/Domin.Rep/2397-7/08 (H5N2) was isolated in 2007 in the Dominican Republic, and is related with two LPAI Mexican strains, A/Chicken/Hidalgo/28159-232/1994 (H5N2) (94.2% identity), and A/chicken/Hidalgo/232/94 (H5N2) (94.1% identity). Interestingly, among strains compared, the next virus closest to the Dominican strain is A/chicken/Taiwan/1209/03 (H5N2) (93.7% identity). In turn, A/chicken/Taiwan/1209/03 (H5N2) and these two Hidalgo strains identities is 98.2% and 98.1% respectively. Others strains isolates in Taiwan in 2008, 2012 and 2013 in poultry are evolutionary more distant of the Dominican strain (91.8 to 90.2%).

The Dominican strain is distant 87.9% and 87.5% respectively from the Guatemala (2000) and El Salvador (2001) isolates.



**Figure 1.** Maximum Likelihood tree with the GTR model for the partial HA gene of H5N2 AIVs. The bar represents the number of substitutions per site and the nodes are the bootstrap values (1,000 replicates). Strain **A/Chicken/Dom.Rep/23977/08** is in bold.

**Table 1. Similarities of A/Chicken/Domin.Rep/23977/08 (H5N2) with North American isolates**

Seq->	A/chicken/Domin.Rep/23977/08(VIR34171H5N2)	CY006040.1 A/chicken/Hidalgo/28159-232/1994(H5N2)	GU052659.1 A/chicken/Mexico/31381-991/1994(H5N2)	GU186565.1 A/chicken/Mexico/31381-3/1994(H5N2)	FJ610108.1 A/chicken/Jalisco/14589-660/1994(H5N2)	GU052651.1 A/chicken/Guanajuato/28159-546/1995(H5N2)	GU186573.1 A/chicken/Mexico/31381-7/1994(H5N2)	AB558474.1 A/chicken/Queretaro/14588-19/1995(H5N2)	GU052690.1 A/chicken/Mexico/28159-541/1995(H5N2)	AB558473.1 A/chicken/Puebla/8623-607/1994(H5N2)	GU052667.1 A/chicken/Puebla/14586-654/1994(H5N2)	U79448.1 A/chicken/Queretaro/7653-20/95(H5N2)	CY014717.1 A/chicken/Chis/15224/1997(H5N2)	GU052596.2 A/chicken/Chiapas/15406/1997(H5N2)	FJ610094.1 A/chicken/Puebla/14587-644/1994(H5N2)	GU052588.1 A/chicken/Mexico/37821-771/1996(H5N2)	GU186557.1 A/chicken/Guatemala/194573/2002(H5N2)	GU052636.2 A/chicken/El Salvador/102711-2/2001(H5N2)	AY497063.1 A/chicken/Hidalgo/232/94(H5N2)
A/chicken/Domin.Rep/23977/08(VIR34171H5N2)	ID	0,942	0,932	0,932	0,926	0,926	0,928	0,919	0,927	0,911	0,911	0,91	0,91	0,914	0,909	0,908	0,879	0,875	0,941
CY006040.1 A/chicken/Hidalgo/28159-232/1994(H5N2)	0,942	ID	0,987	0,985	0,982	0,982	0,979	0,971	0,962	0,959	0,959	0,96	0,958	0,949	0,955	0,954	0,926	0,92	0,998
GU052659.1 A/chicken/Mexico/31381-991/1994(H5N2)	0,932	0,987	ID	0,991	0,986	0,986	0,976	0,977	0,961	0,958	0,958	0,966	0,957	0,947	0,954	0,952	0,925	0,919	0,988
GU186565.1 A/chicken/Mexico/31381-3/1994(H5N2)	0,932	0,985	0,991	ID	0,984	0,984	0,974	0,977	0,959	0,956	0,956	0,964	0,955	0,947	0,952	0,95	0,925	0,919	0,986
FJ610108.1 A/chicken/Jalisco/14589-660/1994(H5N2)	0,926	0,982	0,986	0,984	ID	0,989	0,971	0,978	0,954	0,953	0,953	0,965	0,95	0,941	0,949	0,945	0,921	0,915	0,983
GU052651.1 A/chicken/Guanajuato/28159-546/1995(H5N2)	0,926	0,982	0,986	0,984	0,989	ID	0,971	0,978	0,954	0,953	0,953	0,965	0,95	0,944	0,949	0,947	0,922	0,916	0,983
GU186573.1 A/chicken/Mexico/31381-7/1994(H5N2)	0,928	0,979	0,976	0,974	0,971	0,971	ID	0,96	0,978	0,963	0,963	0,951	0,961	0,951	0,959	0,956	0,932	0,924	0,98
AB558474.1 A/chicken/Queretaro/14588-19/1995(H5N2)	0,919	0,971	0,977	0,977	0,978	0,978	0,96	ID	0,947	0,958	0,958	0,984	0,941	0,931	0,954	0,937	0,909	0,903	0,972
GU052690.1 A/chicken/Mexico/28159-541/1995(H5N2)	0,927	0,962	0,961	0,959	0,954	0,954	0,978	0,947	ID	0,945	0,945	0,94	0,948	0,94	0,943	0,941	0,92	0,911	0,963
AB558473.1 A/chicken/Puebla/8623-607/1994(H5N2)	0,911	0,959	0,958	0,956	0,953	0,953	0,963	0,958	0,945	ID	0,991	0,95	0,94	0,939	0,989	0,943	0,91	0,902	0,96
GU052667.1 A/chicken/Puebla/14586-654/1994(H5N2)	0,911	0,959	0,958	0,956	0,953	0,953	0,963	0,958	0,945	0,991	ID	0,95	0,94	0,942	0,995	0,945	0,91	0,902	0,96
U79448.1 A/chicken/Queretaro/7653-20/95(H5N2)	0,91	0,96	0,966	0,964	0,965	0,965	0,951	0,984	0,94	0,95	0,95	ID	0,934	0,923	0,946	0,928	0,903	0,897	0,961
CY014717.1 A/chicken/Chis/15224/1997(H5N2)	0,91	0,958	0,957	0,955	0,95	0,95	0,961	0,941	0,948	0,94	0,94	0,934	ID	0,928	0,936	0,933	0,958	0,949	0,959
GU052596.2 A/chicken/Chiapas/15406/1997(H5N2)	0,914	0,949	0,947	0,947	0,941	0,944	0,951	0,931	0,94	0,939	0,942	0,923	0,928	ID	0,938	0,951	0,906	0,898	0,95
FJ610094.1 A/chicken/Puebla/14587-644/1994(H5N2)	0,909	0,955	0,954	0,952	0,949	0,949	0,959	0,954	0,943	0,989	0,995	0,946	0,936	0,938	ID	0,941	0,908	0,898	0,956
GU052588.1 A/chicken/Mexico/37821-771/1996(H5N2)	0,908	0,954	0,952	0,95	0,945	0,947	0,956	0,937	0,941	0,943	0,945	0,928	0,933	0,951	0,941	ID	0,902	0,895	0,955
GU186557.1 A/chicken/Guatemala/194573/2002(H5N2)	0,879	0,926	0,925	0,925	0,921	0,922	0,932	0,909	0,92	0,91	0,91	0,903	0,958	0,906	0,908	0,902	ID	0,984	0,927
GU052636.2 A/chicken/El Salvador/102711-2/2001(H5N2)	0,875	0,92	0,919	0,919	0,915	0,916	0,924	0,903	0,911	0,902	0,902	0,897	0,949	0,898	0,898	0,895	0,984	ID	0,921
AY497063.1 A/chicken/Hidalgo/232/94(H5N2)	0,941	0,998	0,988	0,986	0,983	0,983	0,98	0,972	0,963	0,96	0,96	0,961	0,959	0,95	0,956	0,955	0,927	0,921	ID

These two isolates show 98.4% similarity between them having a common ancestor, A/chicken/Chis/15224/1997(H5N2), distant 95.8% and 94.9 % respectively.

The LPAI virus isolated in Belize (2015) was classified as North American LPAI H5N2 virus 98.8% similar to A/CK/Mexico/2012 H5N2 (OIE). The strains of Haiti (2008), Belize (2015) and Sinaloa, Mexico (2015) were not available for this study.

The strains of United States 1982, 1997 and 2004, and the Eurasian-strains of British Columbia 2014 A/chicken/BC/FAV8/2014, A/chicken/BC/FAV9/2014 and A/turkey/BC/FAV10/2014 are distant of the Mexican lineage and the Dominican strain.

The Phylogenetic HA analysis shows that the origin of A/Chicken/Domin.Rep/2397-7/08 (H5N2) is part of H5N2 North American Lineage. The tree relates different strains isolated in poultry in some countries of the Americas region.

The evolutionary relationship of A/Chicken/Domin.Rep/2397-7/08 (H5N2) suggests that it has its origins in a low pathogenic North American lineage virus isolated 22 years ago in Mexico. The molecular epidemiology of H5N2 viruses of North American lineage isolated in different countries of North and Central America, Dominican Republic on the Hispaniola Island in the Caribbean region, and in the island of Taiwan in Asia suggests how low pathogenic strains can be introduced into new regions, islands and/or continents. HA phylogeny demonstrates that LPAI H5N2 has been circulating in poultry population over 30 years evidencing its ability to be introduced in poultry populations giving rise to new sub lineages. Over the years some studies have shown peculiarities of Mexican lineage that support these conclusions. A/chicken/Mexico/CPA-232/94 (H5N2) is the seed strain officially authorized to produce all the commercial inactivated vaccines in Mexico (Villareal-Chavez, 2003; Lee, et al., 2004; Escorcia, et al., 2008 and 2010). The sequences of

A/chicken/Hidalgo/232/94 vaccine strain were tested and compared with A/chicken/Mexico/232/1994 because of nomenclature discrepancy and were found to be similar (Lee, 2004).

At least four distinct clusters were observed within the Mexican lineage isolates, divided in Jalisco, Puebla, A and B sub lineages. One early isolate (A/CK/Puebla/28159-474/95) seemed to be the progenitor strain of the new B sub lineage which includes isolates from Guatemala and El Salvador. A/chicken/Hidalgo/232/94 and A/chicken/Mexico/232/1994 belonged to Jalisco sub lineage, as majority of the earlier isolates (Lee et al, 2004). Viruses circulating in Mexico between 1994 and 1998 were highly divergent to viruses isolated between 2002 and 2008 (Escorcia et al, 2010).

The H5N2 viruses in the Dominican Republic and Haiti were shown to be closely related to the Mexican lineage of H5N2, which has been circulating in Mexico since early 1994 (Seene 2009).

Phylogenetic analyses of H5N2 viruses prevalent in the chicken population in Taiwan show that they were generated by reassortment between an H5N2 virus that originated from Mexico in 1994 and a locally enzootic H6N1 virus lineage. (Lee et al., 2014).

Through over thirty years H5N2 influenza virus has been circulating in some countries in the Americas. Since the Pennsylvania outbreak in 1983 to the Belize and Mexico 2015 outbreaks, North American lineage H5N2 viruses have been reported in poultry birds in eight countries of the Americas region (Canada, United States of America and Mexico in high pathogenic form; Guatemala, El Salvador, Dominican Republic, Haiti and Belize in low pathogenic form) (OIE). Within this period the same lineage was detected in Taiwan with variable level of pathogenicity showing by phylogenetic analyses that they were generated by reassortment between an H5N2 virus originated from Mexico in 1994 and a locally enzootic H6N1 virus lineage.

The reemergence of a North American vaccine strain virus in Asia is a strong indication that a vaccine may be the source of this H5N2 virus (Lee, et al., 2014). Divergence between different strains of LPAI H5N2 isolates in Mexico circulating between 1994 to 1998 and 2002 to 2008 demonstrate by HI assay variable results in titers indicating that the differences may warrant a new vaccine and serosurveillance antigen update (Escorcía et al., 2010).

Different Phylogenetic HA and pathogenicity analysis of North American H5N2 isolates have demonstrated their ability of divergence.

In the subclinical infection occurred in game cocks in the Dominican Republic the transmission rate could not be calculated; seroconversion was not efficient, probably because of the low pathogenic and low transmissibility of the virus, probably because the game cocks were kept separates in cages distanced at least 2 meters from each other. The apparent morbidity in field in the birds was initially 0.87 % and 2.6% at the end of the monitoring and depopulation period which lasted for more than 30 days; during this period the birds did not show any clinical signs or mortality. Despite the serological findings, in this group of birds it was not possible to isolate the virus. (Gomez, personal observation).

This study can lead to evaluate a new perception of risk in the studies of transmission of the low pathogenic North American lineage and the Hidalgo H5N2 sub lineage. We recommend further studies of transmissibility, both in field and laboratory, as well as molecular studies, to detect if changes have occurred in A/Chicken/Domin.Rep/2397-7/08 strain since detected in 2007.

Our study shows the origins of A/Chicken/Domin.Rep/2397-7/08 and their likelihood with the HA genes of H5N2 AIVs North American lineage that have remained circulating in poultry populations, suggesting the ability to spread in some

way over time thus were introduced into other regions. The surveillance of avian influenza in low pathogenic subclinical infection may not be efficient if morbidity and mortality rates are very low; the epidemiology of the virus over the time should be focused, so, we suggest post quarantine surveillance and further tests of biological products; countries carrying out serological surveillance should use those antigens related to strains representing the greatest risk. Furthermore, these conclusions suggest the evaluation of the serological tests currently used in the surveillance in the Dominican Republic and their antigenic relationship with the strain closest to the Dominican virus.

These results can provide better understanding of evolutionary relationship of the viruses in the region and how low pathogenic viruses indefinitely circulating may be involved in selective pressure to mutate into highly pathogenic. The opportunity of mutation for new clades from low pathogenic virus circulating through the years may mean the difference between the magnitude of an outbreak in one country and the risk of introduction and spread of those viruses in different countries in the region. Isolate new strains could collaborate to develop advanced molecular epidemiology studies. Recent 2015 outbreaks of H5N2 North American lineage most likely can offer relevant data on the rate of infection in field and diversity of changes in the isolates.

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## **Database for the Register of Poultry Production in the Dominican Republic.**

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### **Abstract**

The production of data in the poultry sector is very efficient from the individual point of view of each company, due to the intensity and volume of production systems. At the same time the official sectors deserve to implement an agile mechanism to optimize the use of production data in a general and systematic way, so that they can supply the information and at the same time be used as an epidemiological tool. This model of production of statistics is applicable to animal health based on available production data. The applicability of this source of information is directed to studies of the chain of contacts through networks, spatial distribution and models of disease propagation.

**Keywords:** Poultry register, data base, degree of affiliation, estimate population, poultry statistics.

## **Base de dados para o Registro da Produção Avícola na República Dominicana.**

### **Resumo**

A produção de dados no setor avícola é muito eficiente do ponto de vista individual de cada empresa, devido à intensidade e volume dos sistemas produtivos. Ao mesmo tempo, os setores oficiais merecem programar um mecanismo ágil para aperfeiçoar o uso dos dados produtivos de forma geral e sistemática, para que eles possam fornecer a informação e ao mesmo tempo ser usado como um instrumento epidemiológico. Este modelo de estatísticas é aplicável à saúde animal com base em dados disponíveis da produção. A aplicabilidade desta fonte de informação é direcionada aos estudos da cadeia de contatos por meio de redes, distribuição espacial e modelos de propagação de doenças.

Palavras chave: Registro de aves, base de dados, estimativa da população, grau de afiliação, estatísticas de aves.

## **Base de datos para el Registro de la Producción Avícola en la República Dominicana.**

### **Resumen**

La producción de datos en el sector avícola es muy eficiente desde el punto de vista individual de cada empresa, debido a la intensidad y volumen de los sistemas productivos. Al mismo tiempo, los sectores oficiales merecen implementar un mecanismo ágil para optimizar el uso de los datos productivos de manera general y sistemática, para que puedan suministrar la información y al mismo tiempo ser utilizados como herramienta epidemiológica. Estamos presentando un modelo de producción de estadísticas aplicables a la sanidad animal basado en datos disponibles de la producción. La aplicabilidad de esta fuente de información está dirigida a estudios de la cadena de contactos a través de redes, distribución espacial y modelos de propagación de enfermedades.

Palabras clave: Registro avícola, base de datos, estimativa de población, grado de afiliación, estadísticas avícolas.

### **Introduction**

The Database for the Register of Poultry Production in the Dominican Republic was created as a model of production of statistics applied to animal health. According the Sectorial Statistical Plan -PES- (acronym in Spanish) in the country it is necessary to know the demand of statistical information on the part of the users, with the purpose of attending them, guaranteeing timely and quality information. The production of health information for years has escaped the study of these needs. There is a gap both in demand and in the production of this type of information, which is essential for generating epidemiological models and identifying the health status of the animal population.

The animal health law in force in the Dominican Republic, under the authority of the Ministry of Agriculture, declare of public interest the health defense of the cattle, the control and eradication of epizootic diseases, and the prevention of diseases of animals transmissible to man, protecting the source of wealth represented by the national cattle ranch for the production of food of animal origin, and the serious danger posed by the pests of cattle for public health.

The large production capacity of the poultry industry in the Dominican Republic demands the availability of information that is in accordance with the requirements of international trade, Therefore we have outline a Statistical Production Model based on the Poultry Production Register which has relevant information to be applied both to the poultry health and to the animal health in general.

