1   *Epizootic of highly pathogenic H7N3 Avian Influenza in an ecologic reserve in Mexico*

2

3   Roberto Navarro-López[1¶], Mario Solís-Hernández [1¶], Miguel A. Márquez-Ruiz[2¶], Abel

4   Rosas-Téllez[1¶], Carlos A. Guichard-Romero[3&], Gerardo de J. Cartas-Heredia[3&], Romeo

5   Morales-Espinosa[3&], Héctor E. Valdez-Gómez[1*], Claudio L. Afonso[4¶].

6   [1]Mexico-USA Commission for the prevention of F & M Disease and other Exotic Diseases

7   in Animals. DGSA-SENASICA. Av. Cuauhtémoc No. 1230 piso 9, Col. Santa Cruz

8   Atoyac. C.P. 03310 Ciudad de México.

9   [2]Faculty of Veterinary Medicine. UNAM. Ciudad Universitaria, C.P. 04510. Ciudad de

10   México.

11   [3]Regional Zoo "Miguel Álvarez del Toro" SEMAHN. Calzada Cerro Hueco S/N, El

12   Zapotal C.P. 29094. Tuxtla Gutiérrez, Chiapas. México

13   [4]Southeast Poultry Research Laboratory, ARS-USDA, 934 College Station Road Athens,

14   GA 30605 USA

15

16   *Corresponding author

17   E-mail: enriquevaldez@hotmail.com

18

19   [¶]These authors contributed equally to this work.

20   [&] These authors also contributed equally to this work.

21   **Summary**

22   This report includes a 2015 epizootic of highly pathogenic H7N3 avian influenza virus

23   among captive and wild birds at "El Zapotal" ecologic reserve, located in the state of

24   Chiapas, Mexico. Epidemiological control measures were implemented to prevent virus

25   dissemination. The infection with the highly pathogenic H7N3 virus was detected

26   predominantly among Plain Chachalaca (*Ortalis vetula*), with occasional detections in a

27   White-fronted Parrot (*Amazona albifrons*) and a single Clay-colored Thrush (*Turdus grayi*).

28   Here, we describe the characteristics of the outbreak environment, the surveillance strategy,

29   the biosecurity measures, and the evaluation of the site, including external farms. These

30   actions, timely implemented by the veterinary authorities, helped to contain the outbreak

31   beyond the ecologic reserve. This contingency showed the importance of developing a

32   more complete analysis of the existing risks and the challenges to implement minimal

33   biosecurity measures in these facilities.

34

35

36

37

38

39

40

41

Introduction

42

43   Type A influenza viruses (AIVs) typically circulate in migratory and non-migratory wild

44   aquatic waterfowl, gulls, and shorebirds [1]. Geographic barriers have limited

45   intercontinental exchange of AIVs resulting in continental lineages of viral diversity by

46   regions [2, 3, 4]. They are further divided into subtypes based on the antigenic

47   characterization of the virus' surface glycoproteins, hemagglutinin (HA) and neuraminidase

48   (NA). In wild birds, 16 HA and 9 NA subtypes have been identified in many different

49   combinations which have been isolated from a total of more than 125 bird species that

50   belong to 26 different families [1, 2, 5, 6]. AIVs maintained in wild birds periodically spill-

51   over into domestic birds, as wild and domestic mammals, and have been postulated to

52   contribute to pandemic human influenza viruses [7, 8, 9].

53   Based on pathogenicity in young, immunologically naïve chickens (*Gallus gallus*

54   *domesticus*) [10], AIVs are classified as low pathogenicity (LP) or high pathogenicity (HP).

55   LP AIVs can become established in domestic poultry with the potential to become

56   zoonotic. LP AIVs of the H5 and H7 subtypes may develop HP in domestic poultry

57   through the evolution of a polybasic sequence at the HA cleavage site. Domestic birds

58   infected with HP AIVs often-present high morbidity and mortality due to systemic

59   infection and generalized congestive-hemorrhagic condition of diverse intensity. Morbidity

60   and mortality of wild bird infected by HP AIVs may be more variable. HP AIV can be

61   epitheliotropic, endotheliotropic, neurotropic or pantotropic depending on host

62   susceptibility [11, 12]. In domestic chickens, significant lesions that are consistently

63   associated with HP AIV infections include necrosis and inflammation of organs such as

64   heart, brain, spleen, intestinal tract, lungs, and skeletal muscles [11].

3

65   There is limited information about HPAI outbreaks in wild birds, but HPAI infections have

66   shown a range of syndromes that can go from asymptomatic to severe symptomatic

67   conditions. These range from respiratory and/or digestive tract infections to critical lesions

68   in other organs including the heart, brain and pancreas [11]. The reporting of this is type of

69   infection in wild birds has increased during the last 15 years [13, 14, 15].

70   In Mexico, during an epizootic in poultry, a continued transmission of HPAI H7N3 virus

71   was found to occur in *Quiscalus mexicanus* (Fam. Icteridae, Ord. Passeriformes), for at

72   least six weeks. The clinical condition of the *Q. mexicanus* was, prostration, ruffle feathers,

73   diarrhea, anorexia and sudden death [14]. Similar conditions have been reported in the

74   *Corvus macrorhynchos* and in *Pica pica* infected with a HPAI H5N1 virus [11]. In both

75   reports, the lesions described included severe damage of the brain, heart and pancreas and

76   the isolation of the H5N1 and H7N3 viruses from trachea, intestine and heart.

77   Most of the HP AIV identified in wild birds have been detected in typical reservoir species

78   [7, 16, 17], although a diversity of additional species has also been reported to be affected;

79   such is the case of passerine [18,19] and psittacine birds [20]. The role of passerines as

80   virus reservoirs in nature has been gaining terrain, facilitating viral spread between wild

81   and domestic hosts [20, 21].

82   This study describes the initial evidences of mortality in a wild population of Plain

83   chachalacas (*Ortalis vetula*), following an outbreak in a natural reserve in Mexico. We also

84   identified the presence of the HPAIV H7N3 in White-fronted Parrot (*Amazona albifrons*),

85   and Clay-colored Thrush (*Turdus grayi*), which were reported for the first time in an

86   ecologic reserve where a regional fauna zoo is present. We also highlighted the procedures

87   implemented by the veterinary authorities to achieve its control [23, 25].

