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What is new on molecular characteristics of Avian metapneumovirus strains circulating in Europe?

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1   **What is new on molecular characteristics of Avian metapneumovirus strains circulating in**

2   **Europe?**

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4   Molecular epidemiology of aMPV-B in Europe

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Summary

In the present study one hundred and sixteen partial G gene sequences of Avian metapneumovirus (aMPV) subtype B, obtained during routine diagnostics in different European Countries in the last few years (2014-2019), were analysed by sequence and phylogenetic analyses in order to draw an updated picture of the molecular characteristics of circulating strains. Nucleotide sequences were compared with other sequences of European and non-European aMPV-Bs collected prior to that period or retrieved from GenBank. Phylogenetic relationships among the aMPV-B strains, reconstructed using the Maximum Likelihood method implemented in MEGA X, demonstrated that aMPV-B has evolved in Europe from its first appearance, frequently displaying a clear relation with the geographic area of detection. The 40% of aMPV-B viruses analysed were classified as vaccine-derived strains, being phylogenetically related, and showing high nucleotide identity with live commercial vaccine strains licensed in Europe. The remaining 60% were classified as field strains since they clustered separately and showed a low nucleotide identity with vaccines and vaccine-derived strains. The phylogenetic tree showed that the virus has continued to evolve from its first appearance in the '80s since more recently detected strains belonged to clades phylogenetically distant from the older strains. Unlike vaccine-derived strains, field strains tended to cluster according to their geographic origin and irrespective of the host species where the viruses had been detected. In conclusion, the molecular characterization of aMPV-B and the differentiation between vaccines and field strains through G gene sequence analysis can be a useful tool towards correct diagnosis and should be routinely applied in order to better address the control strategies.

Introduction

Avian metapneumovirus (aMPV) infections in Europe have been an issue since its first appearance both in turkeys, chickens and in other minor species (Toquin, Bayon-Auboyer, Eterradossi, & Jestin, 1999; Catelli et al., 2001; Cecchinato, Ferreira, Munir, & Catelli, 2017; Cecchinato et al., 2018), causing economic losses mainly due to respiratory or reproductive problems (Cecchinato et al., 2012), often exacerbated by secondary bacterial infections (Giovanardi et al., 2014).

First aMPV isolations in Europe date back to the second half of the 1980s, when Turkey Rhinotracheitis (TRT) outbreaks appeared in the United Kingdom (McDougall and Cook, 1986) and France (Giraud, Bennejean, Guittet, & Toquin, 1986). As the virus was spreading all over Europe and the clinical problems in poultry farms became increasingly serious, during the early 1990s (Cook et al. 1989a e 1989b) live attenuated vaccines were developed and became commercially available. By analysing strains circulating in Europe, Juhasz and Easton (1994) reported differences in the G gene between aMPV isolates and proposed the classification into A and B subtypes and confirmed the co-circulation of both subtypes. After this initial study, and due to the increasing use of sequence analysis, two further subtypes, named C and D, were identified in France (Bayon-Auboyer, Arnauld, Toquin, & Eterradossi, 2000; Toquin et al., 2006).

Despite these reports, molecular data on aMPV strains circulating in Europe are still poor and scattered (Catelli et al., 2004; Cecchinato et al., 2013a; Listorti et al., 2014; Franzo et al., 2017; Tucciarone et al., 2017 a 2018; Ball, Forrester, & Ganapathy, 2018; Andreopoulou et al., 2019), as most of them originate from few countries. In the last decades, subtype B has been generally the most frequently encountered subtype in Europe, although subtype A has been sporadically reported (Lupini et al., 2011).

In order to update the epidemiological picture of circulating strains, the present study was designed to molecularly characterize, by partial G gene sequencing, aMPV subtype B strains detected in Europe in the last years.

Material and Methods

Sample collection

The survey covered the time period from 2014 to 2019 and targeted aMPV subtype B detected during routine diagnostic activity performed in Italy by the Universities of Bologna and Padua or in Spain by the Centre de Sanitat Avícola de Catalunya i Aragó (CESAC).

Samples originated from different European countries and were usually collected during outbreaks of respiratory disorders referable to aMPV infections in turkey, chicken or guinea fowl flocks. Sampling was also performed for epidemiological purposes in absence of clear clinical signs.