The model combines the production of statistics in the productive sector, population dynamics of poultry and health information using analysis tools to favor epidemiological studies, and consequently the adoption of timely measures before health and emergency events, as part of the referrals addressed to the Directorate General of Livestock (DIGEGA) of the Ministry of Agriculture of Dominican Republic.

## Objective

The main objective is to create a categorized and ordered data bank with information on poultry production that will serve as a statistical production model applied to animal health. By means of statistical analysis tools secondary objectives will be achieved: identification of the chain of contacts in local commerce and affiliation between owners, understand the bird movement, updating of population figures, optimizing surveillance strategies for prevention and control of diseases in animals.

The model is based on a data structure that links the production chain according to the owners, the type of production system, the geographical location of farms and population distribution, the origin and destination of the production chain as well as the health status of the birds. Other variables such as the name of the responsible veterinarian, hatchery plants, food plants, target markets and movement routes can be easily associated in the database.

## Materials

As a source of information we used the surveys conducted by the DIGEGA through the Division of Birds Diseases during the period 2011-2013. The form raises information about the name of the owner or the company, the existing population and the capacity of the farm, the geographic coordinates and location, and the purpose or type of production. As a source of comparative data of the population we use the National Register of Agricultural Producers<sup>3</sup> (SEA, 1998), document also contained in the Institutional Programming Office (OIP) of DIGEGA<sup>4</sup>; the National Census of Light Laying Hens (CONAPROPE, 2011)<sup>5</sup> and the data of the Broiler Chickens Study (CONAPROPE 2009)<sup>6</sup>. In addition we use the list of associated companies that produce grandmothers, hatcheries, incubators, slaughterhouses, chickens and eggs, from the Dominican Poultry Association (ADA).

**Table 1. References of official data**

Indicators	Values	Reference
<b>Official data*</b>		
Population	15.198.865	<i>Censo Agropecuario 1998 (SEA , 2000)</i>
Layers population	6.088.617	CONAPROPE (2011)
Installed capacity in hens units	13.471.029	CONAPROPE (2011)
% Of occupation of installed capacity in hens units	66%	CONAPROPE (2011)
Relationship of associated companies		ADA**

\*Data published by institutions of the Dominican State

\*\* Data provided from private poultry sector

## Methods

### *Registered population data:*

The available information was organized and classified according to the owner's name and the category of the production as registered in the survey. All the geographical coordinates were registered and verified through satellite images, duplicates and not related information was excluded; the farms identified as having the highest production system were verified according to the list of associates. For ease of the data base search we include new variables in complement to the owner name, first we assign an identifier number to each property, then we assign a unique owner code that allows to identify companies registered as the same owner, and based on this code we create an individual code for each farm. According to the number of farms that each owner owns, we classify the farms in another variable denominated degree of affiliation. In addition to the code of owner and the code of the farms, the distribution of farms by provinces, municipal districts and municipalities was classified according to the Territorial Code of the National Office of Statistics<sup>7</sup> (ONE). Other variables whose information was not complete at the time of the survey include the population and the category of production. Data editing was stored and analyzed in an Excel spreadsheet, finally statistical and graphic analysis was performed in the language and programming environment R®.

### *Unregistered population estimated:*

Given the lack of relevant information of the population we determined the importance to represent the population size by means of estimates. The unregistered population was estimated by calculating the likely population density through the use of satellite tools. From data available in the survey we use the mean value of a sample of the population (n=20 farms); this number represents farms in which the installed capacity and the capacity used was identified. Using the coordinates we represent these 20 farms in the satellite image, we count the number of sheds, measure the area of each shed excluding the eaves area and represent the number of birds registered in each of these farms distributed into the sheds. Dividing the amount of m<sup>2</sup> of the shed by the number of birds expected we assume a density of 10 birds/m<sup>2</sup> to estimate unregistered population; this is compatible with the characteristics of the production system of the country in which the sheds are usually built for ten thousand birds for broiler system in an equivalent area. We assumed the density of the population on the farm based on chicken production because compared to the census of layers (see table 1) the largest proportion of layers and layers-integrated had been recorded in the survey (see table 2). According to studies, the occupancy of the installed capacity in laying hens is 66% (CONAPROPE, 2011). For the calculation of estimated area of the warehouses we use image tools CNES Astrium/image Digital Globe, Google earth image 2015, US Dept of State Geographer 2015 Google, Image Landsat 2009 GeoBasis, Image NASA Digital Globe.

Using these tools we estimate the area to define the population in each of the farm without registration and proceeded to the verification and re-definition of coordinates in some establishments facilitating the location and measurement of the geographic distance.

The population was classified according to the number of birds by farm or production unit and according to the number of farms by owner or affiliated company, the population, the category of the production

**Table 2. Results of the registration of poultry production \***

<b>Indicators</b>	<b>Values</b>	<b>Reference</b>
Total number of farms or production units	951	DIGEGA (2011-2013), Gómez (2017)
Farms with population register	500 (52.58%)	DIGEGA (2011-2013), Gómez (2017)
Farms without population register	451 (47, 42%)	DIGEGA (2011-2013), Gómez (2017)
Farms with categorized production**	519 (54, 57%)	DIGEGA (2011-2013), Gómez (2017)
Farms without categorized production	432 (45, 43%)	DIGEGA (2011-2013), Gómez (2017)
**Farms with categorized production and known population	434 (83, 62%)	DIGEGA (2011-2013), Gómez (2017)
**Farms with categorized production and unknown population	85 (16, 37%)	DIGEGA (2011-2013), Gómez (2017)

#### ***Classification of the Population***

Total registered	17.005.669	DIGEGA (2011-2013), Gómez (2017)
Layers and layer-integrated	4. 295.000 (25, 26%)	DIGEGA (2011-2013), Gómez (2017)
Broiler	10. 948.235 (64, 38%)	DIGEGA (2011-2013), Gómez (2017)
Grandmothers	70.000 (0, 41%)	DIGEGA (2011-2013), Gómez (2017)
Heavy breeders	569.000 (3, 35%)	DIGEGA (2011-2013), Gómez (2017)
Light breeding	130. 000 (0, 76%)	DIGEGA (2011-2013), Gómez (2017)
Not identified the category	993.434 (5, 84%)	DIGEGA (2011-2013), Gómez (2017)

#### ***Classification of the farms with known population according to category***

Layer farms	101 (19, 46%)	DIGEGA (2011-2013), Gómez (2017)
Broiler farms	319 (61, 46%)	DIGEGA (2011-2013), Gómez (2017)
Grandmothers	2 (0, 39%)	DIGEGA (2011-2013), Gómez (2017)
Heavy breeders	8 (1, 54%)	DIGEGA (2011-2013), Gómez (2017)
Light breeding	2 (0, 39%)	DIGEGA (2011-2013), Gómez (2017)
Integrated	3 (0, 58%)	DIGEGA (2011-2013), Gómez (2017)
Not identified population	84 (16, 18%)	DIGEGA (2011-2013), Gómez (2017)

\* Purpose of this study

## **Results**

To represent the model, the analysis of the results was performed using 15 variables of interest for epidemiological research (not shown). The farms were classified according the population and according the category of production; the farms were classified by number according the category of production in the farms with known population.

According to the population, from the 951 farms registered, 52.58% (500/951) record a population of 17,005,669 birds; the remaining 47.42% (451/951) does not have a population register.

According to the category or purpose of production, in 54.57% (519/951) the category was registered; in the remaining 45.43% (432/951) was not registered. 83.62% of the farms in which the category of production was registered (434/519) the population was registered. In the remain 16.37% of the farms (85/519) that register the category, the population was not registered.

#### *Size of the farms and population*

According the number of birds the size of the farms was distributed within a range of 50 to 1,500,000 birds. Small units considered family-owned were included in the survey.

**Table 3. Registered population**

<b>Size of the farms</b>	<b>Number of farms</b>	<b>% of farms</b>	<b>Population</b>	<b>% of population</b>
50-1.000	19	3,8	10.250	0,06
1.001-4.000	80	16	224.100	1,32
4.001-9.000	84	16,8	499.300	2,94
9.001-20.000	129	25,8	1.783.369	10,49
20.001-50.000	111	22,2	3.658.100	21,51
50.001-100.000	50	10	3.657.000	21,5
100.001-240.000	18	3,6	2.843.500	16,72
240.001-1.500.000	9	1,8	4.330.050	25,46
<b>TOTAL</b>	<b>500</b>	<b>100%</b>	<b>17.005.669</b>	<b>100%</b>

#### *Affiliations*

We represent a degree of affiliation that represents number of farms registered in the name of the same owner or company. According to this, 58.99% of the farms (561/951) have degree 1, which means that it is not affiliated with another; 41.01% (390/951) ranging from 2 to 37 farms by owner, distributed among 108 owners (see table 4). In all the affiliated farms the name of the owner or company was identified. On non-affiliated farms 9.63% (54/561) appear without owner registration, 85.19% of which (46/54) population was not registered, been that only in 14.81% (8/54) population and category was registered.

The proportion of affiliated owners with degree two represent 6.72% (64/951); degree three 1.79% (17/951); degree four 0.95% (9/951); degree five 0.63% (6/951); degree six 0.32 (3/951); degree eight 0.10% (1/951); degree nine 0.10% (1/951); degree 10, 0.31% (3/951); degree 13, 0.10% (1/951); degree 15, 0.21% (2/951); the largest number of affiliate records, 37 farms belongs to a single company, it represents 0.10% (1/951).

**Table 4. Distribution of the affiliation**

Degree of affiliation	Number of owners	Number of farms
1	561	561
2	64	128
3	17	51
4	9	36
5	6	30
6	3	18
8	1	8
9	1	9
10	3	30
13	1	13
15	2	30
37	1	37
<b>Range 1-37</b>	<b>669</b>	<b>951</b>

#### *Population estimates*

In the farms that had no registered population, the estimate was 13, 670,700 which added to the population registered at the moment of the survey result in a total estimate of 30, 676,369.

If we compare the data of population reported in 1998 and the population registered in the survey there is an increase of 111.88%; comparing the total of the population, estimated and recorded, the increase result in 201.83%.

Comparing the layers recorded and the population of laying hens published by CONAPROPE, 2011, we can conclude that 70.54% of the layers and layers-integrated were recorded in the survey. The population for cage production was not considered due to lack of information. The integrated category includes farms that integrate different systems of production as breeding and laying, or inclusive grandmothers and hatcheries.

In the projections the different categories of local production were considered, grandmothers, breeding, broiler chickens, layers and integrated (CONAPROPE, 2009, 2011; DIGEGA, 2011-2013).

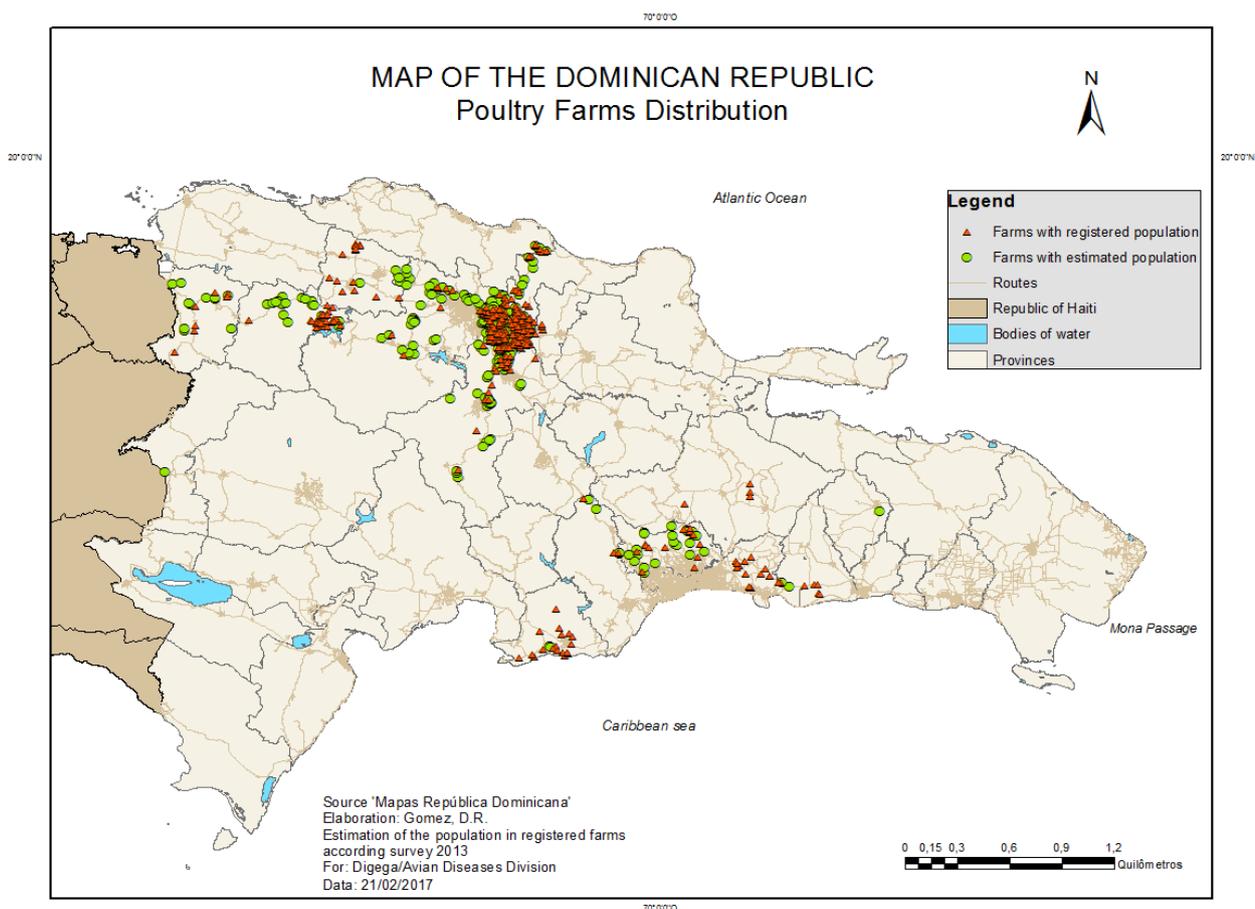
According to the size of the population the largest number of farms registers 9,001 to 20,000 birds, followed by the group 20,001 to 50,000 birds. Both groups represent 48% of the registered population.

**Table 5. Results of the estimates of the total population**

Indicators*	Values	Reference
Population estimated (not registered)	13.670.700	Gómez ( 2017)
Total population (estimated and registered)	30.676.369	Gómez ( 2017)
Total estimates of Layers	4.653.000	Gómez ( 2017)
Total estimates of chickens	17.139.435	Gómez ( 2017)
Total estimates of heavy breeders	1.350.000	Gómez ( 2017)
Total estimates of light breeding	130.000	Gómez ( 2017)

#### *Distribution of farms and population by province*

The largest number of farms is located in the Espaillat province representing 47.84% of the total followed by the provinces La Vega (15, 88) and Santiago (14, 62). Espaillat also has the largest registered population followed by La Vega, Santo Domingo San Pedro de Macorís and Santiago surpassing one million birds. In Elias Piña and Hato Mayor only one small farm was registered in each province. Therefore, in the order of estimated population varies as follows, Espaillat, Santo Domingo, La Vega, Santiago and San Pedro de Macorís followed by other provinces with a lower population.



**Figure 1. Map of the Dominican Republic the showing the poultry farms distribution; the red triangles represent the population registered in a survey and green circles population estimated in this study.**

**Table 6. Distribution of farms and population by province**

Province	Farms	% of farms	Registered population	Total Estimated population
Dajabón	18	1,89	129.000	253.700
Elias Piña	1	0,11	0	2.000
Españat	455	47,84	5.930.689	7.598.189
Hato Mayor	1	0,11	0	4.000
Hermanas Mirabal	6	0,63	79.000	79.000
La Vega	151	15,88	2.894.150	6.384.900
Monte Plata	5	0,53	245.000	310.000
Peravia	20	2,10	425.500	468.500
Puerto Plata	14	1,47	59.000	98.600
San Cristóbal	6	0,63	56.000	96.050
San Pedro de Macorís	8	0,84	2.182.000	2.326.000
Santiago	139	14,62	1.171.000	5.115.100
Santiago Rodríguez	54	5,68	619.830	835.830
Santo Domingo	51	5,36	2.770.000	6.650.500
Valverde	22	2,31	444.500	454.000
<b>TOTAL</b>	<b>951</b>	<b>100</b>	<b>17.005.669</b>	<b>30.676.369</b>

\*Total estimated population sum registered and estimated population

The province Españat concentrates the largest number of farms, followed by La Vega and Santiago. The largest bird population is recorded in the Españat province, followed by the provinces La Vega, Santo Domingo and San Pedro de Macorís.

#### *Distribution and comparison of population by region*

We have compared the results of the different population data in the different regions, the data of the census of 1998, the data of the 2013 survey registered population and the data of the estimated population resulting from this study. When compare the population registered in 2013, represented only in 52.58% of the farms, this proportion exceeded the population registered in 1998, this is to be expected given the growth of the population over the years. When compare population by region, in all cases the largest population is located in the regions North, Central and North central, showing lower values in the central region according the census of 1998, as well as a considerable increase in the population registered and estimated in the east and northwest regions. Even though these results cannot be compared in their entirety, they represent a well-founded basis for gathering information in an upcoming survey.