88   **Material and Methods**

89   Study Area.

90   The natural reserve "El Zapotal" located in the SE portion of Tuxtla Gutiérrez capital of

91   the State of Chiapas, Mexico, (16° 43´ N / 93° 06´ W), comprises 192 hectares of Semi-

92   evergreen forest, Low deciduous woodland and Acacia shrub [26]. The climate was defined

93   as warm, humid and rainy weather (Awo(w)(i)), with a total annual precipitation of 948.2

94   mm and average temperature of 24.7 °C [27]. The decree of protection was established as a

95   preventive measure against the intense urban pressure. The Regional Zoo Miguel Álvarez

96   del Toro (ZooMAT), is located within this natural reserve (Fig. 1). There is also a profusely

97   free-ranging wild animal population, including feral fauna interacting year-round [23, 26,

98   28].
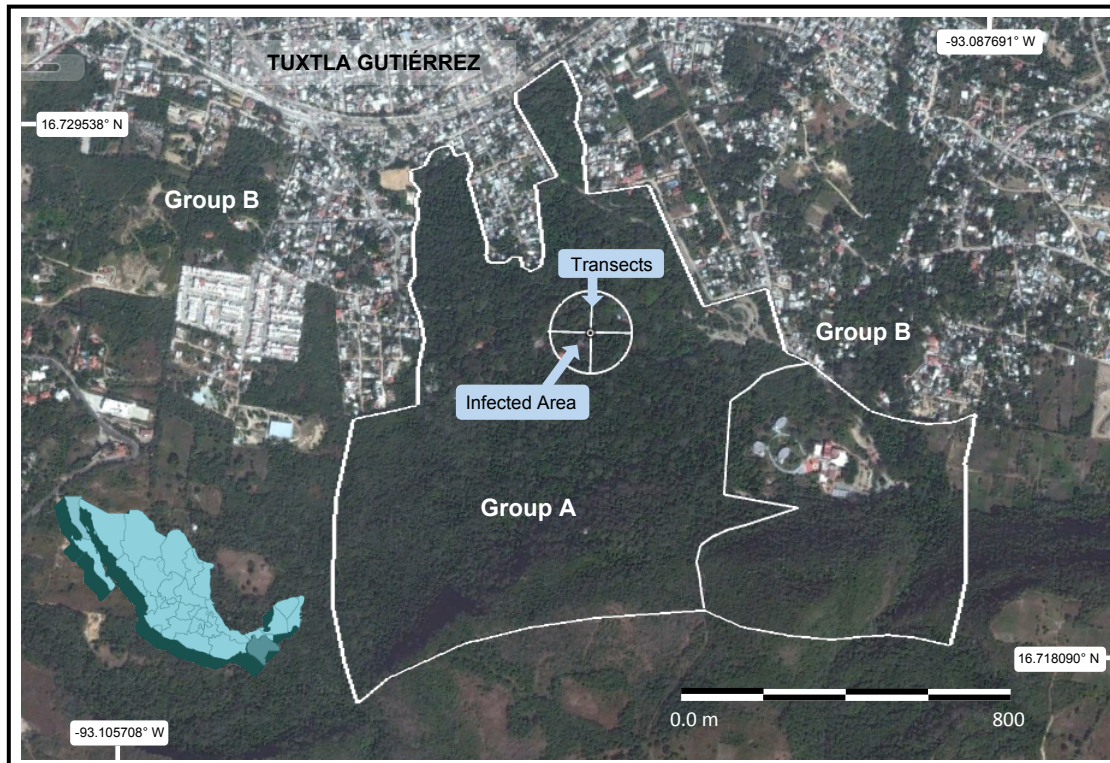
99

100   **Figure 1.** Polygon of the ecological reserve El Zapotal, including working groups zones.

101   Avifauna and target species.

102   El Zapotal, is rich in avifauna with 364 species dominated by Passeriformes. The seasonal

103   behavior shows that 68% are resident breeders, 32% are winter visitors while 34% are

104   considered to be rare [33]. The target species was the Plain chachalaca, whose distribution

105   ranges from South Texas, the Gulf of Mexico, the Yucatan peninsula to North Central

106   America [34, 36]. Within the reserve, the abundance of resources such as fruits, seeds,

107   leaves and insects, comprises the main items present in the diet of the chachalaca [26]. This

108   condition among other factors maintain a relatively high density of individuals [34, 35].

109   The Zoo, where "focal" or infected zone was designated, is surrounded by aviaries mainly

110   populated by Psittacine birds. Some artificial ponds are also present, harboring resident

111   aquatic birds, as well as an important diversity of songbirds [33].

112   Method of Study.

113   In order to address the epizooty and the procedures implemented, the surveillance staff was

114   divided in three groups (A, B and C) following biosecurity protocols [22] to prevent

115   contamination (Figure 1). Group A was responsible for the activities within the reserve

116   consisting of: Attention to AIV suspect infected cases, delimitation of the infected area,

117   stamping out of the birds, postmortem examination, as well as blood and organ extractions.

118   Other activities included cleanliness and disinfection of the facilities, risk evaluation, risk

119   control, in addition to sampling of exhibited birds via tracheal and cloacal swabbing every

120   21 days during three months. Group B was responsible for the communication protocols

121   within the urban and sub-urban (perifocal) zone around El Zapotal (Fig. 2). Group C was in

122   charge of serologic and virologic surveillance of commercial broiler farms located within

6

123   10 km perimeter around El Zapotal (Fig. 2). This group carried out a minimal biosecurity

124   evaluation of the farms, in order to avoid cross-contamination of poultry. During this

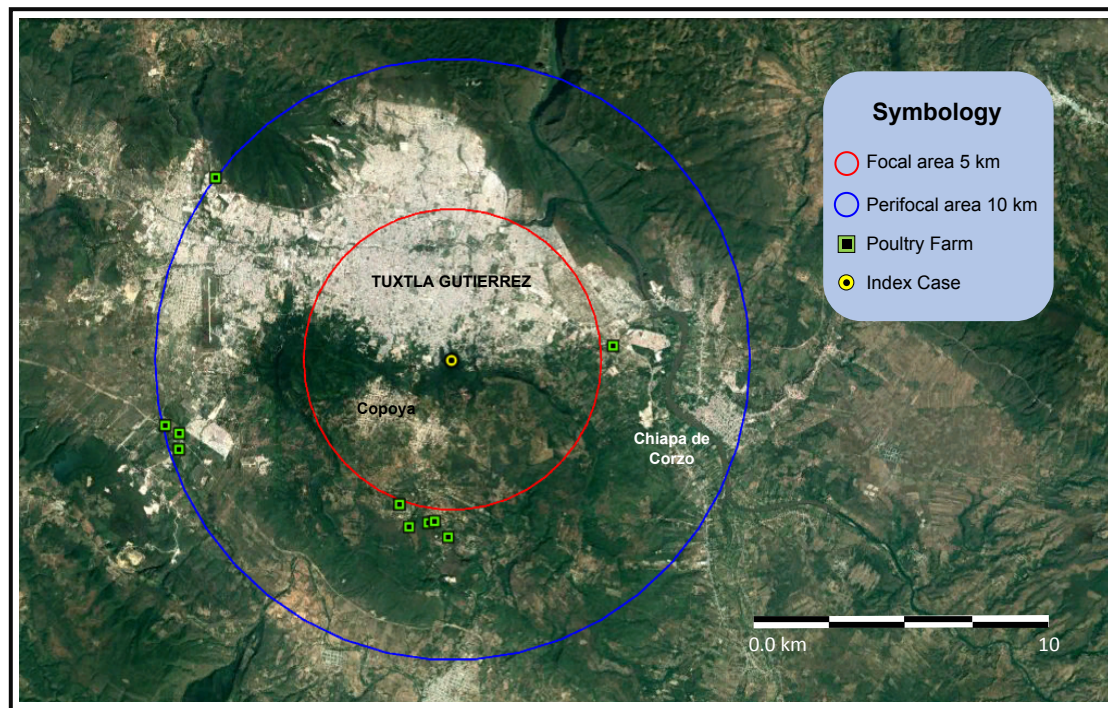125   contingency, the access of official veterinarians to commercial farms was forbidden.