As a rule, samples consisted of pools of 10 rhino-pharyngeal swabs per flock as previously suggested (Catelli et al., 2004).

RNA extraction and PCR analysis

RNA was extracted from each pool of swabs and subjected to a nested RT-PCR targeting the G gene (Cavanagh, Mawditt, Britton, & Naylor, 1999, slightly modified) which allows the simultaneous detection and differentiation of subtypes A and B. The reverse transcription (RT) reaction was primed using oligonucleotide G6- (5'- CTGACAAATTGGTCCTGATT- 3').
Subsequently the same oligonucleotide was used together with oligonucleotide Gstart+ (5'-CAAGTATCCAGATGGGGTC- 3') for the first PCR round. The obtained PCR product was then subjected to a second PCR round with oligonucleotide G5- (5'- CAAAGAGCCAATAAGCCCA-3') in conjunction with oligonucleotides G8+A (5'-CACTCACTGTTAGCGTCATA-3') and G9+B (5'-TAGTCCTCAAGCAAGTCCTC-3'). The predicted amplicon length is of 268 bp for subtype A and 361 bp for subtype B. Depending on the laboratory, this step was preceded by a multiplex real-time RT-PCR (qRT-PCR) screening test targeting the SH gene (Cecchinato et al., 2013b) and only positive samples were subsequently amplified by RT-nested PCR.

Partial G gene sequencing

The partial G gene amplicons (361 bp-long) obtained were purified using ExoSAP-IT™ *Express* PCR Product Cleanup (Thermo Fisher Scientific, Massachusetts, USA) according to the manufacturer's instructions and sequenced in both directions using RT-nested PCR primers G5- (5′-CAAAGAGCCAATAAGCCCA-3′) and G9+B (5′-TAGTCCTCAAGCAAGTCCTC-3′), by a commercial sequencing service (Macrogen Europe, Amsterdam, The Netherlands). The obtained sequences were named using the following nomenclature: aMPV/B/Country of origin/Host species (Turkey: Ty, Chicken: Ck or Guinea fowl)/sample ID number/year of detection.

Sequence and phylogenetic analysis

Nucleotide sequences were edited and assembled using BioEdit software, then, using Clustal W, aligned against and compared with G gene sequences of:

- The most commonly used subtype B vaccines (i.e. aMPV/B vaccine - strain VCO3; aMPV/B vaccine - Strain 11/94; aMPV/B vaccine - strain 1062 and aMPV/B vaccine - strain PL21)

- 82 already available European aMPV B strains (Table S1)

- 59 non-European aMPV-B strains, retrieved from *GenBank* (Table S2).

Phylogenetic relationships among the aMPV-B strains were reconstructed using the Neighbor-Joining method and Kimura 2-parameter model implemented in MEGA X (Kumar et al., 2018). The substitution model was selected based on Bayesian information criterion (BIC), calculated using the same software. The branch support was calculated by performing 1000 bootstrap replicates; only branches supported by bootstrap values ≥ 70% were considered reliable. Complete deletion option was selected before the analysis begins, to remove sites containing missing data or alignment gaps.

118   Within-group mean pairwise genetic p-distance was estimated using MEGA X in order to evaluate

119   the genetic heterogeneity of aMPV population. Overall nucleotide similarity was further estimated

120   by calculating the arithmetic mean of the sequence identity values, obtained using the Sequence

121   Identity Matrix tool on BioEdit software, between field strains or vaccine strains belonging to the

122   same phylogenetic cluster.

123

124   **Results**

125   116 samples tested aMPV positive, all belonging to subtype B (n. 27 from France, n. 18 from Italy,

126   n. 27 from Romania, n. 36 from Spain and n. 8 from the United Kingdom) and 116 partial G gene

127   sequences were obtained. Sequence data have been submitted to the GenBank database under

128   accession numbers MT432826-MT432923 and MT436220-MT436237 (Tables S3 to S7).

129   Phylogenetic analysis was carried out by reconstructing both a comprehensive tree (Figure 1) and

130   five Country- specific trees (Figures S1 to S5). Furthermore, a phylogenetic tree including selected

131   European and non-European aMPV-B sequences was generated (Figure 2). The number of nucleotide

132   positions included in the final datasets subjected to phylogenetic analysis is specified in the legends

133   of the figures.