**Table 7. Distribution of the poultry by region**

Poultry by region	North	Northeastern	Northwest	Norcentral	Central	South	Southeast	East	Total
<b>Poultry registered 2013*</b>	7.160.689	-	1.193.330	2.973.150	3.496.500	-	-	2.182.000	17.005.669
<b>Poultry estimated 2017**</b>	5.651.200	-	350.200	3.490.750	4.028.550	-	2.000	148.000	13.670.700
<b>Poultry total estimates 2017***</b>	<b>12.811.889</b>	-	<b>1.543.530</b>	<b>6.463.900</b>	<b>7.525.050</b>	-	<b>2.000</b>	<b>2.330.000</b>	<b>30.676.369</b>
<b>Poultry census 1998</b>	5.346.300	64.363	214.647	2.514.607	6.884.610	199	7.078	167.051	15.198.855

\* This study

\*\*52.58% of registered farms

\*\*\* registered and estimated

## Conclusions

The final record yielded a total of 951 farms or commercial or small-scale production units, and new records could be incorporated. The study had a real population and an estimated (simulated) population; a quantitative method that validly compares stocks is not applicable. The results showed that poultry sector produce enough information to be used both for epidemiological studies and poultry production studies; we propose this model of data base as a basic tool for the establishment and actualization of the poultry census in the Dominican Republic and other countries. The analysis of the data led to substantial results, proving to be a useful platform and easy to apply. Once you have organized the information and registered the owners and the location of the farms is very easy to complement the rest of the information. The analysis of the information allowed to correct inaccuracies in the data, repetitions, duplication of records and geographical location and to complement some lack information. The comparison of the results of the survey with the existing official information provided more conclusive data.

The generation of statistics applied to animal health contributes greatly to the expansion of health programs. The utility of the data allows us to represent a model of poultry movement network. With this resource it result more easy implement the guidelines for the control of bird traffic and for represent a model of movement of birds in real time.

We conclude that this model database can be used to apply to different areas while it can be used in epidemiological studies focused on disease surveillance and control, it can also be used by different institutions and universities in targeted studies.

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\*Acronyms and references in Spanish.

## Contact networks in poultry productions systems for surveillance and control of avian diseases

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### Abstract

Understanding the dynamics of infection within a host population is essential for the surveillance and control of diseases. Contact networks are commonly used to represent different human infections. In most animal diseases the mechanism of transmission in the field as well as the infection pathways are well known, however, disease spread through contact networks has not yet been represented in all species of production animals. Understand the dynamic movement of livestock and representing it with networks has contributed to surveillance and control of animal diseases, such as Foot and Mouth Disease. Modern systems of poultry production encompass complex mechanism of interaction between the diverse scales of the population involving many partners in the commerce chain. Given the worldwide importance of poultry industry, the use of networks is crucial to furthering research on the transmission and spread of certain diseases, such as avian influenza. In our description of a poultry network each node represents a farm and edges represent the contact between farms. The diverse correlation and distribution between partners and neighbors generates different individual networks that link together resulting a complex dependent-network. The study contributes to the detailed description of a network in a real poultry population.

**Keywords:** Poultry-network, animal models, Individual-agent models, Dependent-network, Epidemic.

### Redes de contato em sistemas de produção de aves para vigilância e controle de doenças aviárias.

### Resumo

Entender a dinâmica da infecção dentro de uma população hospedeira é fundamental para a vigilância e o controle das doenças. As redes de contato são comumente usadas para representar diferentes infecções humanas. Na maioria das doenças animais, o mecanismo de transmissão em campo é bem conhecido, bem como as vias de infecção, mas a propagação através de redes de contacto ainda não foi representada para todas as espécies de animais de produção. Compreender o movimento dinâmico dos animais e representá-lo por redes tem contribuído para a vigilância e controle de doenças animais, como a febre aftosa. Os sistemas modernos de produção avícola implicam um complexo mecanismo de interação entre as diversas escalas da população envolvendo muitos parceiros na cadeia do comércio. Dada à importância mundial da indústria avícola resulta de grande relevância a investigação sobre a transmissão e propagação de certas doenças, como a gripe aviária. Na rede avícola que descrevemos cada nó representa uma fazenda e as arestas representam o contato entre as fazendas. A diversa correlação e distribuição entre parceiros e vizinhos geram diferentes redes individuais que ligadas resultam em uma rede complexa dependente. O estudo contribui para a descrição detalhada de uma rede em uma população real de aves de curral.

**Palavras chave:** Rede de aves, modelos em animais, modelo de agentes individuais, rede dependente, epidemia.

## Introduction

Poultry network have not been yet described profoundly as an animal-based networks model. In modeling of human infectious diseases, many examples have been used to represent contact between individuals and partnerships as *Random*, *Spatial*, *Scale free*, *Lattice*, *Small world* and *individual-based* network models (Keeling and Rohani 2008; Vynnycky and White 2010; Newman 2010). In *animals*, disease dynamics using *individual-based* and explicitly *spatial* models, were used in the 2001 foot-and-mouth epidemic in the United Kingdom, (Keeling et al. 2001b; Morris et al. 2001), based on the known location of the farms (Keeling and Rohani, 2008). In *spatial network* individuals are probabilistically connected based upon the distance between them; the probability is given by a connection kernel which usually decays with distance such that connections are predominantly localized (Danon et al, 2011).

In *individual-agent* models based on animal diseases it is of great importance to represent a realistic situation of the agent, so as in emergent animal diseases. Some animal diseases affect many species making the contact between individuals susceptible and infected difficult to represent through models. Usually the transmission route is known, so that is used to represent the infection time step. Frequently, the population number and their distribution is unknown, and there are no records of contact chain by commerce, affiliation, movement and dynamics between farms and/or from farms to markets.

Nickbakhsh et al., (2013), gained insights of the implications of the dynamics of network links between premises using empirical data from a major catching company in England. They show how particular assumptions for

within-farm dynamics have implications for the pathogen spread at the population-level demonstrating the importance of cross-scale interactions for HPAI in commercial poultry and on the control measures at the farm level. Leibler et al., (2010), used company affiliation and social contacts to estimate risk of avian influenza transmission between farms. Spatial factors and locational data of farms were not explicitly on the model and contact network are not mentioned. Lockhart et. al., (2010) used social network analysis to describe the topology of contacts resulting of a cross-sectional survey between 58% of the members of the New Zealand poultry industry association. They represented the contact structure created from the movement of feed, live bird and hatching eggs, table eggs, and manure and waste litter structure of the networks.

To represent more realistic epidemic models in animals in addition to the population itself, it is important to consider the dynamic of that specific animal population, including the location of the farms and the interactions between farms or at least most of them. We are describing a network model in a realistic poultry population which can be applied to an *agent-based* model such as Avian Influenza or Newcastle Disease, and to *individual-based simulations* network models (Vynnycky and White, 2010), since it explains in detail the network of individuals and their partnerships in poultry.

## Methodology

We performed a comprehensive data analysis to demonstrate the complexity of the contact and the interactions in poultry populations. The poultry network describes different structures (farms) illustrated in graphs by nodes showing the different levels of production according

their contacts. The model represents the contact network formed by different structures in which the principal nodes are connected on a large scale. The structure gives rise to the entire production chain involving the complex production system, from the origin of the progeny to the end of the chain of production, including partners, affiliated and neighbors. In fact, the whole structure is not be described as we excluded in the commercialization phase, slaughterhouses, incubators and markets, due to the lack of this information. Our poultry network describes exclusively the location and interaction between poultry farms.

This *poultry network model* is a dynamic structure built from the database for the Register the poultry Production in the Dominican Republic (Gomez and Ferreira, 2017, unpublished thesis chapter). To create this network it is necessary to have a well-organized data structure codified according to the property, owner and farm. The farms must be classified according to the geographical location, category of production and size of the population. This structure allows for owners and properties to be linked as commercial partners, determining the degree of affiliation between owners or business relationship. Based on this information we create the array of contact forming the network between affiliated farms, and we construct a matrix model for creating a commercial network of contacts between possible partners, according to the production pyramid.

Data was initially stored in a spreadsheet in an excel database, as a .csv file; analysis was performed using R language 3.3.2 statistical platform; *igraph* library was used in the graphs layout. We use the packages *devtools* and *epinemo* for quantitative network analysis, and the software Gephi 0.9.1. Maps were created with the software QGis, ArcGis and Google earth map.

The network was represented based on the total population and the dynamic. We consider

each farm as an epidemiological unit, obviating the internal dynamics of the unit, since the birds in each farm are grouped by lots that in turn are distributed among a certain number of sheds of different capacity. Daily movements between birds are not implicit; the network only identifies relationships between contacts. According to the activity of the poultry industry we referred to the contact between poultry farms, not among the individuals forming the population. This contact is very important for understanding the population dynamics and for identifying transmission rates of highly contagious diseases of rapid spread in birds. Partnerships in dynamic networks may change over time as individuals enter and leave the population.

Vynnycky and White (2010), refer to the importance of small world networks for understanding the rapid spread of infections through human populations.

Describing the complexity of the *poultry network* is likely to provide a more realistic identification of where to focus efforts to reduce the risk and spread of infection.

Networks provide a unified way about the interaction between individuals or populations (Keeling and Rohani, 2008). A matrix from all the interaction strengths specifies all the connection strengths within a network expected to be sparse with the majority of values being zero.

$$\rho_{ij}$$

Assuming  $\rho$  is a measure of the strength of interaction between populations,  $\rho_{ij}$  measures the relative strength of transmission to subpopulation  $i$  from subpopulation  $j$  (Keeling and Rohani 2008). Where  $i$  represents the susceptible and  $j$  the infectious individual.

Networks can be represented as graphs containing *vertices* or *nodes* that are

represented by points, and *edges* or *links* represented by lines (Vynnycky, 2010).

The network of contacts is specified by a graph matrix  $G$ , where  $G_{ij}$  is 1 if individuals  $i$  and  $j$  are connected, or 0 otherwise (Keeling and Rohani, 2008). Directed network or directed graph is a network in which each edge has a direction, pointing *from* one vertex to another (Newman, 2010).

$$G_{IJ}$$

The network is defined as undirected when  $G$  is symmetric and infection can pass in both directions across a contact (Keeling and Rohani, 2008).

$$G_{ij} = G_{ji}$$

A graph can be formed by different values ( $n$ ) of vertices and edges so it may have self edges, single edges, multi edges and both. A network with multi edges is called a multi graph (Newman, 2010).

The degree of a vertex in a network is the number of vertex attached to it. In undirected networks, degree is just a single number. In directed networks on the other hand, vertices have two different degrees, in-degree and out-degree equivalent to the number of edges pointing inward to and outward from those vertices (Newman, 2010).

### Poultry Network structure

In poultry networks each node represents a farm and the edges represent the contact between them. Production farms are composed by houses, and houses in turn have different populations of birds. Despite the population distribution we are assuming each farm as a node. So, each node will be composed by different number of individuals, and inclusive, different levels of production which represent a complex scheme in the transmission network, based on the etiological agent. We represented the contact network

based on the type of contact or interaction between farms. Therefore, a *node* will have as many *edges* as it has contact with other nodes.

According to the graph definition, the *links* between poultry farms are *directed*, if the contact between two nodes though the edges occurs only in one direction, and *undirected*, if the contact between two nodes through the edges occurs in both directions. The type of contact in the network depends on the distribution of the production system, the direction of movements between the different categories of birds *from* one farm (node) to another, and the degree of affiliation between companies of the same owner. In addition, spatial networks represent neighboring farms that share a common boundary and those that form a cluster; in the poultry network these represent a big cluster based on the density, location and distance between the farms.

According the scale of interaction between farms we describe three principal structures of contact in the poultry network: A) Breeding or genetic farms; B) production farms C) Affiliated farms.

**A)** The poultry network is based initially on the pyramid structure that begins with the **breeding** lines. In some developed countries, the structure of poultry production begins with the great-grandparent lines and is formed by two lines, laying-hens and broilers, that give rise the respective grandparent lines. Our model starts representing the structure of broiler grandparent line. The laying grandparent lines are not represented in the network because in the country actually there are no these farms since their origin is importation. We will describe the three principal contact structures that involve breeder farms, which form a *direct contact* chain in the pyramid of production, and we use the terms *from-to* to refer in to the origin and course of the contact in these networks:

**1. First structure:** contact from **grandparents** to **breeders**:

The contact from grandparent farms to breeding farms involves a big number of

partners. Hatcheries are in the middle of this contact chain. Fertile grandmother-layed eggs go into incubators, and once they hatch, day-old chicks are distributed to the different breeding farms. In most cases, hatcheries are a separate structure from the grandparent farms, in some cases they are part of the same epidemiological unit, which is not recommended by animal health standards. Due to the complexity of the network, we should consider hatcheries as part of the network, as a string contact. Hatcheries could be excluded in models in which transmission comes from progeny and not from the hatchery as a structure in itself; they can be included in risk models when the origin and destination are known. The biosecurity and disinfection measures applied to the eggs during the incubation process minimize the risk of transmission of infections. We assume that the network contact from grandparents to breeding broiler occurs as *direct contact*. The intermediation of incubators is omitted due to lack on the actual flow between them.

**2. Second structure, from **breeding** (broilers and laying) to **production farms**:**

a) There is a directed contact from **breeding broiler** to chicken farms.

b) There is a directed contact from **breeding laying hens** to laying hens.

In these structures hatcheries are also involved in the contact chain, and may or may not be included in the contact network.

In these two structures we represent the contact in the network as directed occurring movements in a single direction.

**3. Third structure, *integrated farms*** in which breeders are produced in the same area or unit with other productive categories, for example: broiler breeders and/or broiler production; laying-hens breeders and layers; broiler breeders and laying-hens breeders; broiler breeders, laying-hens breeders and layers. In this structure contact can occur from

integrated-to-integrated, from integrated to broilers and/or from integrated to layers. Due to the complexity of this structure many cases these farms often end up forming undirected networks.

**B)** The poultry network finally converge in the structure composed by the **production** or **commercial** farms, made up by the chicken farms, laying farms, or farms that combine these two categories. They are involved in the production of chicken and eggs, and may or may not be affiliated; they can also represent self edges, multiedges or both.

**C)** Affiliated farms representing any A or B structure that have a degree of interaction between them. They are characterized by the fact that the activity occurs specifically between farms of the same owner with a range or *degree of affiliation* greater than two units. According to the type of structure, the contact between these farms can be represented as a directed or undirected graph. To represent contact by affiliation we assume to use the undirected graph and assign an exponential Gaussian kernel corresponding to the distance between the farms.

If the contact occurs by affiliation, the network can be undirected or directed depending on the type of productive system and movements between farms.

The contact with neighbors is determined in A, B or C by a distance matrix which will depend on the distance allocated. Clusters are commonly observed in the nearest neighbors. The probability of contact between neighbors is the same; therefore the graph is represented as undirected.

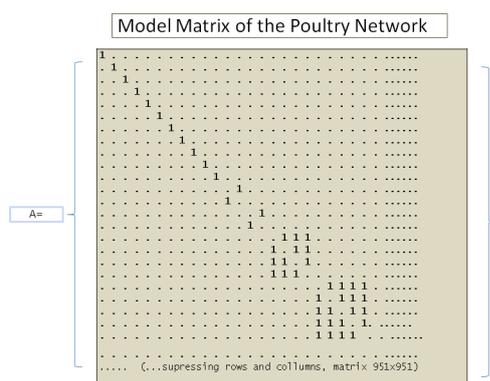
### **Adjacency matrix in poultry network**

In the poultry network model, the adjacency matrix is 1 if there is an edge between vertices  $i$  and  $j$ , and 0 otherwise. This is true whether

the contact happens by affiliation or through the productive chain.

## Neighboring

The contact between neighboring farms is represented by a distance matrix;  $d_{ij}$  represents the distance between the susceptible individual  $i$  and the infectious individual  $j$  (not shown).



The adjacency matrix shows the distribution of non-affiliated and affiliated farms. Number One (1) represent contact, points represent no contact. Gomez et al. 2017.

Model of sparse Matrix class “ngCMatrix” resulting from the distribution of non-affiliated and affiliated farms. Single farms appear on the diagonal; affiliation degree two, four and five are shown in the matrix. Diagonal “zero” is observed in affiliation contact (in R language).

## Results and discussion

The results show the structure of the poultry network, and the type of contact between the different categories that integrate the productive pyramid; first we describe the structure of grandparents and breeders which represent the origins of the progeny, the principal *nodes*. We describe their contact relationship, *edges*, with the production farms, the particular integrated system and the combined system of both. In addition we involved the productive categories, commercial layers and chickens, and conclude describing the affiliation existing between all of them, representing *multi edges*. Contact network can occur in two ways, affiliation or neighboring. Our results permit us describe the different

networks that make up this complex network which although it has a small number of nodes, can be represented in a large number of contacts:

The **poultry network** is formed by 951 nodes represented by the same number of farms, different forms of contact occur between them, resulting in a complex network that we describe in three models:

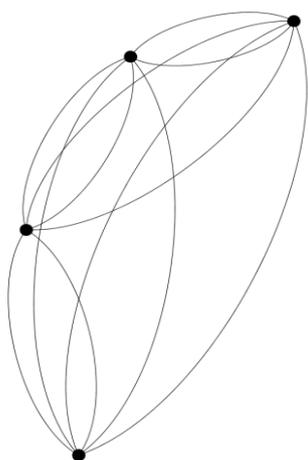
1) A *Spatial network*, represented by the contiguous or neighboring 951 farms that share common boundary and those which form a cluster. In spatial networks, a kernel is often used to calculate the probability of two individuals being connected depending on the distance between them (Watts 1999; Read and Keeling 2003; Keeling 2005a).