127   **Figure 2.** Focal area and Perifocal area around El Zapotal.

128   Virologic surveillance was carried out by sampling daily mortality in poultry. Tracheal and

129   cloacal swabbing performed by official veterinary were obtained from carcasses placed in

130   containers outside the farms. The serologic surveillance was carried out by non-official

131   veterinarians accredited by the authority.

132   The infected area was delimited considering the index case (IC) as the center of a circle of

133   100 m radius. All chachalacas and birds showing symptomatology where shot with two air

134   rifles type PCP Cal. 5.5. Additionally, two transects of two hundred meters each, were

135   implemented toward the four cardinal points, by placing the IC at its intersection. Along

7

136   these transects active vigilance on wild birds took place (Fig. 1). These actions were

137   endorsed by the Articles 8, 11, and 160 of the General Law of Animal Health (24).

138   We calculated the minimal number of samples by implementing the Cannon & Roe formula

139   [31]. Population density for *O. vetula* were assumed considering previous censuses [35]

140   with a prevalence of 3%. A total number of commercial birds within the perifocal zone was

141   applied considering a prevalence of 10% for intra-farms, with a level of confidence of 97%

142   for both cases [29].

143   Protocols such as Guidelines to the Use of Wild Bird in Research were considered [30].

144   Poultry flock surveillance strategies, clinical examination, serologic and virologic sampling

145   were carried out as described in the Terrestrial Animal Sanitary Code of OIE [22, 32, 42].

146   All field activities were implemented according to the Manual of Procedures for the

147   prevention and eradication of HPAI virus [22].

148   <u>Phylogenetic analyzes</u>.

149   To examine the relationship between the HPAI H7N3 from ZooMAT, 16 full-length HA

150   nucleotide sequences were aligned (isolated from ZooMAT, Puebla, Oaxaca, Jalisco and

151   two additional low pathogenicity viruses). Next, a maximum likelihood tree was

152   constructed using the Tamura-Nei model. Initial tree (s) for the heuristic search were

153   obtained by using the Maximum Composite Likelihood (MCL) approach, and then

154   selecting the topology with superior log likelihood value. A discrete Gamma distribution

155   was used to model evolutionary rate differences between sites. The tree was drawn to scale

156   with branch lengths measured in the number of substitutions per site. Evolutionary analyzes

157   were conducted in MEGA7.

158   <u>Diagnostics tests</u>.

159   The samples were processed according to the Catalogue of Procedures and Services of the

160   Laboratory Network of the DGSA [38, 39]. Samples were subjected to virus isolation in

161   embryonated chicken eggs and reverse transcription polymerase chain reaction in real time

162   (rRT-PCR). Virus subtype and pathogenicity were assessed by hemagglutination

163   inhibition, sequencing, and intravenous pathogenicity index (IVPI), OIE´s method adopted.

164   For the determination of the nucleotides at that portion of the HA gene coding for the

165   cleavage site region of the haemagglutinin has been used the Applied Biosystems® 3130

166   Genetic Analyzer. The completed haemagglutinin sequence was performed by using the

167   conserved AIV primers to amplify a product that was sequenced by the Illumina Nexgen

168   MiniSeq™ System.

169   <u>Databases</u>.

170   The informatics platform used for the collection and analysis of the data was obtained from

171   the Mexican National System of Information for Exotic and Emergent Diseases –SINEXE-

172   (39). The number and origin of people visiting the ZooMAT along this study was taken

173   from to the zoo's own data.

174   **Results**

175   The epidemiologic analysis showed that the IC, was the primary case (PC) detected on

176   April 20 2015, in two *Ortalis vetula* by RT-PCR technique; both birds had died on April

177   16. On April 29, viral isolation and identification confirmed the existence of AIV H7N3

178   subtype. On April 30, this virus was sequenced and on May 4, the results of Intravenous

179   Pathogenicity Index (IVPI) exposed 2.91-2.99 (100% mortality) indicating a HPAI H7N3

9

180   virus. The clinical signs of inoculated chicken exposed to the disease for the IVPI were

181   hyperacute. These included ruffled feathers, respiratory distress, nasal discharge,

182   conjunctivitis, torticollis, prostration and sudden death. The macroscopic lesions observed

183   corresponding to petechial hemorrhages in the comb, tarsi and barbels, lung congestion,

184   hemorrhagic tracheitis, proventricular hemorrhage, and hemorrhages in the muscles of the

185   breast.

186   Group A collected 1,853 virologic samples for diagnosis. Among them 511 were cloacal

187   and tracheal swabs, 198 organs, 1,135 dragging swabs, and 9 sera (Table1). From these

188   samples, 64 birds were positive to virus isolation. Positive samples belonged to three

189   species grouped in three taxonomic families (Table 2).

190   **Table 1**. Number of samples collected, positive to Highly Pathogenic Avian Influenza

191   H7N3, per group.

Team	Number of Samples	HPAI H7N3
Group A	1,853	64
Group B	702	0
Group C	2,523	0
Total	5,078	64

192

193   The minimal sample size calculated suggested 145 individuals with Finite Population

194   Correction. The infected area had 3.14 ha where a total of 193 birds were sacrificed

195   (depopulated). 174 *Ortalis vetula*, 6 *Turdus grayi*, 5 *Molothrus aeneus*, 4 *Egretta thula*, 2

196   *Coragyps atratus* 1 *Quiscalus mexicanus* and 1 *Amazona albifrons*. One hundred percent of

197   the birds were sampled for AIV, 64 were positive for H7N3: 62 *Ortalis vetula*, 1 *Turdus*

198   *grayi* and 1 *Amazona albifrons* (Table 2).

199   **Table 2**. Taxonomic description and proportions of infected wild birds.

Order	Family	Species	Number of infected birds	Infected dead naturally	Infected stamped out apparently healthy
Galliformes	Cracidae	*Ortalis vetula*	62 (97%)	21 (34%)	41 (66%)
Psittaciformes	Psittacidae	*Amazona albifrons*	1 (1.5%)	1 (100%)	0
Passeriformes	Turdidae	*Turdus grayi*	1 (1.5%)	1 (100%)	0
Total			64 (100%)	23 (36%)	41 (64%)

200

201   Group B. The surveillance teams detected 3,141 locations with backyard birds,

202   predominately represented by *Gallus gallus domesticus*, although, pigeons, ducks,

203   pheasants, peacocks, turkeys, parrots and songbirds were also identified. Through

204   interviews and visual inspection, it was determined that *O. vetula* was able to come in

205   contact with poultry and backyard birds in the neighboring sites that surround the "El

206   Zapotal" natural reserve. During the research, we received 53 notifications of ill birds by

207   different causes. A total of 702 samples were collected: 45 organs, 657 tracheal and cloacal

208   swabs, however none of the samples collected had the A H7N3 virus (Table 1).

209   Group C. This group carried out biosecurity evaluation of the farms. Around the ecologic

210   reserve, we identified 11 commercial farms (Fig. 2), 8 broilers and 3 rearing farm

211   operations. Under the dead-bird sampling system, 1,743 tracheal and cloacal swabs were

212   obtained in 83 visits and the accredited veterinarians also collected 780 blood samples

213   (Table 1). No evidence of H7N3 virus presence and/or exposure was detected. To verify

214   compliance with minimal biosecurity practices, 29 farms were audited, 10 in the perifocal

215   area (Fig 2) and 19 in the area where they wanted to improve the biosecurity conditions.