134   Regardless of the Country of origin, aMPV strains detected from 2001 to 2019, herein referred to as

135   "recent strains", were distinguished from those clustering with vaccines and field strains detected

136   prior to the 1990s, referred to as "older strains" (from which the vaccines were established by

137   attenuation) and those forming independent clades.

138   As a rule, aMPV strains were referred to as "vaccine-derived strains" if the maximum nucleotide

139   sequence identity with a reference vaccine strain was greater than or equal to 99 % (2 or less

140   nucleotide differences) and if they fell into the same phylogenetic cluster. The remaining strains were

141   classified as "field strains".

On the above basis, 70 out of 116 aMPV-B strains were labelled as field strains (60%); the remaining 46 strains (40%) were identified as vaccine-derived strains.

A certain degree of geographic clustering was observed among Romanian, Spanish, French and Italian field strains (Figure 1). Some Italian strains also clustered together with some recent Greek strains.

French aMPV field strains (Figure S1) were divided in two clusters. The largest cluster displayed a within-group mean p-distance of 0.003 (overall nucleotide similarity of 99.7%) and included chicken, turkey and guinea fowl strains detected from 2017 to 2019, while the smallest had a within-group mean p-distance of 0.000 (overall nucleotide similarity of 100%) and contains turkey strains detected in 2018. Vaccine-derived strains clustered respectively with vaccine strain PL21, 11/94 or VCO3 and were mostly detected in homologous-vaccinated turkeys or chickens, but also in unvaccinated guinea fowls. The cluster containing vaccines, vaccine-derived strains and older French strains displayed a within-group mean p-distance of 0.006 (overall nucleotide similarity of 99.4%)

Italian field strains were grouped in two main clusters (Figure S2): the larger one was composed of several sub-clusters including field strains detected from 2010 to 2019 in turkey, chicken, and guinea fowl flocks and showed a within-group mean p-distance of 0.010 (overall nucleotide similarity of 99.0%). The smaller one included field strains detected from 2001 to 2009 and in 2016 in turkeys and chickens, with a within-group mean p-distance of 0.004 (overall nucleotide similarity of 99.6%). Vaccine-derived strains clustering with aMPV-B vaccine strains VCO3 or 1062 were detected from vaccinated turkeys. The cluster containing vaccines, vaccine-derived strains and older Italian strains displayed a within-group mean p-distance of 0.007 (overall nucleotide similarity of 99.3%).

Romanian aMPV-B field strains (Figure S3), detected from turkeys or chickens, fell within a single phylogenetic group showing a within-group mean p-distance of 0.001 (overall nucleotide similarity of 99.9%). The vaccine-derived strains, all detected in vaccinated turkey flocks, clustered with

166 vaccine strains PL21, 11/94 or VCO3. The cluster containing vaccines and vaccine-derived strains

167 displayed a within-group mean p-distance of 0.007 (overall nucleotide similarity of 99.3%).

168 In the Spanish tree (Figure S4) field strains fell within two main clusters: a larger one (within-group

169 mean p-distance: 0.006 and overall nucleotide similarity of 99.4%) containing strains detected from

170 2014 to 2017; a smaller one (within-group mean p-distance: 0.003 and overall nucleotide similarity

171 of 99.7%) containing viruses detected from 2014 to 2015. Spanish vaccine-derived strains clustered

172 with vaccine strain 11/94 or PL21. The cluster containing vaccines, vaccine-derived strains and older

173 strains displayed a within-group mean p-distance of 0.008 (overall nucleotide similarity of 99.2%).

174 The British phylogenetic tree (Figure S5) exclusively displayed vaccine-derived strains, clustering

175 with vaccine strain PL21, 11/94 or VCO3 (within-group mean p-distance: 0.007 and overall

176 nucleotide similarity of 99.3%).