2) A *Scale-Free network*, represented by the contact between farms that belong to the same owner according the affiliation. *Scale-Free networks* connect individuals with a probability that is directly proportional to their current number of partners, distributing according to a power law (Vynnycky and White 2010).

3) In general we describe the poultry network as an *animal-based network model* that we denominate *DEPENDANT NETWORK MODEL* as it depends on the type of contact between partners. This network represents the complexity of contacts existing in the poultry systems. There is a group of individuals that form a unique population as an internal condition of each farm with an internal and particular dynamic between them. Some of these farms can form clusters or be linked as neighbors with spatial dependence, and there may be interactions between farms of the same owner; likewise, among all of them exist a general contact by trade relationships. Throughout the structure, a contact dynamic converges constantly. This is a simple way to understand the complex structure of the poultry network.

The model describes the characteristics of a realistic poultry population and is applicable to animal disease spread and to individual-agent transmission models. It is an *animal-based network* model with dependence of the interaction. Individual-based simulation network models are created by modeling of the entire network of individuals and their partnerships. These models are widely used in complex ecological systems and are becoming more common in epidemiology (Vynnycky and White, 2010).

The spatial network results in a big matrix where more distanced farms are 300 km apart. The network can be described based on two types of contacts represented as scale-free network: the affiliated matrix that resulted in a network with 951 nodes and 1,427 edges and the production matrix which results in 281 nodes and 350 edges. The whole structure of the Network has 951 nodes and 3,192 edges. All of them result in a sparse matrix with most of the values being zero. The results had shown a complex network with self edges, single edges, multi edges forming a multi graph of directed and undirected mode.

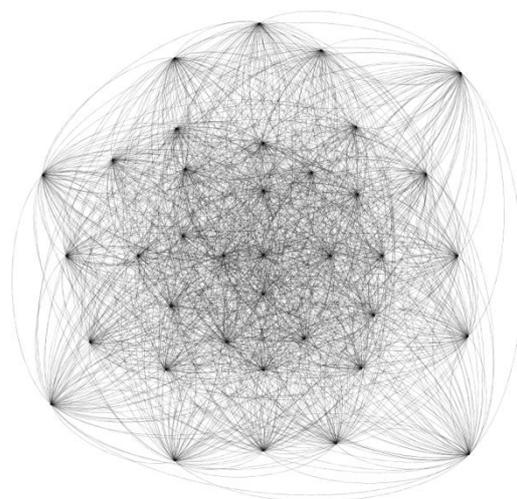


**Figure 1.** Undirected graph represent how is the contact by affiliation between four farms of the same owner in a matrix 4x4. Layout Force Atlas distribution in Gephi.

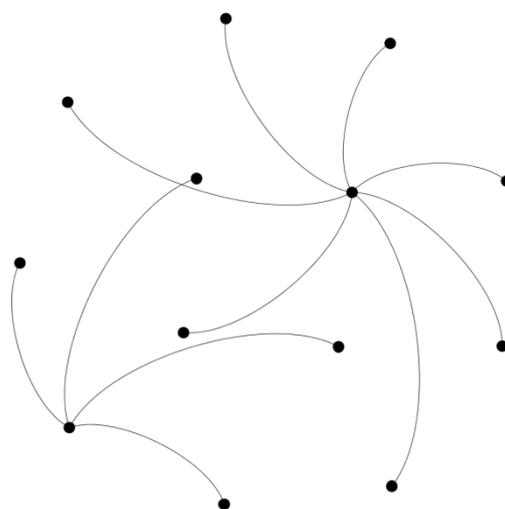
A graph can be formed by different values ( $n$ ) of vertices and edges so may have self edges, single edges, multi edges and both. A network with multi edges is called a multi graph

(Newman, 2010). In a multi graph the contact network can be directed or undirected, according to the type of contact between partners. In affiliation we represent undirected graph, which means that contact occurs in both directions.

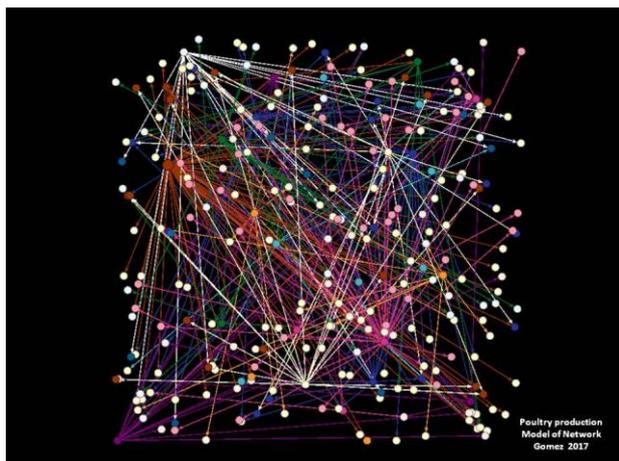
In the production chain, we considered the contact as directed, so the graph represents only one direction.



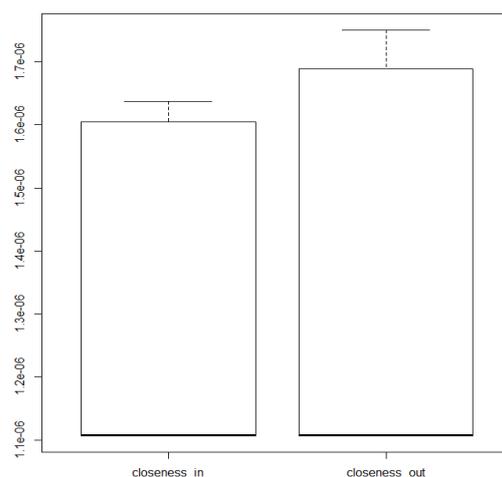
**Figure 2.** Undirected graph representing contact by affiliation between 37 farms of the same owner in a matrix 37x37. Force Atlas2 distribution, mode LinLog mode prevent overlap, approximate repulsion, in Gephi.



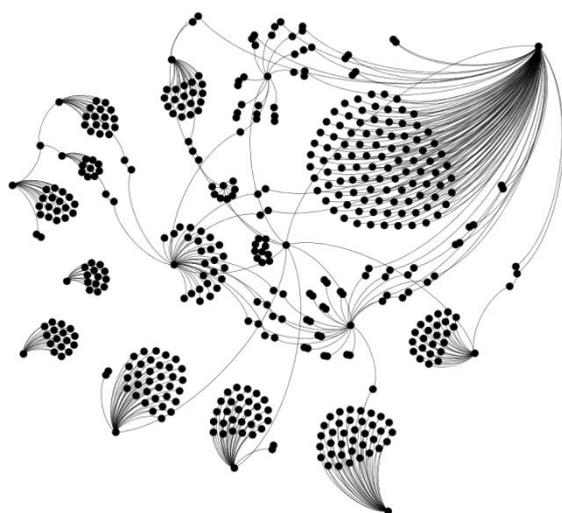
**Figure 3.** Directed graph represents the contact in the production structure from two grandparent's farms and their respective breeders. Two directed graph are shown, corresponding out degree is four and seven respectively.



**Figure 4.** Directed graph representing the production structure with 350 nodes and 935 edges. Contraction distribution in Gephi.

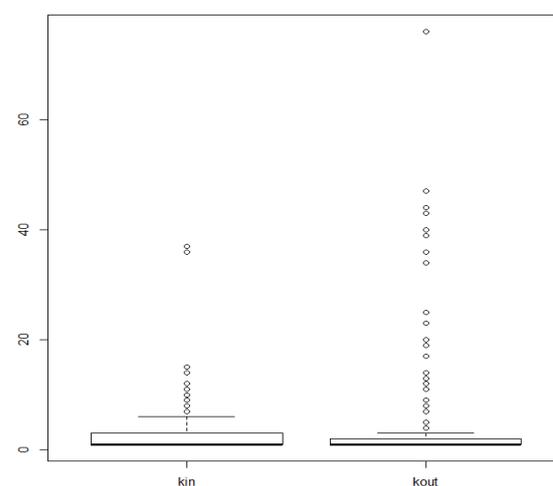


**Figure 6.** Closeness in and closeness out in the poultry network.

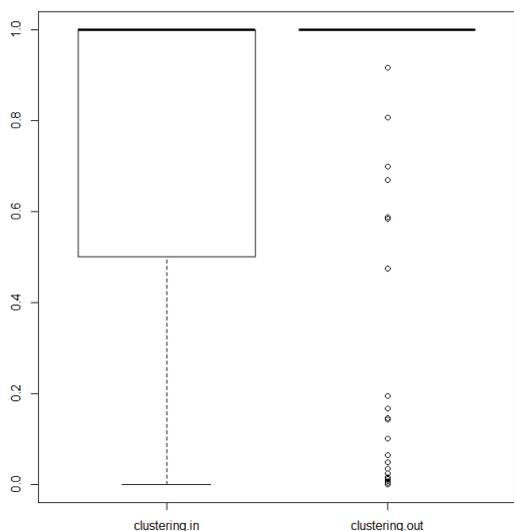


**Figure 5.** Directed graph representing the contact from integrated farms to broilers-farms and layers-hens. Different degree distribution is observed; multiple edges are shared between the contacts.

Centrality measures are associated to the structure of the network. We describe two principal contacts in the Poultry network, the production and the affiliated; both generate individual networks, in the first. We show centrality measures, closeness in and out, in and out-degree, clustering coefficient and page rank of the production individual network.

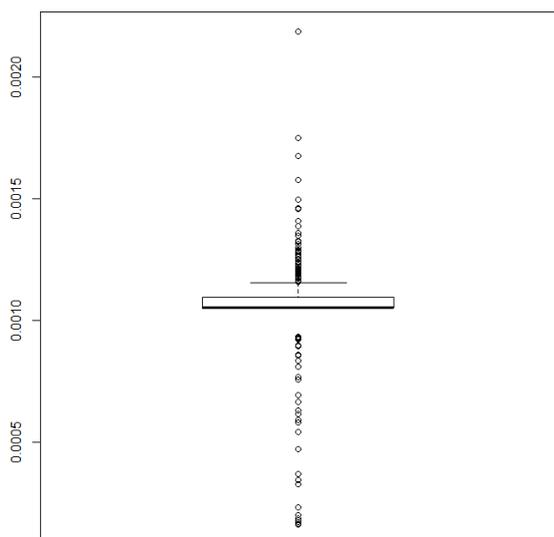


**Figure 7.** In-degree (kin) and out-degree (kout) in the poultry network.



**Figure 8.** Clustering coefficient in the poultry network

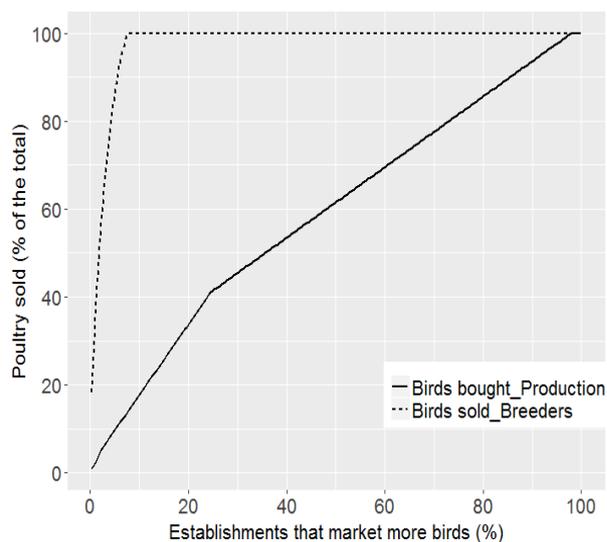
In degree an out degree helps to understand the relation between degree of entrance and degree of exit in each property representing the contact between partners in the production network. In figure 11 a small number of farms send birds to a big number of farms. The contact represents the breeders to the production system.



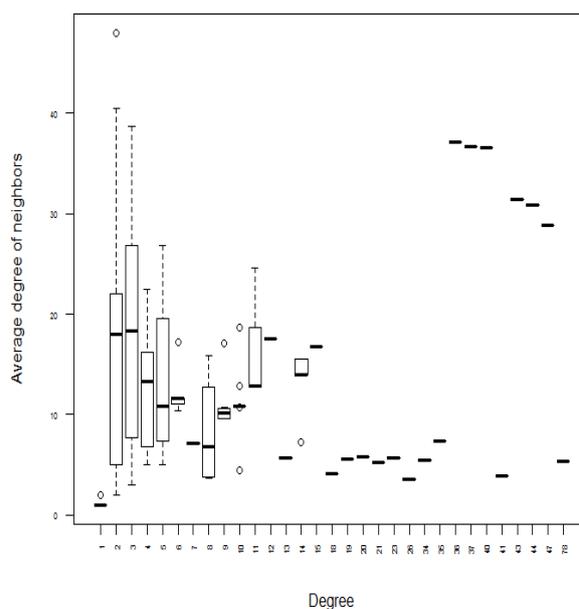
**Figure 9.** Pagerank in the poultry network.

The pagerank is an adjustment of the centrality according to the number of links.

For representing distribution of the population the Pareto principle can be calculated. For demonstrating we compare the distribution in each network, the production and the poultry network.



**Figure 10.** In the production structure 100% of the birds sold by the breeders are proportionally distributed in the total of the production according the Pareto principle.

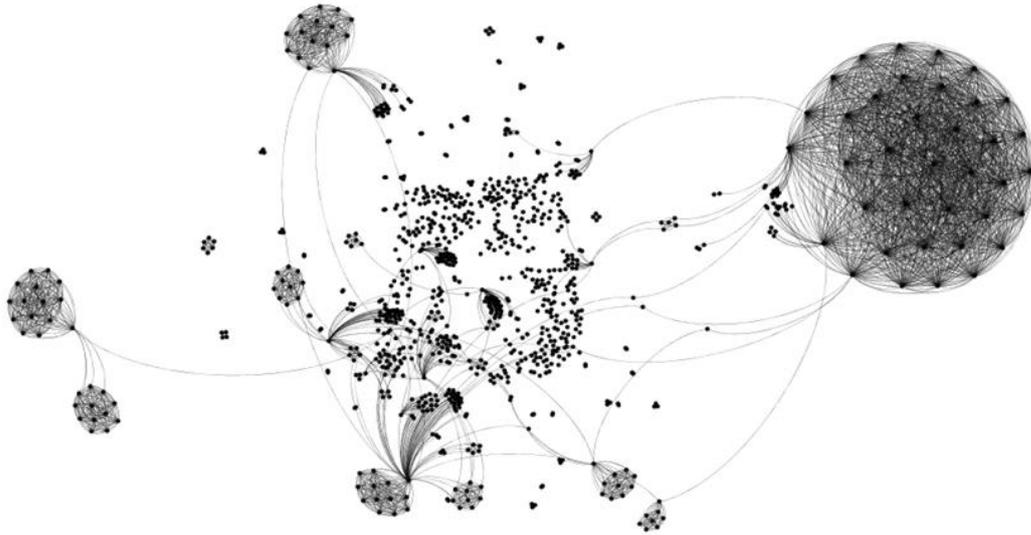


**Figure 11.** Average degree of neighbors in the poultry network.

Another way to evaluate assortativity is to calculate the average of the average degree of neighbors for each grade category.