216   Among these farms, 21 were considered to have in place appropriate biosecurity measures,

217   whereas 8 were required to improve their preventive infrastructure.

218   64% of the cases were found by controlled depopulation and 36% were detected through

219   passive surveillance with carcasses. The ratio of infected males *vs* females in *O. vetula* was

220   3 to 1. The epizootic lasted 6 weeks from April 17 (IC) to May 23, which corresponds to

221   epidemiologic weeks 16 to 21. The sampling of *O. vetula* started on week 18 and finished

222   on week 26. (Fig. 3).

223
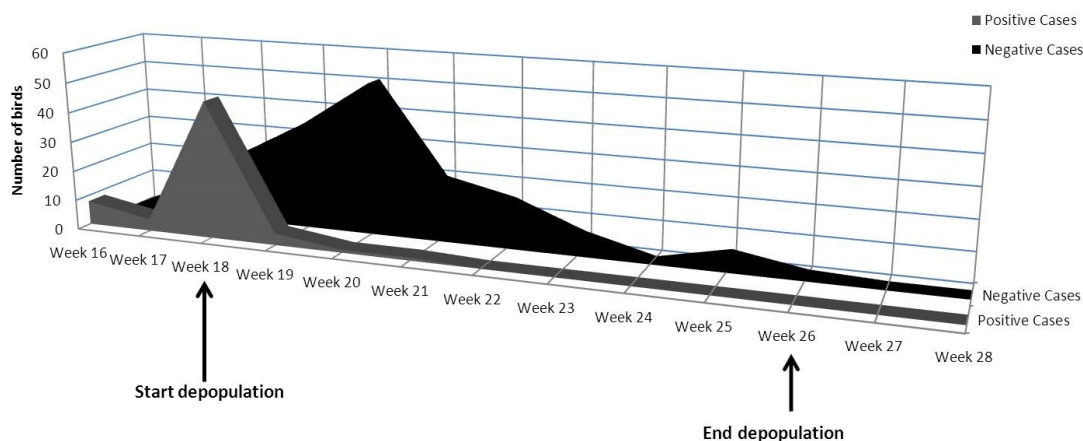
224   **Figure 3**. Chronology of the outbreak of Avian Influenza H7N3 HP in the ecological

225   reserve El Zapotal.

226   Clinical signs and post-mortem findings.

227   Most of the infected birds with the HPAI H7N3 virus died rapidly in less than 24 hours

228   (Fig. 4B). Clinically ill birds showed signs of depression, ruffled feathers, nasal discharge,

229   anorexia, dehydration, yellow diarrhea, fever (43ºC), most likely due to severe systemic

230   infection (Fig. 4A). During post-mortem examination a generalized congestive-

12

231   hemorrhagic condition was observed with the following findings: air sacs opacity, heart

232   necrosis, necrotic foci in liver, tracheal congestion and hydropericardium, gastroenteritis,

233   hemorrhagic enteritis, and splenomegaly, presence of bloody fluids in the coelomic cavity

234   (Fig. 4 C-D).

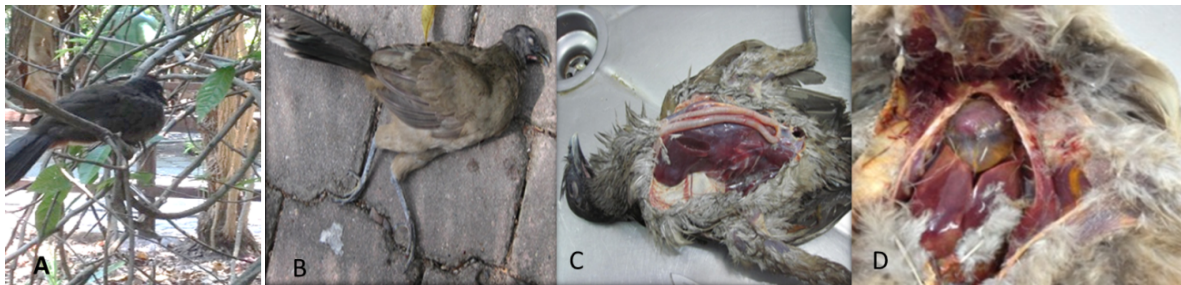

236   **Figure 4**. *Ortalis vetula* affected with HPAI H7N3 virus.

237   Sources of the outbreak.

238   According to the SINEXE, there were no receptions on the database of dead birds in the

239   ZooMat since April 1-15, suggesting that the virus was probably not present on the

240   premises before the IC was detected. Based on the list of visitors 27,294 people attended

241   the zoo during April 1-15, 80% originated from Chiapas and the rest from other Mexican

242   states.

243   Control of the outbreak.

244   ZooMAT implemented a stringent monitoring and control program based on clinical and

245   virological surveillance, previously verified by the veterinarian authorities according to

246   OIE as a consequence of a Newcastle Disease outbreak [40, 41]. The actions implemented

247   at the zoo were the following: Temporary closure and depopulation of the *O. vetula* in the

248   focal area, in order to curtail the transmission chain. In addition, since 2008 a program of

249   surveillance for Avian Influenza and Newcastle viruses has been established using PCR

250   molecular tests and viral isolation on birds that come to the zoo through donations,

251   confiscation or birds that die within the zoo.

252   The infected area was cleaned with water and soap and disinfected with citric acid (2%,

253   commercial name Desfan-100) using a pressure pump. Organic materials were sprayed with

254   Desfan-100 during 14 days and then were composted in situ. At the two zoo entrances,

255   pumps and footbaths with disinfectant for footwear were placed and vehicles were

256   disinfected with active neutral (pH 0.006%) chloraldehyde (1%). Footbaths were also

257   placed in the aviaries using the same disinfectant and concentration. Same actions were

258   applied at the three points around infected area inside the zoo. Covered garbage cans were

259   used to replace those without cover, in order to prevent feral birds and O. vetula from

260   having contact with trash and leftover food at the two restaurants of the zoo. High-risk

261   materials, such as organic waste from the restaurants and dead birds were destroyed in the

262   incinerator located within the zoo. During the 90 days of active surveillance, 100% of the

263   exhibition aviaries were sampled (1,264 swabs obtained from 500 clinically healthy birds)

264   with negative results for both rRT-PCR and virus isolation.

265   The tree with the highest log likelihood (-3494.0680) is shown. The percentage of trees in

266   which the associated taxa clustered together is shown next to the branches. The discrete

267   Gamma distribution used to model evolutionary rate differences between 5 sites (5

268   categories: + G, parameter = 1.0429). The analysis involved 16 nucleotide sequences. All

269   positions containing gaps and missing data were eliminated. There were a total of 1683

270   positions in the final dataset (Fig. 5).

14

271

Figure 5. Evolutionary relationships of taxa from HPAI H7N3 isolated from *Ortalis vetula*, located in ZooMAT, Chiapas.