177 The tree reconstructed with representative aMPV-B sequences from all over the world (Figure 2)

178 confirmed the Country-specific clustering trend seen for the European strains. Well-defined clusters

179 were identified for field strains circulating in Brazil, Iran, Israel and Turkey. Vaccines, vaccine-

180 derived and older strains clustered together and were showed in the figure compressed in two

181 subtrees. Within-group mean p-distance and overall nucleotide similarity of the cluster containing

182 vaccine PL21 were respectively 0.007 of 99.3%,

183

184 **Discussion**

185 In the present study, one hundred and sixteen aMPV-B partial G gene sequences, obtained during

186 routine diagnostics in different European Countries in the last few years, were analysed by sequence

187 and phylogenetic analyses in order to molecularly characterize them.

188 The G gene, which harbours mutations at variable positions between aMPV-B strains, was

189 conveniently amplified by the routine diagnostic RT-PCR protocol employed in the study, and its

sequencing proved useful for epidemiological purposes. Furthermore, its variability can give an

indication of whether vaccine or field strains are present, making the differentiation relatively easy

and cheap to perform.

Live attenuated aMPV vaccines are widely administered to prevent disease in turkeys and chickens,

and the recovery of vaccine-derived strains is not unusual both in vaccinated or in unvaccinated

flocks (Banet-Noach et al., 2009; Lupini et al., 2011; Chacon et al., 2011; Cecchinato et al., 2013a;

Listorti et al., 2014; Arafa et al., 2015; Bayraktar et al., 2019; Andreopoulou et al., 2019).

The 40% of the aMPV-B strains detected in the present study were classified as vaccine-derived

strains, being phylogenetically related and showing high nucleotide identity with live commercial

vaccine strains licensed in Europe. As expected, vaccine-derived strains formed separate clusters

depending on the vaccine strain of origin and were detected in all tested European countries.

A large part of the vaccine-derived strains analysed in the present study was detected in

homologous-vaccinated birds, from approximately two to four weeks after vaccination, and only

occasionally in unvaccinated birds. Reversion to virulence of aMPV subtype A or B has been

previously demonstrated (Catelli et al., 2006; Brown et al., 2011, Cecchinato et al., 2014).

Therefore, the detection of vaccine-derived strains closely related to the applied vaccine,

concurrently with respiratory signs, could be reliably linked to the vaccine reversion to virulence.

The detection of the strain aMPV/B/France/GuineaFowl/1060/18 in unvaccinated guinea fowls is

noteworthy, since its partial G gene sequence shared 100% nucleotide identity with the vaccine

strain 11/94. It could be speculated that the vaccine virus could had been introduced as a

contaminant by personnel, fomites, vehicles movement or airborne from neighbouring premises.

The field veterinarian reported the presence of a turkey farm at approximately 500 meters from the

guinea fowl one. Vaccinal strain 11/94 was applied in the turkey flock and the use of the litter from

the turkey farm for fertilization of the surrounding crops was reported. The ability of vaccine-

derived aMPVs to spread beyond the administration site is well known and it has been proven by

215 Lupini et al. (2011) following a turkey rhinotracheitis outbreak caused by aMPV subtype A in

216 unvaccinated turkeys.

217 The remaining 60% aMPV-B viruses analysed in the present study were classified as field strains

218 since they clustered separately and showed a low nucleotide identity with vaccines and vaccine-

219 derived strains.

220 The phylogenetic tree reconstructed with European sequences showed that the virus has continued

221 to evolve from its first appearance in the '80s. In fact, more recently detected field strains belonged

222 to clades phylogenetically distant from the older field strains, confirming the previously-reported

223 aMPV tendency to evolve over time (Cecchinato et al., 2010).

224 Unlike vaccine-derived strains, field strains tended to cluster according to their geographic origin,

225 with few exceptions. Distinct clusters were observed for French, Italian, Romanian and Spanish

226 strains, yet some Italian and Greek field isolates clustered closely together, indicating a potential

227 transmission route between these two countries (Tucciarone et al., 2017; Andreopoulou et al.,

228 2019).

229 The molecular epidemiology of aMPV within each country was analysed in detail reconstructing

230 country-related phylogenetic trees. Heterogeneous field strain populations seemed to co-exist within

231 single European countries, with the only exception of Romania, where all identified strains were

232 part of just one clade. This last finding could be explained by keeping into account that all the

233 processed samples came from different sites within the same company in which viral circulation

234 could be compartmentalized (Franzo et al., 2020).