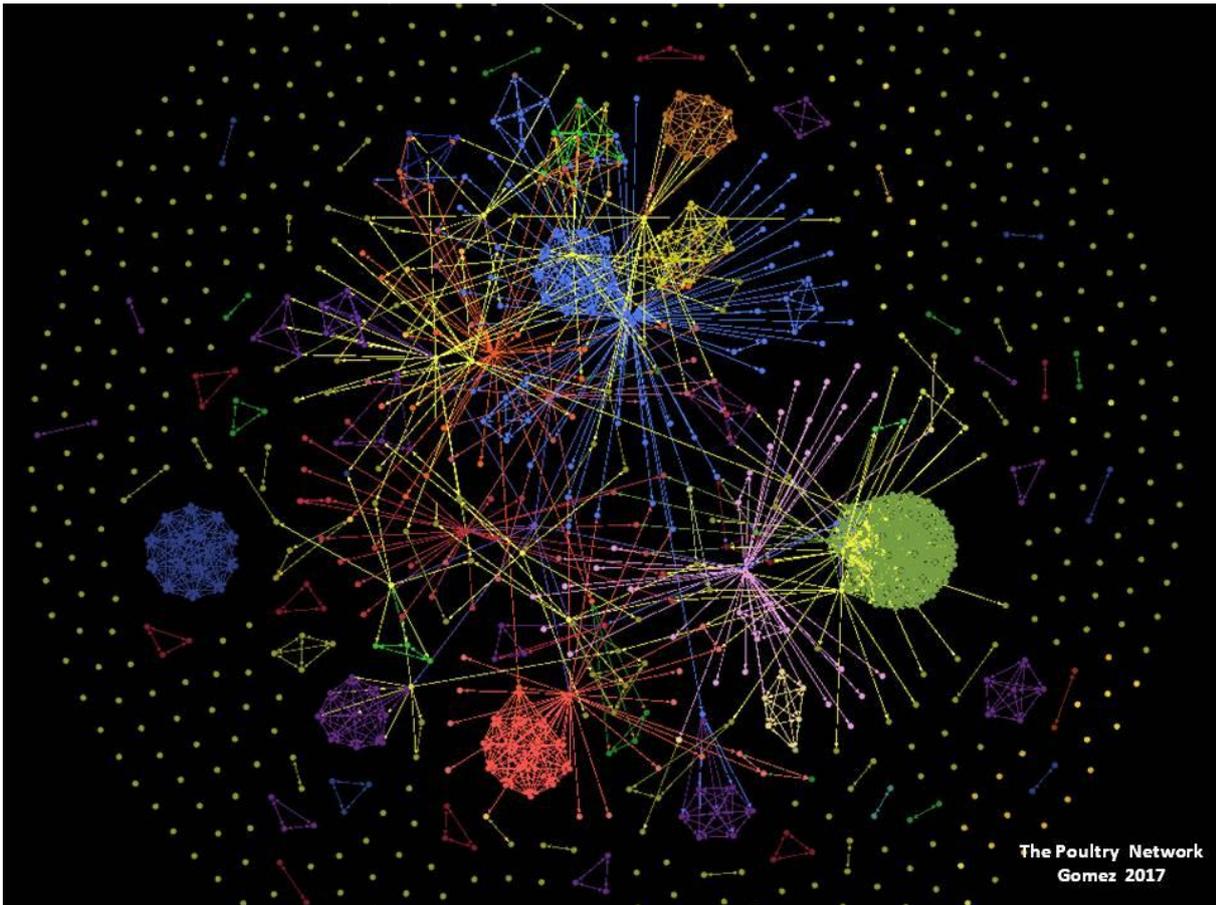
## **Conclusions**

The main characteristic of the poultry network is the design of the contact matrix. The complexity of productive and commercial activities between farms was explicitly demonstrated in the graphs. We consider this to be the first detailed description of a poultry network in a real population. Other animal networks have been described without emphasizing the construction of the data structure. This network describes interactions that are not described in other ecological works. We recommend that future studies include incubators as an important point of transmission between the breeders and production birds. Slaughterhouses and markets could also be an important reference for studies of animal and human transmissible diseases.

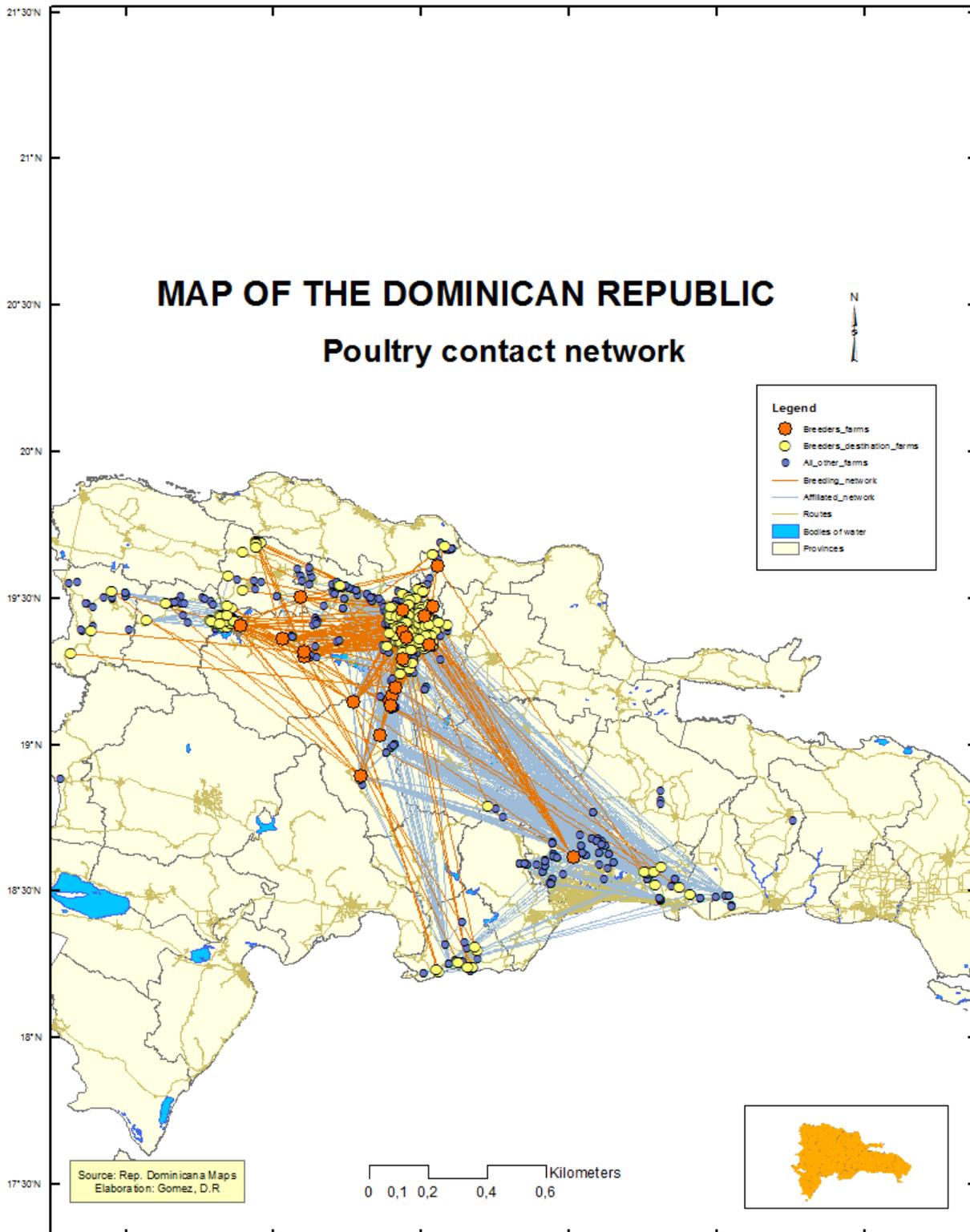


Poultry Contact Network  
Gomez 2017

**Figure 12. Poultry network.** Graph shows the degree distribution according to the owner's affiliation; undirected mode. 951 nodes and 1,427 edges are shown. Non-affiliated farms appear in the middle of the distribution.



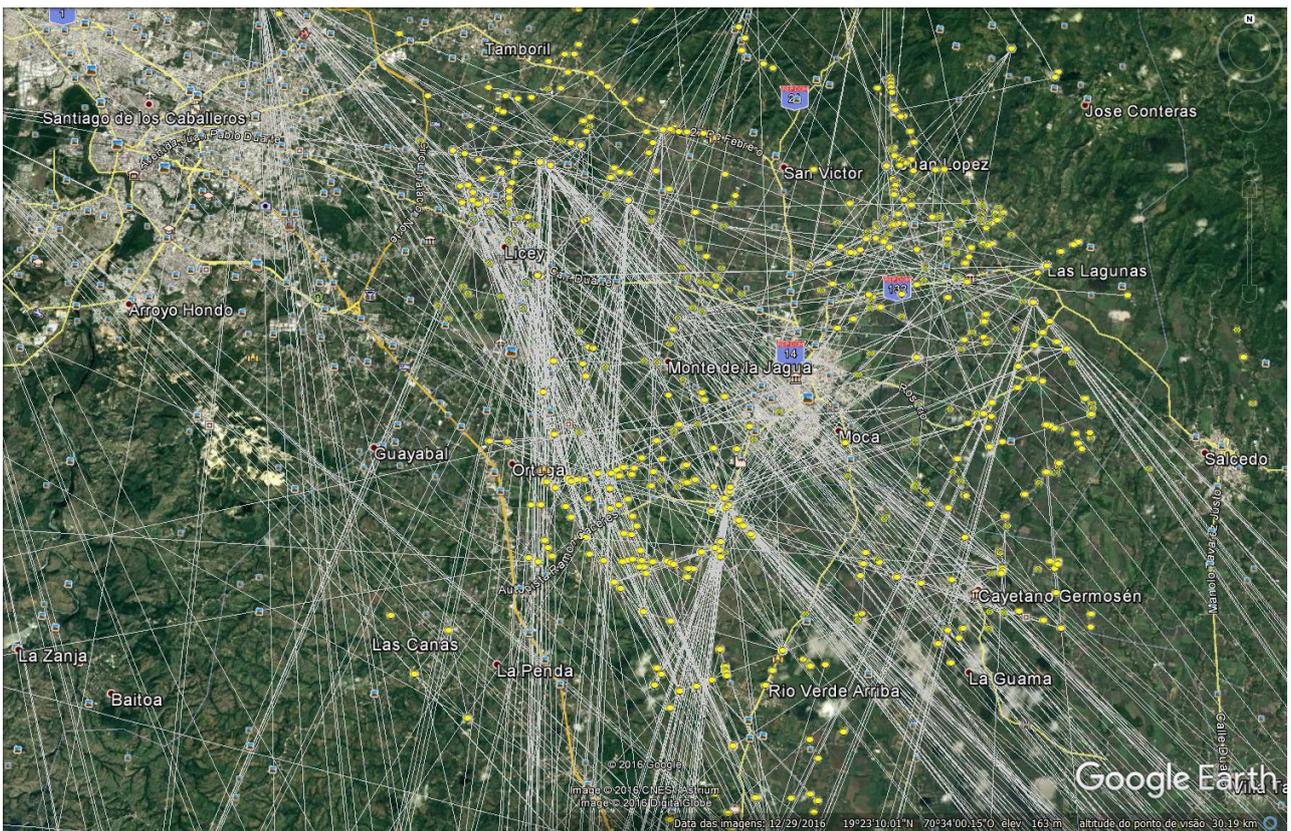
**Figure 13. Poultry network.** The graph represents the network in all the structure, 951 nodes and 3,192 edges colored according the contact and/or the nearest neighbors (within 2 km). The poultry network is an animal-based model dependant of the different mechanisms of interaction between the contacts, which may be either, directed or undirected mode, or both.



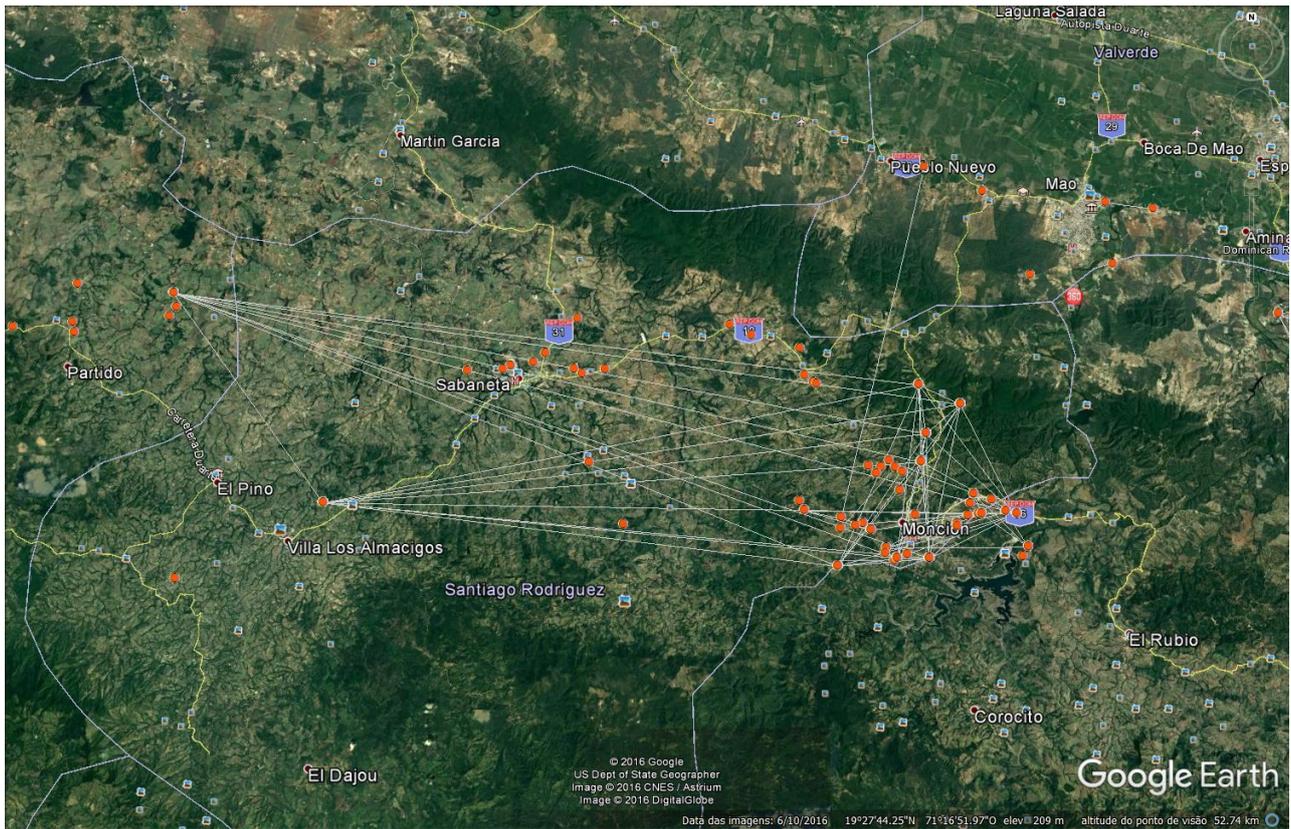
**Figure 14.** Map of the Dominican Republic representing the poultry network structure. Blue points and lines represents nodes and edges of the owner's affiliation; orange points represents the nodes of origins of genetic lines, grandparents and breeding farms; orange lines represent the contact from breeders farms to the production chain; yellow points represent the destination of breeders.



**Figure 15.** Map showing a visualization of the network in the province Espailat, the greater in population.



**Figure 16.** Google earth image showing a visualization of the network in the provinces Espailat and Santiago.



**Figure 17.** Google earth image showing the contact by affiliation between farms in the Santiago Rodriguez province.

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## **Propagation Models of high and low pathogenic Avian Influenza in poultry operations in the Dominican Republic.**

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### **Abstract**

Different subtypes of avian influenza (AI) virus have been circulating in many countries around the world in their pathogenic and non-pathogenic forms. In North America, H5N2 subtypes, emerging from low pathogenic strains have caused important outbreaks in poultry in Canada, United States and Mexico. Strains of low pathogenicity have been reported to cause clinical or sub-clinical signs in birds through the Americas. In 2007 the Dominican Republic reported sub clinical infection with H5N2. More recently the H5 outbreaks in the United States caused by a Eurasian-North American reassortant have led neighboring to establish mechanisms to improve surveillance in poultry and wild birds. Developing a model in a dynamic population is the best way to represent the complex phenomenon of influenza virus spread. By setting up a stochastic *SIR* model we estimated within-flock transmission in different sizes and levels of the population. We modeled transmission between premises based on a network structure considering the realistic contact between poultry farms and the interaction between different production sectors and owners. We used transmission parameters of experimental infection with low (LP) and high pathogenicity (HP) AI North American lineage H5N2 for modeling propagation. These results represent viral propagation of 6 LPAI and 6 HPAI scenarios through a poultry contact network without the implementation of control strategies for during 100 days. In both LPAI and HPAI scenarios the epidemics peaks at approximately 34 days infecting about 700 farms. Both stages of infection result in large outbreaks in very short time.

**Keywords:** Avian Influenza, propagation, SIR, transmission, dependent-network model.

### **Modelos de propagação da gripe aviária em operações de frangos em República Dominicana**

#### **Resumo**

Diferentes subtipos de vírus de influenza aviária (IA) têm circulado por vários países do mundo em suas formas patogênicas e não patogênicas. Na América do Norte, subtipos H5N2 emergentes de estirpes de baixa patogenicidade têm causado surtos importantes em aves de curral no Canadá, Estados Unidos e México. Muitos outros países nas Américas relataram vírus não patogênicos com sinais clínicos ou subclínicos. República Dominicana em 2007 relatou infecção subclínica por causa de H5N2. Mais recentemente surtos nos Estados Unidos por causa de vírus H5 recombinante linhagem Eurasiática-Norte Americana levou aos de países da região para estabelecer mecanismos para melhorar a vigilância nas aves de produção e selvagens. Desenvolver um modelo em uma população dinâmica é a melhor maneira de representar o complexo fenômeno da propagação do vírus da gripe. Através da criação de um modelo estocástico SIR estimamos transmissão intra-rebanho em diferentes tamanhos e níveis da população. Com base em uma estrutura de rede modelamos a transmissão entre as instalações de aves considerando o contato real entre fazendas e segundo a interação entre diferentes setores da produção e proprietários. Utilizamos parâmetros de transmissão de infecção experimental de vírus BP e AP H5N2 linhagem norte-americana para modelagem da propagação. Os resultados representam a propagação em seis cenários de baixa e seis de alta patogenicidade infectados durante 100 dias através de uma rede de contato de aves sem programar estratégias de controle. Em ambos os cenários a epidemia atinge picos no dia 34 aproximadamente infectando cerca de 700 fazendas. Ambos os estágios da infecção resultaram em grandes surtos em tempo muito curto.

Palavras chave: Gripe aviária, propagação, SIR, transmissão, modelo dependente de rede.