Discussion

Here we reported the first case of infection of *Ortalis vetula*, *Turdus grayi* and *Amazona albifrons* with HPAI H7N3 virus, in the "El Zapotal" natural reserve. In addition, the implementation of established procedures was used to control poultry infections in order to protect wild bird populations. The AIV epizootic was eliminated in the natural reserve, complying with the "90 days period without any new case", required by OIE, in order to declare free a zone of Avian Influenza [42] and did remain free for at least 105 days (week 41) since the end of the campaign. Particularly significant is the fact that *O. vetula* came in contact with backyard birds in the neighboring sites around the natural reserve. These arguments are visually based or through interviews. The high prevalence observed in

15

284   infected area, demonstrates the capacity of this species to be infected by H7N3 and to

285   efficiently transmit the virus under field conditions.

286   In this particular case, the biosecurity measures undertaken to control the AIV resemble

287   poultry farms in Mexico. A vaccination program under official control is used to prevent

288   AIV infection and spreading, accompanied by quarantine and stamping out policies

289   according to the requirements established by the veterinarian authorities [22, 40, 43].

290   Extending stamping out practices to non-endangered wild bird species within their natural

291   environment proved effectiveness in controlling the spread of the HPAI H7N3 virus in *O.*

292   *vetula*, thus preventing the potential spill over into other endangered species. That is case of

293   Resplendent Quetzal (*Pharomachrus mocinno*); Horned Guan (*Oreophasis derbianus*),

294   parrots and macaws (*Ara* spp.). The involvement of some of these species would have

295   resulted in a serious animal health conflict, where public opinion plays a very important

296   role pressure. Upon early detection, the key action implemented at the ZooMAT was the

297   rapid depopulation of *O. vetula* in the focal area. By reducing its population in this critical

298   spot of the zoo, it may help to reduce the transmission chain. The epizootic lasted six weeks

299   due to the implemented actions undertaken by the official veterinary services (Figure 2).

300   During controlled depopulation, it was discovered that many apparently healthy birds were

301   infected. The cleaning actions, disinfection, waste handling and control, the worker´s

302   training and the improvement of biosecurity in general, were all key actions that

303   contributed to the contention of the disease. These actions prevented wild birds to act as

304   "bridge birds" by infecting other animal populations.

305   Our data further emphasizes the delicate balance that exists at the wild bird/poultry

306   interphase (44, 45).

16

307 The ecological consequences of this process in which highly virulent viruses develop in

308 poultry and return to the wild are unknown.

309 This outbreak represents yet another case in which an AIV started as a LPAI virus in wild

310 birds becoming highly pathogenic in poultry and then circling back as HPAI virus into wild

311 birds. The HPAI H7N3 virus was reported for the first time in Mexico in June 22, 2012

312 [48], in the state of Jalisco at layer hens, in places where wild migratory birds come

313 together from different migratory routes during wintertime [46, 47].

314 The sequence of the hemagglutinin cleavage site had 100% similarity with the sequence of

315 HPAIV isolated from Jalisco in 2012. As shown in the image 1, the inclusion of 24

316 nucleotide described by Maurer-Stroh et. al., clearly shows a widespread anchor associated

317 with the host 28S ribosomal RNA (rRNA). The host range expansion of a HPAIV H7N3

318 virus was previously detected with the presence of viruses of this serotype in poultry in

319 Mexico.

320 The state of Jalisco, located in west-central Mexico, provides the largest production of table

321 eggs in the country, followed by the state of Puebla [51]. In these states, vaccination against

322 avian influenza H7 is authorized. From 2013 the H7N3 HP virus has extended its

323 geographical distribution [49, 50, 52, 53, 54, 55,].

324 The *O. vetula* population is abundant and possible overpopulated in this reserve due to

325 plentiful food and to predator´s absence. Considering that there must be more than 1,100

326 individuals (5.9 individuals/ha) [34, 35]. We believe that the AI infection in *T. grayi* and *A.*

327 *albifrons*, was not progressive, and that these are spillover from the infection in *O. vetula*,

328 since we did not find evidence of ill birds or even dead ones. However, other exhibited

17

329   Cracids such as Great Curassow (*Crax rubra*) and Crested Guan (*Penelope purpurascens*),

330   closely living with *O. vetula* within the infected area, did not show clinical signs. Some of

331   these birds were sampled with cloacal and oral swabs, showing negative results to AIV.

332   It is also possible, that other avian species may have been infected as result of spillovers

333   and not detected by the established active surveillance system. The complexity of the

334   vegetation structure, may reduce the detection of ill birds. Also, the presence of

335   opportunistic carnivores could have easily killed weak birds, including scavengers.

336   Although we do not know how the virus reached the reserve, we have established some

337   possible routes of entrance. The first one is migratory birds in which many cases are the

338   most common way of AIV entry into a bird population. The south bound neotropical

339   migrants in El Zapotal, begins in July, reaching its peak during December and January [26].

340   During this migration there are no records of Anseriformes and Charadriiformes, the

341   predominant reservoirs of AIV [26]. Also, during April 1-15, there was no accounts of

342   mortalities, donations, confiscations or any other bird mobilizations in the ZooMAT.

343   The records of the visitors that attended the zoo during April 1-15, did not provide any

344   useful information.

345   We do not discard that the interaction agent-bird could occur in ZooMAT in the food court

346   area, where *O. vetula*, comes close to the people looking for food. The epizootic happened

347   during the Holy Week period in which the number of visitors is very high. Because of this,

348   it is possible that transmission might have occurred through fomites, mainly, shoes, clothes

349   or contaminated material such as egg boxes that could have introduced the virus into the

350   reserve [54]. Additionally, it is also possible that the holiday period incremented

18

351   considerably the food consumption and sales in the restaurant, mainly eggs, originating

352   product in the states under vaccination against H7 virus, recalling that the egg can be a

353   source of infection [56, 58, 59, 60,].

354   During the breeding period, *O. vetula* may have had the chance to feed on cooked eggs and

355   even egg shells from the garbage cans, becoming infected, considering garbage and

356   containers, did not have an adequate manage [37]. Other possibility, could be the fights

357   between males during the reproductive period, considering male-female ratio was 3 to 1.

358   Once a bird is infected, the horizontal transmission of the virus is initiated.

359   Another form of transmission could have been related through foraging behavior of many

360   birds. Some of them included an ample range of tropical fruits eaten by *O. vetula*, *T. grayi*.

361   Although, considering the captivity of *Amazona albifrons*, the possibility of transmission

362   could have happened due to human contamination. No hypothesis could be proved in this

363   particular case. However, the results of the phylogenetic analysis inferred that the VIA

364   H7N3 detected has an origin in the Mexican state of Puebla, second producer of egg for

365   plate [60].

366   In 2004, a low pathogenic H5N2 influenza virus was identified in a psittacine bird for the

367   first time in the United States under the Mexican lineage H5N2 viruses [20].

368   In addition, wild birds interact with poultry in a close vicinity. If these birds were AIV

369   carriers, they contaminated the environment through oral and fecal excretions, and possibly

370   carcasses [2, 7]. This environmental situation also increases the exposition and viral

371   transmission risks to resident wild birds, known as "bridge birds" (BB). The BB can be

372   infected by direct contact with feces or contaminated water, since the AI virus can survive

373 in water during weeks or months [8, 57, 58, 59, 61]. Later, the BB will disseminate the

374 virus by mechanical or biological means into the backyards or directly to poultry farms

375 lacking of biosecurity (protections against synanthropic birds) [4, 8, 44, 58, 62].