235 French, Italian, and Spanish trees showed a rather heterogeneous field strain population, as the

236 strains fell into more than one cluster, divided in several sub-clusters. The heterogeneity was further

237 deduced from the within-group mean p-distance values. The highest value, indicating the highest

238 heterogeneity of nucleotide sequence, was observed for Italian field strains, presumably because of

239   the larger number of sequences available over a broader time period and coming from different

240   commercial poultry companies.

241   Furthermore, time-related clustering was visible for Italian and Spanish field strains, as strains

242   detected in the last few years tend to form separate clusters or sub-clusters.

243   As a common finding, recent field aMPV-B strains, belonging to different and distant phylogenetic

244   clades, were detected in the presence of respiratory signs in vaccinated flocks. Due to the G gene

245   variability observed in recent aMPV-B strains, and knowing that the encoded surface G

246   glycoprotein is a key antigen for vaccine-induced immune protection (Naylor et al., 2007) and can

247   evolve in order to avoid vaccine-induced immunity (Catelli et al., 2010; Cecchinato et al., 2010),

248   re-assessment of protection conferred by commercially available vaccines against currently

249   circulating aMPV field strains might be necessary to improve disease control strategies.

250   The obtained phylogenetic data revealed that aMPV-B strains clustered together irrespective of the

251   host species where the viruses had been detected. A recent experimental challenge study showed

252   that both chickens and turkeys are susceptible to aMPV-B infection with the same virus isolate

253   (Brown et al., 2019). Moreover Cecchinato et al. (2018) reported an outbreak of respiratory disease

254   in guinea fowls caused by aMPV-B strain identical to the ones circulating in the surrounding turkey

255   flocks. Therefore, no evidences are currently available to support a host-specific adaptation of

256   aMPV variants.

257   As a final remark, the tree reconstructed on European and non-European strains further confirmed

258   the geographic clustering of field strains. As a rule, field strains grouped together for country

259   location and time period, suggesting a local evolution tendency of the virus that might have taken

260   place after a single introduction event. The majority of recently detected European field strains were

261   located in a big subtree indicating a certain genetic similarity and supporting the wide circulation of

262   a quite homogeneous aMPV subtype B clade in European countries, with the only exception of two

263   clusters of phylogenetically-distant French and Spanish strains.

In conclusion, the molecular characterization of aMPV subtype B and the differentiation between vaccines and field strains through G gene sequence analysis can be a useful tool towards a correct diagnosis and should be routinely applied in order to better address the control strategies. In this respect, current vaccine research is focused on reducing the issues connected to live attenuated vaccine reversion to virulence or the selection of potentially virulent subpopulations (Franzo et al., 2015). Therefore, considering the associated risks, further efforts should be directed at improving administration and biosecurity measures, in order to reduce their prolonged circulation and spreading.

Several research groups have attempted to develop efficacious, more stable and safer next-generation vaccines but, despite this effort, conventional live attenuated vaccines still provide the greatest protection after homologous challenge (Qingzhong et al., 1994; Tarpey et al., 2001; Kapczynski and Sellers, 2003; Kapczynski, 2004; Chary, Njenga, & Sharma, 2005; Yu et al., 2013; Hu, Roth, Zsak, & Yu, 2017).

Promising reverse genetics systems for aMPV have been developed in recent years for A, B and C subtypes (Naylor et al., 2004; Yu et al., 2010; Laconi et al., 2016) other than being an exceptional tool for the study of the virus properties (Brown et al., 2011), can be used for the development of rationally modified aMPV vaccines (Naylor, Lupini, & Brown, 2010) or recombinant vaccines (Falchieri et al., 2013) expressing foreign genes.

Conflict of Interest Statement

The authors declare no conflict of interest.

Ethical approval

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as the original research data of this

article was obtained from oro-pharyngeal swabs collected for routine diagnostics purpose by field

veterinarians.

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Figure legends

Figure 1. Phylogenetic tree based on G gene nucleotide sequences of 60 selected European aMPV-B strains detected in the present study (identical sequences within a country were removed), including the most commonly used subtype B vaccines and European aMPV-B strains previously obtained or retrieved from GenBank. The evolutionary history was inferred using the Neighbor-Joining method in MEGA X. All nucleotide positions containing gaps and missing data were eliminated, there were a total of 246 positions in the final dataset. The two subtrees containing vaccines and vaccine-derived strains have been compressed. Field aMPV-B strains clusters are included in square brackets and coloured by country of origin. Only bootstrap values ≥ 70 are reported.