## INTRODUCTION

Low pathogenic (LP) and highly pathogenic avian influenza virus (HPAIV) strains of the H5N2 subtype have been reported in poultry in the Americas for over 30 years. Some LP strains have been found to cause sub-clinical signs in poultry populations, such as the H5N2 virus isolated in the Dominican Republic in 2007 (OIE). Over the years the odds of propagation in the poultry industry have been studied in many countries. Due to the impact of H5 LPAIV on trade and the risk for poultry farming, it is crucial to identify the phylogeny of the virus and follow preventative measures. Early detection of subclinical infection with LPAIV is challenging in poultry populations. Field morbidity and mortality rates are important to simulate models of transmission however they are not readily available due to lapses in time between outbreaks or due to lack of information. Recent outbreaks can offer robust information, if data is available. Currently, the most effective method for recognizing detection of the first occurrence of AIV infection within a flock is periodic laboratory testing on the flock, and immediate testing of ill and dead birds (Spickler et al. 2008). Compared to HPAIV, LPAI viruses are considered to be of lower risk because they are associated with limited mortality in poultry, but they may be of risk to humans. Their circulation in poultry flocks provides a setting for viral evolution and human exposure (Leibler et al. 2010). The spread of avian influenza intra-campus was more often of interest in modeling studies (Bos et al, 2007, 2009, 2010; Savill et al, 2006b, 2008; Tiensin et al, 2007).

One of the most important benefits of models is their ability to analyze complex subjects. These models then become a logical structure of the real world

(Vynnycky and White 2010). The number of mathematical models to predict the epidemic dynamics is increasing (Keeling and Rohani 2008).

The objective of the present study is model propagation of AIV in the poultry population of the Dominican Republic using transmission parameters from low and high pathogenic H5N2 viruses of North American lineage. Modeling different stages of infection reveals how AIV infection can spread within the population during the first 100 days without implementing control measures.

## MATERIALS AND METHODS

We use data from the Poultry Producers Register on Dominican Republic (PPR-DR)\* (*Unofficial acronym*), a Statistical model of the Avian Diseases Surveillance Program (ADSP) of the Ministry of Agriculture (Gomez and Ferreira, 2017, in course). The data represent the poultry farms registered in the country, linked through geographical positioning, owners, companies, and commerce interactions. We modeled propagation of infection between 951 premises with different scale and types of production through a contact matrix that represents the connections within the *Poultry Network* (Gomez et al, 2017). Two matrixes were used: a distance matrix and an affiliation matrix. The distance matrix has a dimension of 951 rows per 951 columns and each cell stores the geodesic distance between the properties. The affiliation matrix is a sparse array of the same size, storing value 1 in the properties that are in contact because they belong to the same owner.

**Model:** The built model considers intra-property and inter-property transmission. The population within each property was divided in three categories: susceptible (S), infected and infectious (I) and recovered (R). Disease transmission

between susceptible and infected occurs at a rate of  $\beta I / N$ , where  $N$  represents the total population ( $N = S + I + R$ ). Once infected, the individual can die of the disease at a rate  $\alpha$  or recover at a rate  $\delta$ . The possibility of loss of immunity was not considered because the simulations were performed for periods of 100 days. All individuals are subject to a natural mortality rate at the end of the cycle equal to  $\mu$  and are replaced by susceptible at rate  $a$ . Two different values of  $\mu$  and  $a$  for chickens and reproduction/laying properties were considered according to table 1.

The equations below represent the dynamics of the disease within each property.

$$\begin{aligned}\frac{dS}{dt} &= aN - \beta S \frac{I}{N} - \mu S \\ \frac{dI}{dt} &= \beta S \frac{I}{N} - (\alpha + \delta + \mu) I \\ \frac{dR}{dt} &= \delta I - \mu R \\ \frac{dN}{dt} &= aN - \mu N - \alpha I\end{aligned}$$

The values of the parameters used are shown in table 1. The disease dynamics was simulated using the Gillespie Stochastic Simulation Algorithm performed with R language 3.3.2 statistical platform, we use the packages *rgdal*, *sp*, *foreach*, *Matrix*, *doMC*, *ggplot*, *mapproj*, *rgeos*, *geosphere* and *GillespieSSA*.

Transmission between properties was considered possible by two mechanisms: (1) due to spatial proximity and (2) resulting from contacts at greater distances between properties of the same

owner due to the sharing of inputs, labor, vehicles, or other.

Spatial proximity transmission was allowed for distances shorter than two kilometers. It was considered that the probability of transmission would drop exponentially, reaching a negligible probability, arbitrarily set at 0.00001, for infected properties 2 km away from uninfected property. Equation (1) presents the probability of transmission as a function of distance ( $x$ ) in km.

$$(1) \quad P_1(x) = e^{-5.76x}$$

Thus, each day infected properties were identified within a 2 km radius of each susceptible property, and a random numbering was drawn between zero and one. If the number drawn was less than or equal to the transmission probability for the distance between the properties, the transmission would occur generating infection of 10 animals in the susceptible property (this number was chosen arbitrarily).

The second mechanism of transmission between properties states that properties belonging to the same owner could contact every 10 days indirectly by trucks, labor, supply of inputs, etc. Such contact could result in infection at distances of up to 200 km. Again, an exponential decay function was used to estimate the probability of infection of each contact as a function of distance. In this case, the probability of transmission was considered negligible (0.00001) for contacts between properties distant 200km. Equation 2 presents this function:

$$(2) \quad P_2(x) = e^{-0.057x}$$

Six scenarios different scenarios were simulated twice each, once for HPAIV infection and once for LPAIV infection. Twelve simulations were performed total,

seven times each. The six scenarios differed in the property initially infected. The initial condition was selected with a different criterion according to the population, category of production, density and degree of affiliation between farms. The infection started in four different properties, in which ten birds were initially infected. The rest of the 951 properties were susceptible individuals; in each property the population size was obtained from the Dominican Republic database.

Scenarios criteria:

1. **Small farms or backyard:** farms with population sizes of less than 100 birds located in provinces with high farms density.
2. **Greater farms:** With a population size greater than 240,000 birds, different degree of affiliation considering the smallest and the largest range and belongs to different owner.
3. **Single units:** Farms not affiliated with other farms, with population between sizes between 20,000 and 240,000 and known type of production; we excluded breeders.
4. **Affiliated units:** Farms from owners with a degree of affiliation equal to two (2), population size less than 20,000, known purpose of production and belonging to different owners.
5. **Genetic line:** Included grandmothers, breeder-layer and breeder broiler, belonging to different owners.
6. **Density zone:** We select the province with greater density of farms, and farms with lower degree of affiliation and low population.

Each one was simulated for high and low pathogenic, which result in 12 scenarios.

## RESULTS AND CONCLUSIONS

This study characterizes one of the most thorough network models of AIV to spread

in a realistic poultry population. Figure 1 represents the 12 scenarios; HPAI infection in scenarios 1 to 6 and LPAI infection in scenarios 7 to 12.

In the HPAI scenarios, the epidemic curve is similar in scenarios 1, 2 and 6, with an increase of infection in days 6 to 10 resulting in proximately 100 farms infected in scenarios 1 and 6. Then the spread keeps increasing until 19 to 21 days with 200 farms infected and at day 25 the number of infected farms is up to 500. The epidemic reaches its peak approximately at day 34 infecting almost 700 farms and decreases at day 100, remaining at about 600 infected farms. The epidemic curve of the second scenario shows a different behavior at the beginning, the first ten days the epidemic is maintained in about 15 farms and thereafter propagates in the same way as in scenarios 1 and 6. The initial infected farms in scenarios 1 and 6 were farms with lowest population and or low degree of affiliation; in scenario 2, which correspond to largest farms, the infection remains in those units for the first 10 days before spreading. In scenario 4 the epidemic propagates similar to scenarios 1 and 6 with certain variations between 12 to 21 days during which it infects 350 farms. Initially infected farms were farms with population below 20,000 birds. In scenarios 3 and 5 the epidemic curves have a different initial distribution among infected farms propagating from day 10 to 38 in the scenario 3 and through day 60 in the scenario 5. In all HPAIV scenarios the epidemic reaches its peak, then decrease.

In LPAIV scenarios the epidemic shows a similar distribution curve, however the epidemic is sustained over time after reaching the peak. The number of initial infected farms in both scenarios suggests similar spread conditions.

The number of infected and dead birds are different when we considering viruses of high and low pathogenicity. It is important to highlight that birds infected with HPAIV die, and as a consequence, the epidemic curve declines. The peak of the epidemic is observed about 38 days, extending to day 50 in stage 3 and day 65 in stage 5. The LPAIV epidemic curve remains steady with a slight increase, suggesting that the virus continues to propagate slowly throughout populations. None of the two scenarios contemplated control measures during the 100 days.

The model uses arbitrary estimates for some parameters due to the absence of data in the literature. Despite this limitation, the model has great potential to assist in the study of disease dynamics and the effect of control strategies, highlighting the effect of long-distance transmission.

Proper registration and documentation are becoming increasingly frequent in this disease. This will allow a more accurate and precise calculation of the parameters and thereby improved the accuracy of the model. There is still a great need for improvement in the quality of records of animal populations and records of bird movements in the Dominican Republic.

We modeled different scenarios of propagation of low and high pathogenic avian influenza in the poultry population of the Dominican Republic. We use transmission parameters from LP and HP H5N2 viruses of North American lineage, according to the origins the LPAI Dominican strain (Gomez, Brandão and Ferreira, 2017, unpublished). Our data shows that infection with both LPAIV and HPAIV H5N2 can lead large scale outbreaks in a very short time and remain within poultry population without rapid control measures implemented.

The model has the potential to represent influenza epidemics in the Dominican Republic and in other countries and may represent an important tool to assist in the structuring of surveillance and control strategies for the disease.

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## CONCLUSION

This study consists of four parts, each focusing on a different theme, but among all there is a common point, poultry, which represent an important part of livestock production worldwide. We use several scenarios for model transmission of avian influenza involving different epidemiological aspects in the transmissibility in a total susceptible population. The study was developed using modern mathematical and computational tools. We modeled different scenarios of propagation for low and high pathogenic avian influenza represented in the Dominican Republic. We use parameters from low and high pathogenic H5N2 viruses of North American lineage.

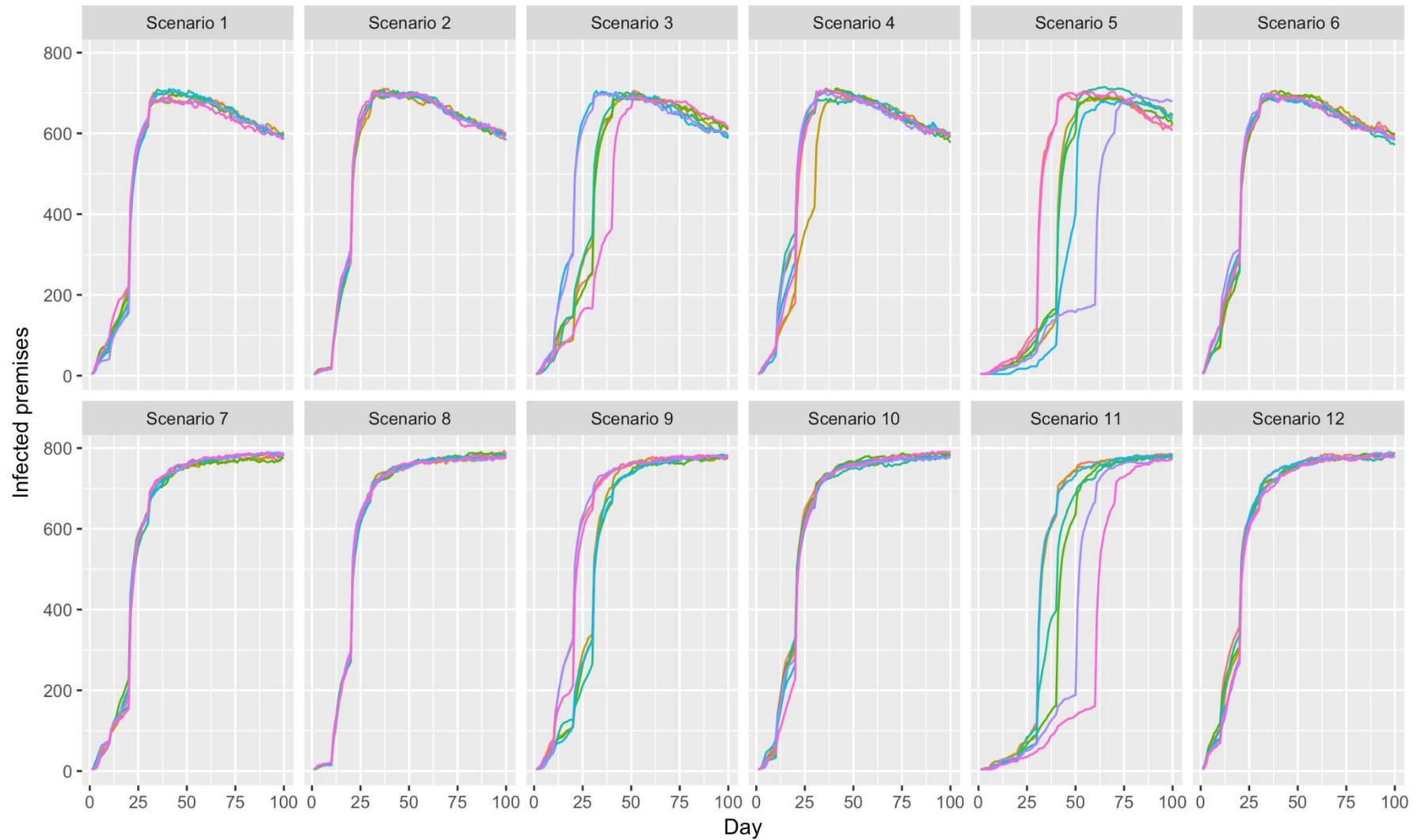
This is a particular model in a realistic population, which has the potential to represent influenza epidemics in any scenario and could be applied in other countries as an important tool to assist in the structuring of surveillance and control strategies for the avian influenza.

We conclude that epidemic can spread in all population if control measures are not implemented during the first ten days. Results suggest that low and high avian influenza virus continues to propagate in populations during the 100 days of the simulation.

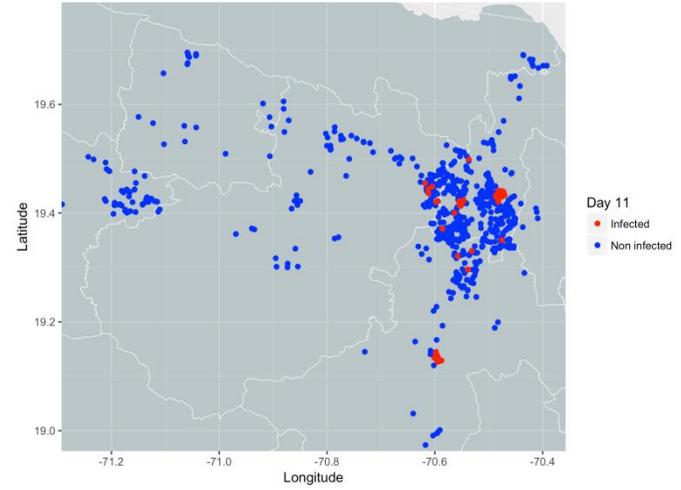
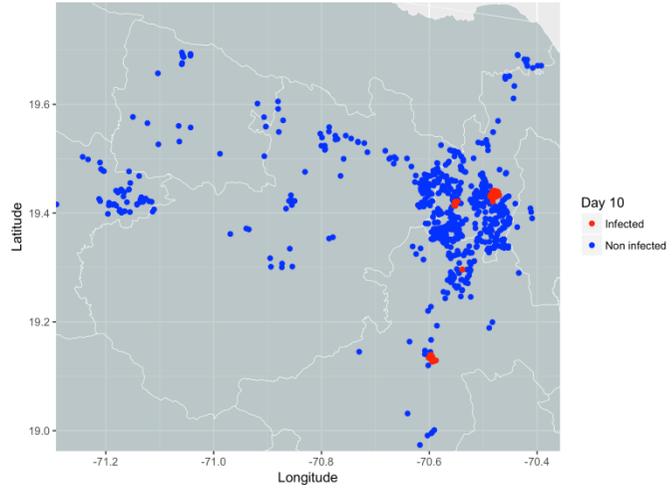
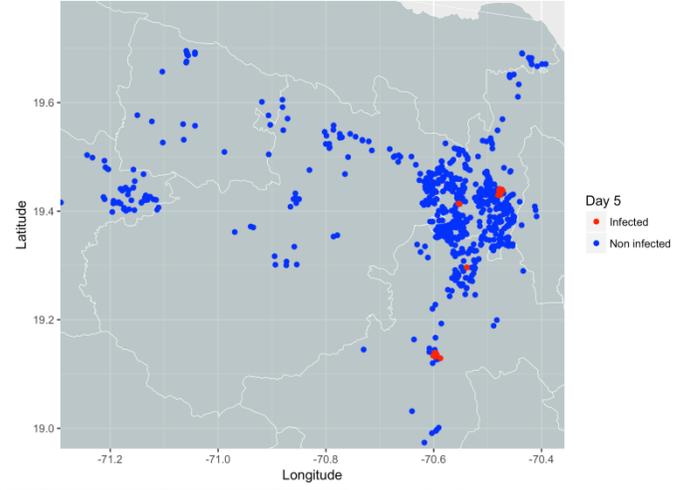
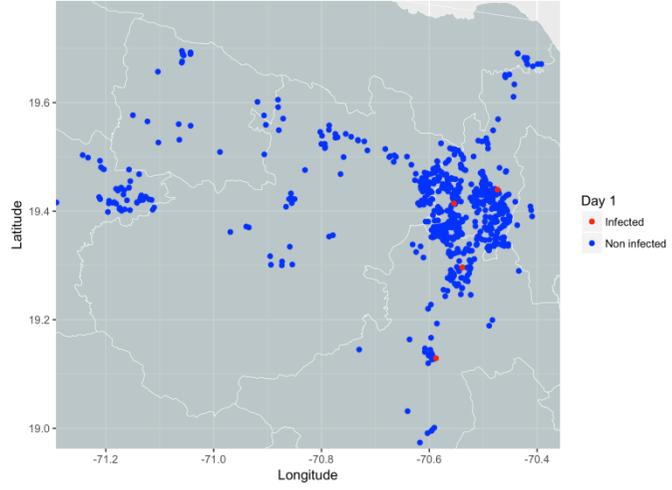
## APÊNDICE