376 This outbreak is significant because it shows the vulnerability of sites devoted to the

377 protection and conservation of avian species, some of them under a risk category [28]. It is

378 crucial to develop more complete analysis of the existing risks, and of the challenges to

379 implement minimal biosecurity measures in these facilities. If the epizootic control had

380 been unsuccessful, the viruses could have caused additional damage to other wild birds'

381 species as well as to the regional poultry industry with the subsequent loss of food

382 production and jobs in one of the poorest states of Mexico.

383 Because of the susceptibility shown by *O. vetula*, its particular characteristics and wide

384 distribution in the tropical and subtropical zones in Mexico and Central America, we

385 recommend that this species, performs the role "as sentinel". So, any event of mortality in

386 this species, should be analyzed for Avian Influenza.

387 Finally, it is pivotal that veterinarians, wildlife managers and biologists notify to the

388 veterinary authority, any suspicious activity of ill or event of mortality in wild birds, to be

389 investigated [20, 25, 26].

390

Acknowledgments

394

References.

1. Webster RG, Krauss S, Hulse-Post D, Ramirez S. Evolution of Influenza A viruses in wild birds. J Wild Dis. 2007; 43(3): S1-S6.

2. Krauss S, Obert CA, Franks J, Walker D, Jones K, Seiler P, et al. Influenza in migratory birds an evidence of limited intercontinental virus axchange. PLoS Pathog. 2007; 3(11): 1684-1693.

3. Rejmanek D, Hosseini PR, Mazet JAK, Daszak P, Goldsteina T. Evolutionary dynamics and global diversity of Influenza A virus. J. Virol. 2015; 89(21): 10993-1001.

4. Vijaykrishna D, Deng Y-M, Su YCF, Fourment M, Iannello P, Arzey GG, et al. The recent establishment of North American H10 lineage Influenza Viruses in australian wild waterfowl and the evolution of australian Avian Influenza viruses. J. Virol. 2013; 87(18): 10182–10189.

5. Olsen B, Munster VJ, Wallensten A, Waldenström J, Osterhaus AD, Fouchier RA. Global patterns of Influenza A Virus in wild birds. Science. 2006; 312: 384-388.

6. Artois M, Bicout D, Doctrinal D, Fouchier R, Gavier-Widen D, Globig A. Outbreaks of highly pathogenic Avian Influenza in Europe: the risks associated with wild bird. Rev. sci. tech. 2009; 28(1): 69-92.

7. Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. Evolution and ecology of influenza A viruses. Microbiol. Rev. 1992; 56(1): 152-179.

8. Chen R, Holmes EC. Avian Influenza virus exhibits rapid evolutionary dynamics. Mol. Biol. Evol. 2006; 23(12): 2336-2341.

417   9. Reperant LA, Rimmelzwaan GF, Kuiken T. Avian influenza viruses in mammals.

418   Rev. Sci. Tech. 2009; 28(1): 137-159.

419   10. Fumihito A, Miyake T, Takada M, Shingu R, Endo T, Gojobori T, et al.

420   Monophyletic origin and unique dispersal patterns of domestic fowls. Proc Natl

421   Acad Sci. 1996; 93(13): 6792–6795.

422   11. Pantin-Jackwood MJ, Swayne DE. Pathogenesis and pathobiology of avian

423   influenza infection in birds. Rev. Sci. Tech. 2009; 28(1): 113-136.

424   12. Bröjer C. Pathobiology of avian influenza in wild bird species. PH. D. Thesis,

425   Swedish University of Agricultural Sciences. 2012. Available from:

426   https://pdfs.semanticscholar.org/1ddd/60bd818347ca4425f75db0f948879a53afcb.pd

427   f

428   13. OIE. Event summary: Highly pathogenic avian influenza. 2012 June 22 [cited18

429   Aug 2015]. In: World Animal Health Information System (WAHIS) [Internet].

430   México. Available from:

431   https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=M

432   apFullEventReport&reportid=12067

433   14. Navarro-López R, Vázquez-Mendoza LF, Villarreal CL, Casaubon MT, Márquez

434   MA. Highly pathogenic avian influenza A/H7N3 in great-tailed grackles (Quiscalus

435   mexicanus) in the Altos de Jalisco region of Mexico. JMM Case Rep. 2014; 1(4):

436   e001461 doi: 10.1099/jmmcr.0.001461

437   15. Sleeman J. Detection of highly pathogenic Avian Influenza Viruses H5N2 and

438   H5N8 in wild birds of the United States. 2014; Wildlife Health Bulletin Dec-2014.

439   Available from: https://www.usgs.gov/centers/nwhc/science/wildlife-health-

440   bulletins?qt-science_center_objects=0#qt-science_center_objects

441   16. Sleeman J. Detection of novel highly pathogenic Avian Influenza Viruses in wild

442   birds. Wildlife Health Bull. 2015; 1. Available from:

443   https://www.usgs.gov/centers/nwhc/science/wildlife-health-bulletins?qt-

444   science_center_objects=0#qt-science_center_objects

445   17. Hénaux V, Samuel MD. Avian Influenza shedding patterns in waterfowl:

446   Implications for surveillance, environmental transmission and disease spread. J.

447   Wildlife Diseases. 2011; 47(3): 566-578.

448   18. Nestorowicz A, Kawaoka Y, Bean WJ, Webster RG. Molecular analysis of the

449   hemagglutinin genes of Australian H7N7 influenza viruses: role of passerine birds

450   in maintenance or transmission. Virol. 1987; 160(2): 411-418.

451   19. Perkins LE, Swayne DE. Varied pathogenicity of a Hong Kong-origin H5N1 avian

452   influenza virus in four passerine species and budgerigars. Vet. Pathol. 2003; 40: 14-

453   24.

454   20. Pillai SPS, Suarez DL, Pantin-Jackwood M, Lee CW. Pathogenicity and

455   transmission studies of H5N2 parrot avian influenza virus of Mexican lineage in

456   different poultry species. Vet Microbiol. 2008; 129: 48–57.

457   21. Fuller TL, Saatchi SS, Curd EE, Toffelmier E, Thomassen HA. Mapping the risk of

458   avian influenza in wild birds in the US. BMC Infectious Diseases. 2010; 10(187).

459   Available from: http://www.biomedcentral.com/1471-2334/10/187

460   22. Sagarpa, Senasica. Manual de procedimientos para la prevención, control y

461   erradicación de la influenza aviar de alta patogenicidad. 1st ed. México:

462   SENASICA; 2011.

463 23. Martin V, Forman J, Lubroth J. Preparándose para la influenza aviar altamente

464   patógena. Rome: FAO; 2007. Available from:

465   http://www.fao.org/3/a0632s/a0632s.pdf

466 24. Sagarpa. Ley Federal de sanidad animal. 2018 Feb 16 [cited 11 January 2020]. In:

467   [Internet]. México. Available from:

468   http://www.diputados.gob.mx/LeyesBiblio/pdf/LFSA_160218.pdf

469 25. Honhold N, Douglas I, Geering W, Shimshoni A, Lubroth J. Good emergency

470   management practice: The essentials. Rome: FAO; 2011. Available from:

471   http://www.fao.org/3/a-ba0137e.pdf

472 26. Smahn. Programa de manejo centro ecológico recreativo El Zapotal. 1st ed. Tuxtla

473   Gutierrez: Gobierno del Estado de Chiapas; 2013.

474 27. Peel MC, Finlayson BL, Mcmahon TA. Updated world map of the Köoppen-Geiger

475   climate classification. European Geosciences Union. 2007; 11(5): 1633-1644.

476   Available from: https://hal.archives-ouvertes.fr/hal-00305098

477 28. Semarnat. Lista de especies en riesgo de la Norma Oficial Mexicana NOM-059-

478   SEMARNAT-2010, protección ambiental especies nativas de México de flora y

479   fauna silvestres; categorías de riesgo y especificaciones para su inclusión, exclusión

480   o cambio. 2010 Dec 30. México. Available from:

481   http://www.dof.gob.mx/nota_detalle.php?codigo=5578808&fecha=14/11/2019

482 29. Daniel WW. Biostatistics: A foundation for analysis in health sciences. 7th ed. New

483   York. John Wiley & Sons. 1999.

484 30. Fair J, Paul E, Jones J. Guidelines to the use of wild birds in research. 3rd ed.

485   Washington. Ornithological Council. 2010.

486   31. Cannon RM, Roe RT. Livestock disease surveys. A field manual for veterinarians.

487   Canberra: Bureau of rural science, department of primary industry. Aust. Gov. Publ.

488   Cerv. 1982.

489   32. OIE. Informe de Seguimiento N° 11. Actualización sobre la Influenza Aviar

490   Altamente Patógena en Animales (Tipo H5 y H7). 2014 May 19 [Cited 27 July

491   2015]. España. Available from: http://www.oie.int/es/sanidad-animal-en-el-

492   mundo/actualizacion-sobre-la-influenza-aviar/2014/.

493   33. Ramírez-Albores JE. Avifauna de sitios asociados a la selva tropical en la

494   Depresión Central de Chiapas, México. A. Zool. Mex. 2010. 26:3

495   34. Conabio. (*Ortalis vetula*) Chachalaca vetula, residencia permanente. Distribución

496   potencial. in: Catálogo de metadatos geográficos. Agosto 19, 2009. [Cited: Julio 27,

497   2015.] Abailable from:

498   http://www.conabio.gob.mx/informacion/metadata/gis/orta_vetugw.xml?_httpcache

499   =yes&_xsl=/db/metadata/xsl/fgdc_html.xsl&_indent=no.

500   35. López RR, Silv NJ, Peterson M, Baur EH, González-García F, Brooks DM.

501   Chachalacas, hocofaisanes y pavas. In: Valdez R, Ortega-S JA, editors. Ecología y

502   Manejo de la Fauna Silvestre en México. Texcoco: COLPOS; 2014. pp. 121-145.

503   36. Peterson R, Chalif EL. Aves de México. Mexico: Diana, 2008.

504   37. Molina-García AA, Maldonado CR, Oliveras A, Rojas-Soto OR. Primer reporte de

505   nidos depredados por la chachalaca vetula (Ortalis vetula). Revista Mexicana de

506   Ornitología. 2008; 9(2): 32-34.

507   38. Senasica. Catálogo de Tramites y Servicios de la red de Laboratorios de la DGSA-

508   CPA. México: SENASICA; 2015.

25

509   39. Senasica. SINEXE: 2013. [Cited: October 13, 2015.] Available from:

510   http://www.senasica.gob.mx/?id=4689.

511   40. Sagarpa. Establecimiento de cuarentenas para animales y sus productos. 1998 Jun 8

512   [Cited 2 Sep 2015]. In: Diario Oficial de la Federación [Internet]. México.

513   Available from:

514   http://www.ordenjuridico.gob.mx/Publicaciones/CDs2007/.../pdf/50NOM.pdf.

515   41. Cárdenas-García S, Navarro-López R, Morales R, Olvera MA, Márquez MA,

516   Merino R, et al. Molecular Epidemiology of Newcastle Disease in Mexico and the

517   Potential Spillover of Viruses from Poultry into Wild Bird Species. Appl. Environ.

518   Microbiol. 2013; 79(16): 4985-4992.

519   42. OIE. Influenza Aviar. 2014. [Cited July 27 2015]. In: Código sanitario para los

520   animales terrestres [Internet]. México. Available form:

521   http://www.oie.int/doc/ged/D13948.PDF.

522   43. Villarreal CL. Avian Influenza in México. Rev. Sci. Tech. 2009; 28(1): 261-265.

523   44. Valdez-Gómez HE, Navarro-López R, Vázquez-Mendoza LF, Zalapa-Hernández

524   M, Guerrero-Hernández I, Fonseca-Delgado V, et al. Risk factors for the

525   transmission of infectious diseases agents at the wild birds - commercial birds

526   interface. pilot study in the region of the altos de Jalisco Mexico. Bull. Acad. Vét.

527   France. 2017; 170(2): 142-150.

528   45. Mohamad AA, Moore BR, Pérez AM. Phylodynamics of H5N1 Highly Pathogenic

529   Avian Influenza in Europe, 2005–2010: Potential for Molecular Surveillance of

530   New Outbreaks. Viruses. 2015; 7(6): 3310-3328.

531   46. Lu L, Lycett SJ, Leigh AJ. Determining the Phylogenetic and Phylogeographic

532   Origin of Highly Pathogenic Avian Influenza (H7N3) in Mexico. PLOS ONE.

533   2014; 9. Available from: https://doi.org/10.1371/journal.pone.0107330

534   47. Maurer-Stroh S, Lee R, Gunalan V, Eisenhaber F. The highly pathogenic H7N3

535   avian influenza strain from July 2012 in Mexico acquired an extended cleavage site

536   through recombination with host 28S rRNA. Virol. J. 2013; 10(139). Available

537   from: https://doi.org/10.1186/1743-422X-10-139

538   48. OIE. Reports AGS. Informes de seguimiento No 1. Referencia del informe (OIE):

539   13090. [cited 16 January 2013]. [Internet]. México. Available from:

540   https://www.oie.int/wahis_2/temp/reports/es_fup_0000013090_20130228_141421.

541   pdf

542   49. —. Reports GTO. Informe de seguimiento No 2. Referencia del informe (OIE):

543   13091. [cited 18 february 2013]. [Internet]. México. Available from:

544   https://www.oie.int/wahis_2/temp/reports/es_fup_0000013091_20130228_141531.

545   pdf

546   50. —. Reports JAL. Informe de seguimiento No 5. Referencia del informe (OIE):

547   13252. [cited 17 April 2013]. [Internet]. México. Available from:

548   https://www.oie.int/wahis_2/temp/reports/es_fup_0000013252_20130418_165608.

549   pdf

550   51. —. Reports PUE. Informe de seguimiento No 7. Referencia del informe (OIE):

551   13422. [cited 7 May 2013]. [Internet]. México. Available from:

552   https://www.oie.int/wahis_2/temp/reports/es_fup_0000013422_20130508_174939.