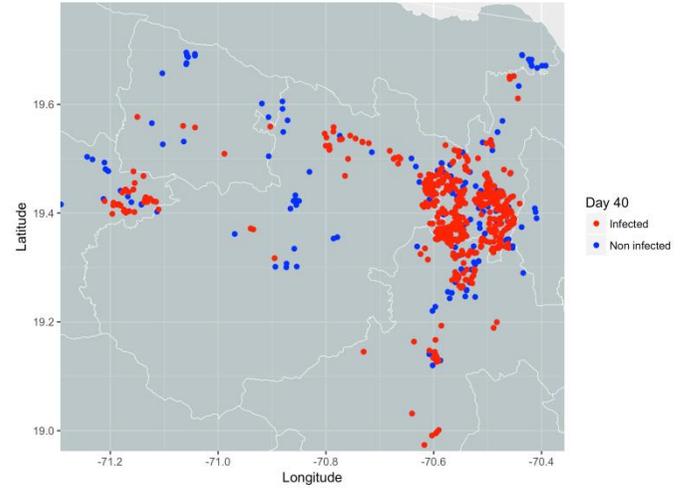
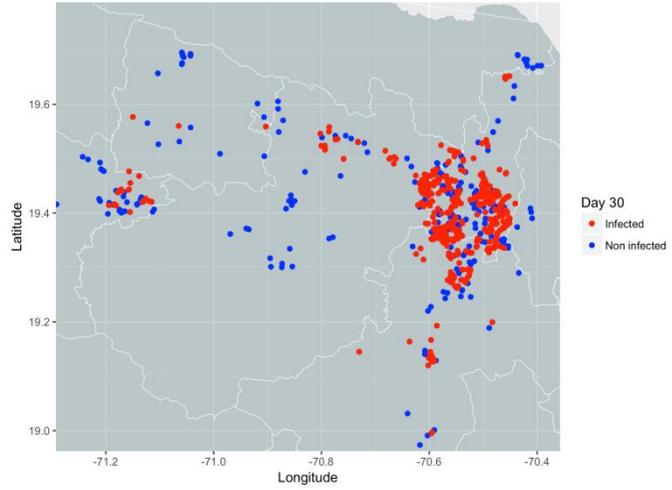
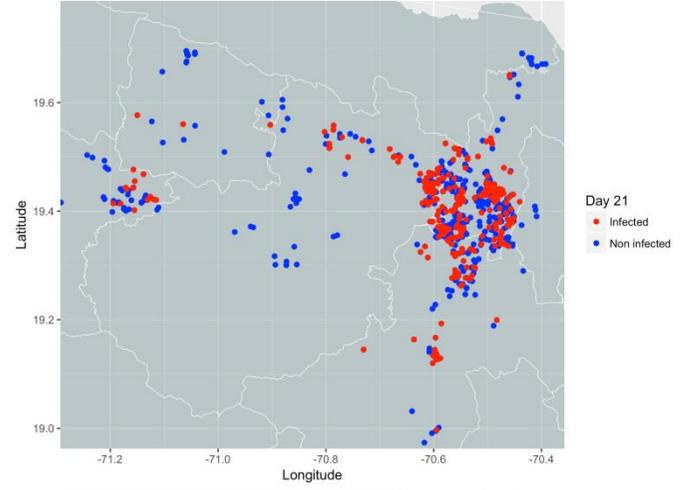
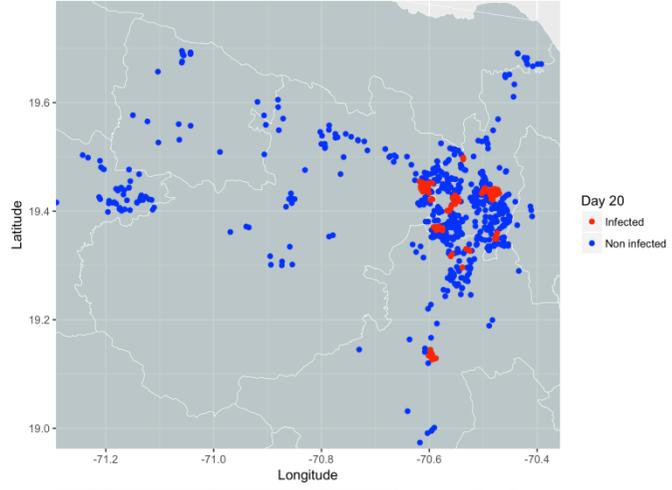
**Table 1. Parameters of the model**

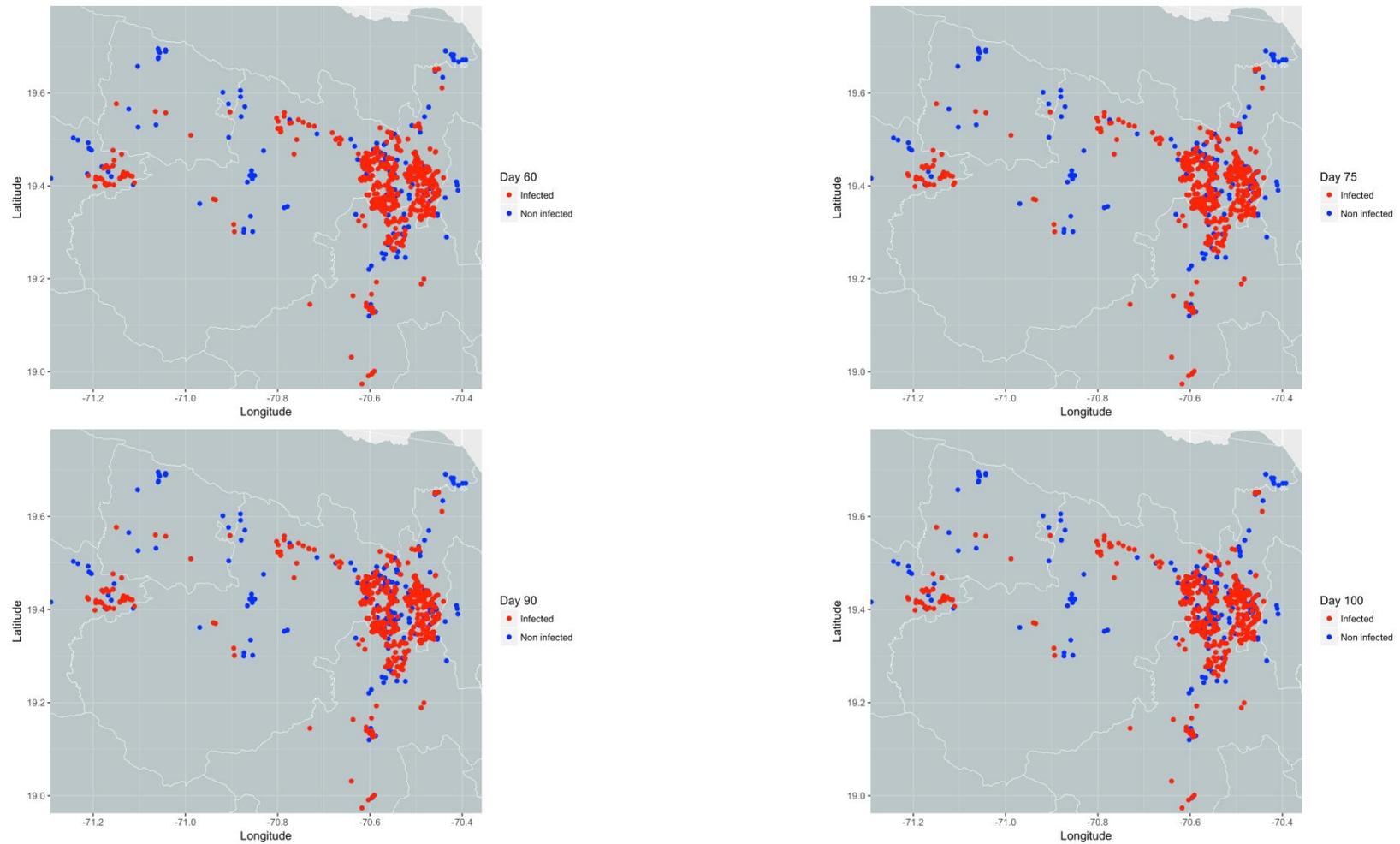
Total of farms	951	MA, 2017 (Gomez, 2016)	
N = Population on farms	50 -1.500.000	Gomez and Ferreira, 2017, (unpublished)	
Time of simulation	100 days		*
<b>a= expectancy of life</b>			
a=Layers/breeders/grandmothers	490 days		**
a= chickens and not defined category	42 days		
<b><math>\mu</math> = Natural mortality rate</b>			
$\mu$ _Layers/breeders/grandmothers	1/490 days		**
$\mu$ _chickens	1/42 days		**
<b><math>\beta</math> = Transmission rate</b>			
High pathogenic	4.66	van der Goot et al., 2003	
Low pathogenic	0.45	van der Goot et al., 2003	
<b><math>\gamma</math> = Infectious period in days</b>			
High and low pathogenic	6.8	van der Goot et al., 2003	
<b><math>\alpha</math> = Mortality rate due to infection</b>			
High pathogenic	90%	Alexander et al, 1986	
Low pathogenic	6%	Swayne et al, 1997	
<b><math>\delta</math> = Survival rate</b>			
High pathogenic	10%	Alexander et al, 1986	
Low pathogenic	94%	Swayne et al, 1997	
<b>Mean death time</b>			
High pathogenic	5.2 days	Alexander et al, 1986	
Low pathogenic	7 days	Swayne et al, 1997	
Number of farms initially infected	4		*
Number of birds that become infected for contact	10		*
Distance between farms	as distance matrix		*
Maximum distance between affiliated	200 km		*
Maximum distance by neighbor contact	2 km		*



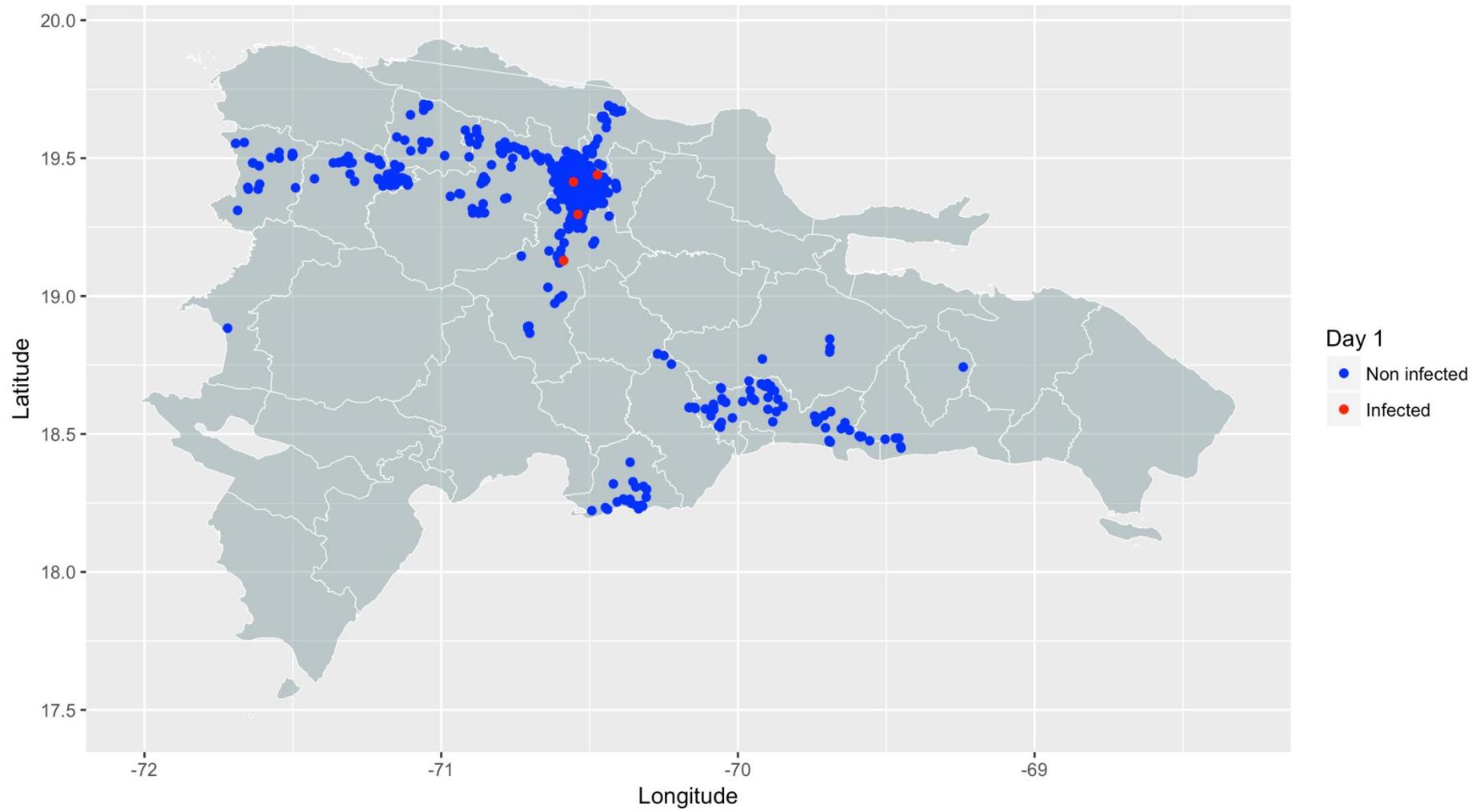
**Figure 1.** The twelve scenarios showing the development of the epidemic over 100 days. Scenarios 1-6 represent high pathogenic infection; scenarios 7-12 represent low pathogenic infection. All scenarios were simulated seven times. The epidemic curve declines from day 34 because of mortality in HPAI, while it remains in epidemic course in LPAI.