553   pdf

27

554   52. —. Reports OAX. Informe de notificación inmediata. Referencia del informe (OIE):

555   17489. [cited 8 April 2015]. [Internet]. México. Available from:

556   https://www.oie.int/wahis_2/temp/reports/es_imm_0000017489_20150409_172153

557   .pdf

558   53. —. Reports CHIS. Informe de notificación inmediata. Referencia del informe

559   (OIE): 17686. [cited 8 May 2015]. [Internet]. México. Available from:

560   https://www.oie.int/wahis_2/temp/reports/es_imm_0000017686_20150512_122351

561   .pdf

562   54. Chmiellewski R, Swayne DE. Avian Influenza: Public Healt and Food Safety

563   Concerns. Annu Rev Food Sci Technol. 2011; 2: 37-57. doi: 10.1146/annurev-food-

564   022510-133710.

565   55. Van der Berg T. The rol of the legal all illegal trade of live birds and avian products

566   in the spred of avian influenza. Rev. sci. tech. Off. Int. 2009; 28(1): 93-111

567   56. Kilany WH, Arafa A, Erfan AM, Ahmed MS, Nawar AA, Salim AA, etal. Isolation

568   oh higlhy pathogeneic avian influenza H5N1 from table eggs after vaccinal breack

569   in comercial layer flock. Avian dis. 2010; 54(3): 1115-1119. doi:10.1637/9369-

570   041310-Case.1

571   57. Horm VS, Gutiérrez RA, Nichols JM, Buchy P. Higlhy pathogenic influenza A

572   (H5N1) virus survival in complex artificial aquatic biotipes. Plos One. 2012; 7.

573   DOI: 10.1371/journal.pone.0034160.

574   58. Song H, Wan H, Araya Y, Pérez DR. Partial direct contact transmission in ferrets of

575   a mallard H7N3 influenza virus with typical avian-like receptor specifity. Virol. J.

576   2009; 6. DOI: 10.1186/1743-422X-6-126.

577   59. Stallknecht DE, Brown JD. Tenacity of avian influenza viruses. Rev sci tech Off int

578   Epiz. 2009; 28(1): 59-67.

579   60. Una. Indicadores Económicos: Principales Estados Productores de Pollo. 2014

580   [cited 27 July 2015]. México. Available from:

581   http://www.una.org.mx/index.php/component/content/article/2-uncategorised/19-

582   indicadores-economicos.

583   61. Ictv. Virus Taxonomy: 2014 Release. 2014 July [cited 28 July 2015]. Available

584   from: http://www.ictvonline.org/virustaxonomy.asp.

585   62. Alexander DJ. A review of avian influenza in different bird species. Vet Microbiol.

586   2000; 74 (1-2): 3-13. doi:10.1016/s0378-1135(00)00160-7

587   63. Max V, Herrera J, Moreira R, Rojas H. Avian Influenza in Chile: a successful

588   experience. Avian dis. 2007; 1: 363-365.

589   64. Stegeman A, Bouma A, Elbers AR, De Jong MC, Nodelijk G, De Klerk F, et al.

590   Avian influenza A virus (H7N7) epidemic in The Netherlands in 2003: course of the

591   epidemic and effectiveness of control measures. J. Infect. Dis. 2004; 190(12): 2088-

592   2095.

593   65. Li XI, Shi J2, Guo J1, Deng G, Zhang Q, Wang J, et al. Genetics, receptor binding

594   property, and transmissibility in mammals of naturally isolated H9N2 Avian

595   Influenza viruses. PLoS Pathog. 2014; 10(11): e1004508. doi:

596   10.1371/journal.ppat.1004508.

597   66. Pedersen K, Swafford SR, DeLiberto TJ. Low pathogenicity avian influenza

598   subtypes isolated from wild birds in the United States, 2006–2008. Avian Dis. 2010;

599   Vol. 54, No. s1, pp. 405-410.

600 67. Ito T, Goto H, Yamamoto E, Tanaka H, Takeuchi M, Kuwayama M, et al.

601   Generation of a Highly Pathogenic Avian Influenza A Virus from an avirulent field

602   isolate by passaging in chickens. J. Virol. 2001; 75(9): 4439–4443.

603 68. Soda K, Asakura S, Okamatsu M, Sakoda Y, Kida H. H9N2 influenza virus

604   acquires intravenous pathogenicity on the introduction of a pair of di-basic amino

605   acid residues at the cleavage site of the hemagglutinin and consecutive passages in

606   chickens. J. Virol. 2011; Doi: 10.1186/1743-422X-8-64.

607 69. Pasick J, Berhane Y, Hisanaga T, Kehler H, Hooper-McGrevy K, Handel K, et al.

608   Diagnostic test results and pathology associated with the 2007 Canadian H7N3

609   highly pathogenic avian influenza outbreak. Avian Dis. 2010; 54(1 Suppl): 213-219.

610 70. Lebarbenchon C, Stallknecht DE. Host shifts and molecular evolution of H7 avian

611   influenza virus hemagglutinin. Virol. J. 2011; 8(328). https://doi.org/10.1186/1743-

612   422X-8-328

613 71. Webster RG, Hulse DJ. Microbial adaptation and change: avian influenza. Rev. Sci.

614   Tech. Off. Int. Epiz. 2004; 23(2): 453-465.

615 72. Alexander DJ. A review of avian influenza in different bird species. Vet. Microbiol.

616   2000; 74(1–2): 3-13. Doi.org/10.1016/S0378-1135(00)00160-7

617 73. Alexander DJ, Brown IH. History of highly pathogenic avian influenza. Rev. Sci.

618   Tech. Off. Int. Epiz. 2009; 28(1): 19-38.

619 74. OIE. Informe de Notificación Inmediata. Actualización sobre la Influenza Aviar

620   Altamente Patógena en Animales (Tipo H5 y H7) Ref. OIE 12067. 2012 Jun 21

621   [Cited: 27 July 2015]. España. Available from: http://www.oie.int/es/sanidad-

622   animal-en-el-mundo/actualizacion-sobre-la-influenza-aviar/2012/.

623 75. —. Informe de Notificación Inmediata. Actualización sobre la Influenza Aviar

624   Altamente Patógena en Animales (Tipo H5 y H7) INF. REF. 12839. 2013 Jan 8.

625   [Cited 27 July 2015]. España. Available from: http://www.oie.int/es/sanidad-

626   animal-en-el-mundo/actualizacion-sobre-la-influenza-aviar/2013/.

627 76. —. Informe de Notificación Inmediata. Actualización sobre la Influenza Aviar

628   Altamente Patógena en Animales (Tipo H5 y H7) REF. OIE 17489. 2015 Apr 8

629   [Cited 27 July 2015]. España. Available from: http://www.oie.int/es/sanidad-

630   animal-en-el-mundo/actualizacion-sobre-la-influenza-aviar/2015/

631 77. —. Informe de Notificación Inmediata. REF. OIE 17686. 2015 May 8 [Cited 27

632   July 2015]. España. Available from: http://www.oie.int/es/sanidad-animal-en-el-

633   mundo/actualizacion-sobre-la-influenza-aviar/2015/.

634 78. Global avian influenza situation OIE. Actualización sobre la IAAP en animales

635   (TIPO H5 y H7). 2004-2015. 2015 Oct 15 [Cited 27 July 2015]. España. Available

636   from: http://www.oie.int/es/sanidad-animal-en-el-mundo/actualizacion-sobre-la-

637   influenza-aviar/2015/.