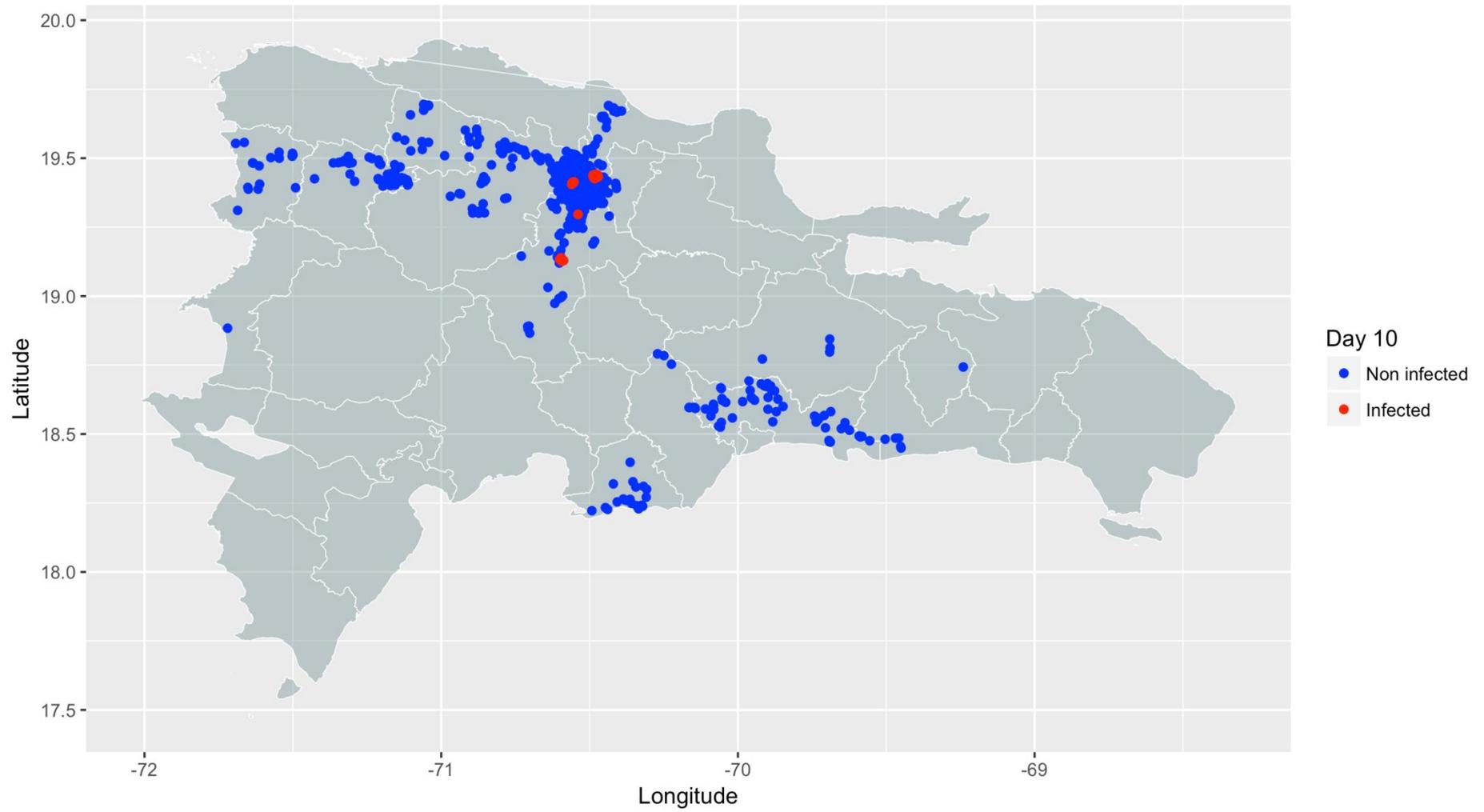




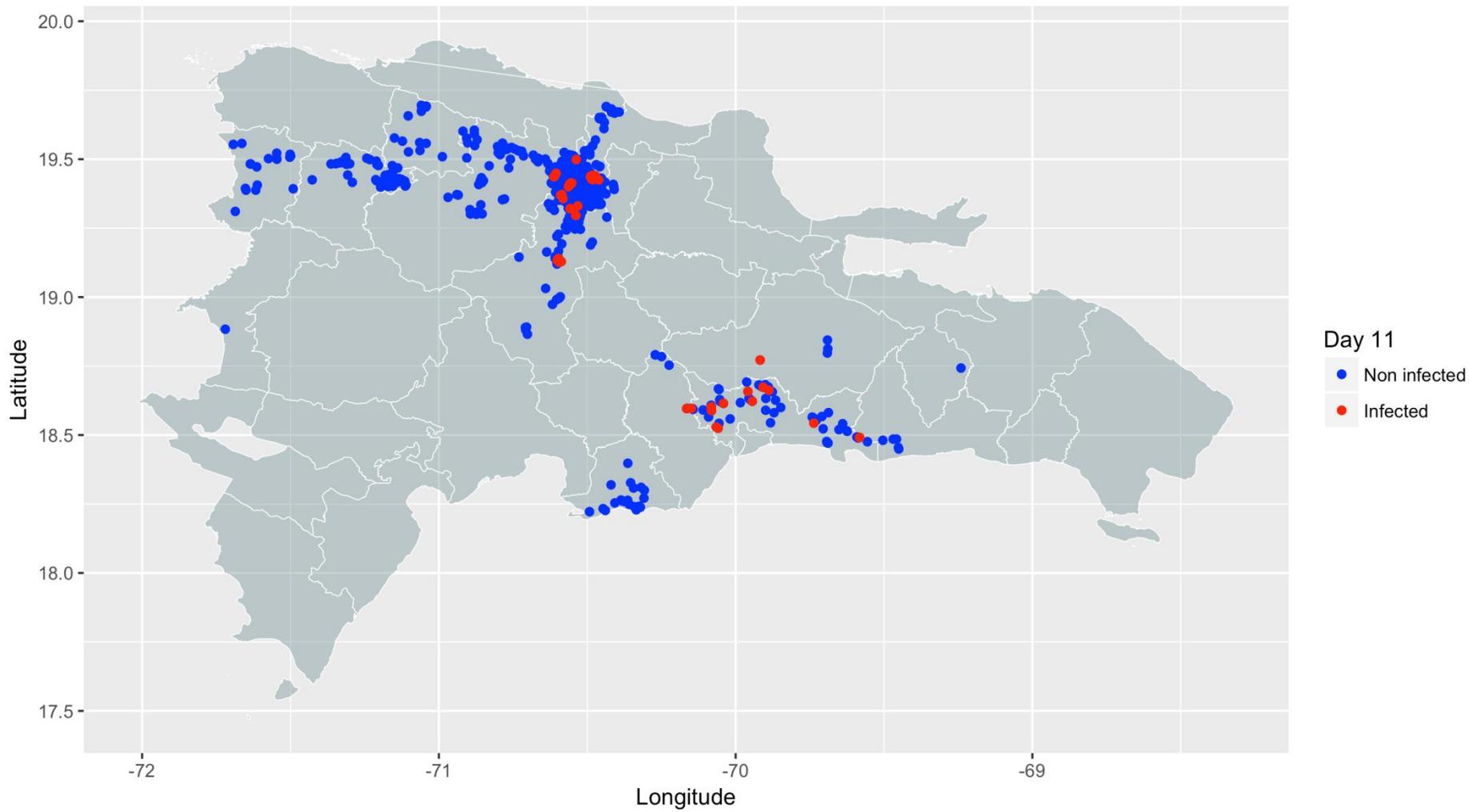
**Figure 2.** The sequence of images shows in the scenario the development of the epidemic at different days of high pathogenic infection. From days 1 to 10 transmission spread locally; an explosion occurs for other distant farms from day 11. The infection expands locally up to the 20<sup>th</sup> day, expanding openly from day 21 to 30 and up to 100 days.



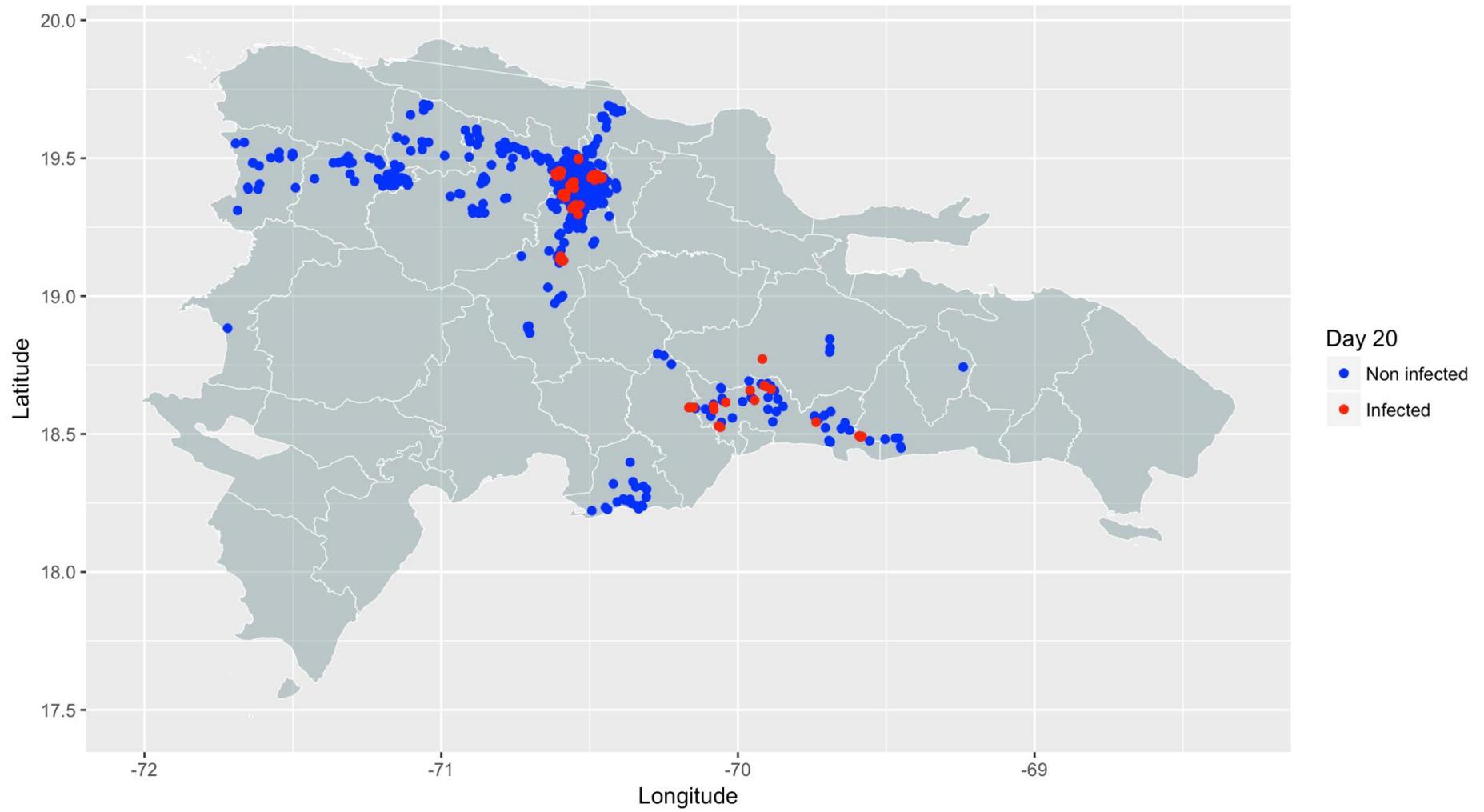
**Figure 3.** Scenario 7 for low pathogenic at day 1 showing initial conditions.



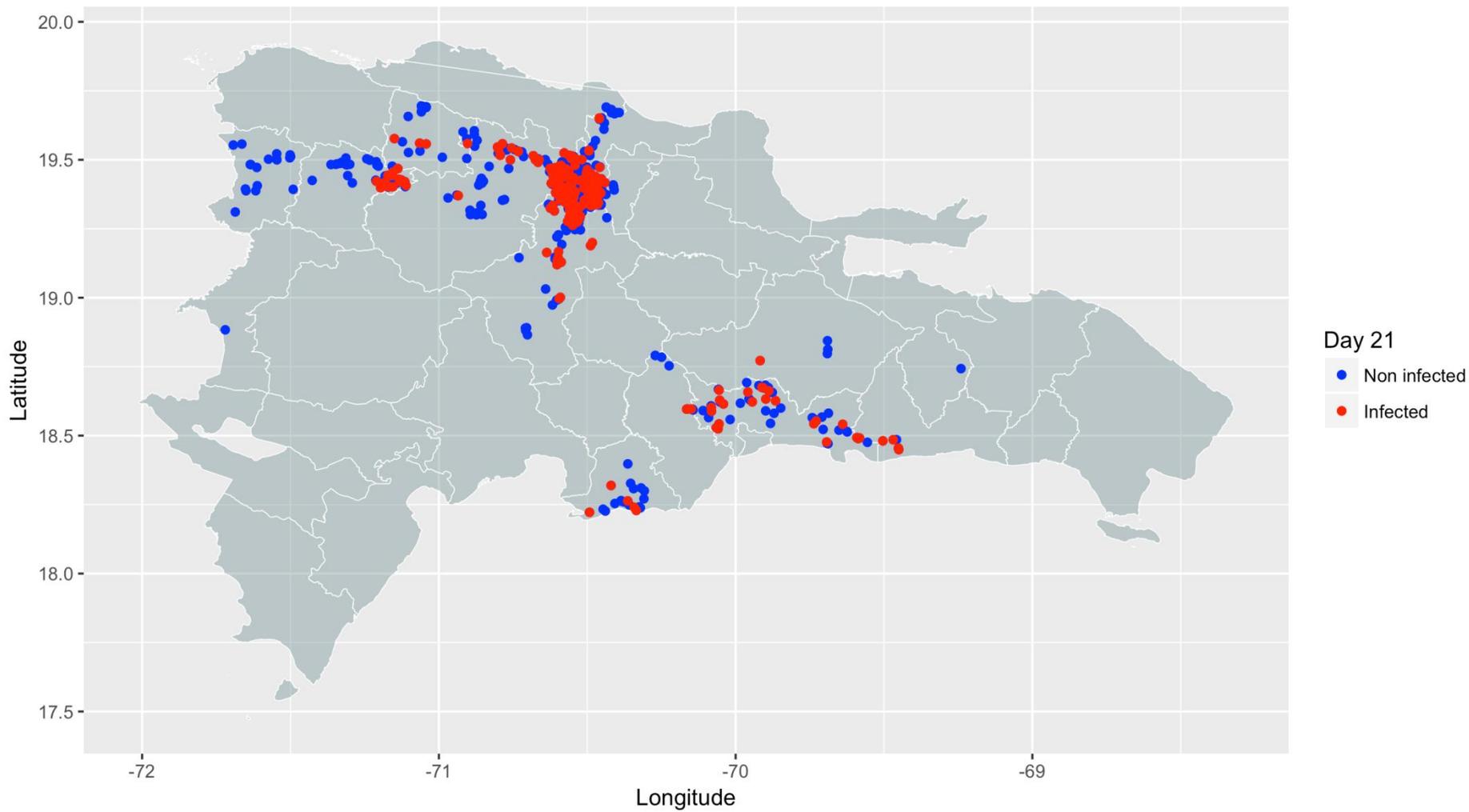
**Figure 4.** Scenario 7 for low pathogenic at day 10, the infection spreads locally by neighbor.



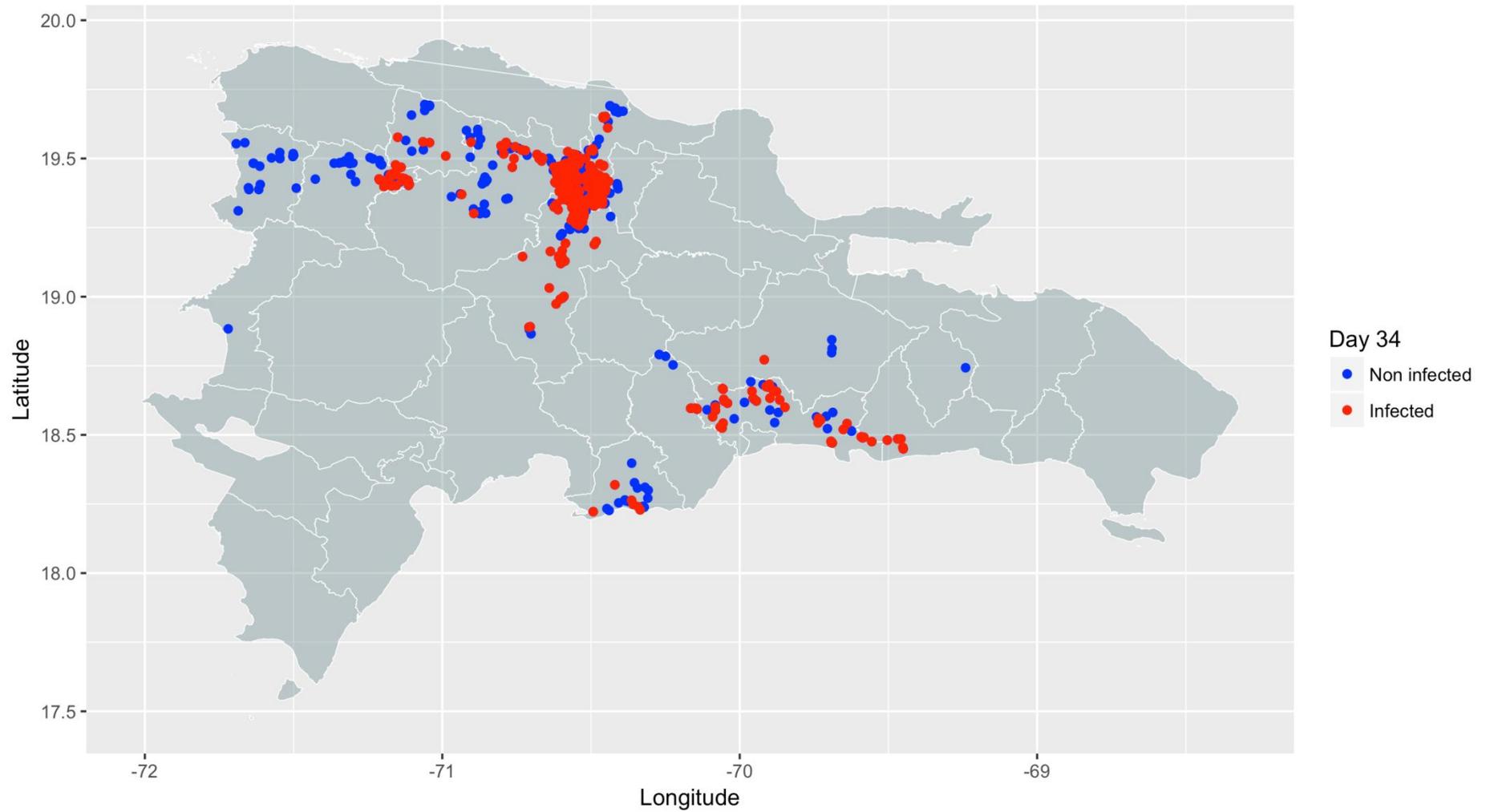
**Figure 5.** Scenario 7 for low pathogenic at day 11. The infection reaches distant farms though contact by affiliation.



**Figure 6.** Scenarios 7 for low pathogenic at day 20 showing the infection spreading locally in the neighbor of the affiliated recently infected.



**Figure 7.** Scenario 7 for low pathogenic at day 21, infection continue expanding, a big cluster of infected farms represent the high density province, almost 21% of the farms are infected at this day.



**Figure 8.** Scenario 7 for low pathogenic. At day 34 infections reaches almost 70% of the farms. Sequence of images show the infection expands openly from 11th until 34th.