Figure 2. Phylogenetic tree based on G gene nucleotide sequences of selected European and non-European aMPV-B strains, previously obtained or retrieved from GenBank. The evolutionary history was inferred using the Neighbor-Joining method in MEGA X. All nucleotide positions containing gaps and missing data were eliminated, there were a total of 171 positions in the final dataset. The two subtrees containing vaccines and vaccine-derived strains have been compressed. Field aMPV-B strains clusters are coloured by country of origin. The country of origin was added in brackets next to the strain name when it was not clearly specified in the very name. Only bootstrap values ≥ 70 are reported.

Supplementary figure legends

Figure S1. Phylogenetic tree based on G gene nucleotide sequences of 27 French aMPV-B strains detected in the present study, including the most commonly used subtype B vaccines (marked with a green triangle) and 2 French aMPV-B strains previously obtained. The evolutionary history was inferred using the Neighbor-Joining method in MEGA X. All nucleotide positions containing gaps

494   and missing data were eliminated, there were a total of 253 positions in the final dataset. Field

495   aMPV-B strains clusters are included in square brackets. Only bootstrap values ≥ 70 are reported.

496

497   **Figure S2.** Phylogenetic tree based on G gene nucleotide sequences of 18 Italian aMPV-B strains

498   detected in the present study, including the most commonly used subtype B vaccines (marked with

499   a green triangle) and 42 Italian aMPV -B strains, previously obtained or retrieved from GenBank.

500   The evolutionary history was inferred using the Neighbor-Joining method in MEGA X. All

501   nucleotide positions containing gaps and missing data were eliminated, there were a total of 252

502   positions in the final dataset. Field aMPV-B strains clusters are included in square brackets. Only

503   bootstrap values ≥ 70 are reported.

504

505   **Figure S3.** Phylogenetic tree based on G gene nucleotide sequences of 27 Romanian aMPV-B

506   strains detected in the present study and the most commonly used subtype B vaccines (marked with

507   a green triangle). The evolutionary history was inferred using the Neighbor-Joining method in

508   MEGA X. All nucleotide positions containing gaps and missing data were eliminated, there were a

509   total of 282 positions in the final dataset. Field aMPV-B strains clusters are included in square

510   brackets. Only bootstrap values ≥ 70 are reported.

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512   **Figure S4.** Phylogenetic tree based on G gene nucleotide sequences of 36 Spanish aMPV-B strains

513   detected in the present study and the most commonly used subtype B vaccines (marked with a green

514   triangle). The evolutionary history was inferred using the Neighbor-Joining method in MEGA X.

515   All nucleotide positions containing gaps and missing data were eliminated, there were a total of 303

516   positions in the final dataset. Field aMPV-B strains clusters are included in square brackets. Only

517   bootstrap values ≥ 70 are reported.

518

519 **Figure S5.** Phylogenetic tree based on G gene nucleotide sequences of 8 British aMPV-B strains

520 detected in the present study, including the most commonly used subtype B vaccines (marked with

521 a green triangle) and 10 British aMPV -B strains, previously obtained or retrieved from GenBank.

522 The evolutionary history was inferred using the Neighbor-Joining method in MEGA X. All

523 nucleotide positions containing gaps and missing data were eliminated, there were a total of 302

524 positions in the final dataset. Only bootstrap values $\geq$ 70 are reported.

aMPV/B/Spain/Ck/14898/17
aMPV/B/Spain/Ck/17248/17
aMPV/B/Spain/Ck/13993/17
aMPV/B/Spain/Ck/7514/17
aMPV/B/Spain/Ck/1514/17
aMPV/B/Spain/Ck/12079/16
aMPV/B/Spain/Ck/1852/16
aMPV/B/Spain/Ck/11929/17
aMPV/B/Romania/Ck/1/16
aMPV/B/Romania/Ty/18/17
aMPV/B/Romania/Ty/62/17
aMPV/B/Romania/Ty/766/17
aMPV/B/Spain/Ck/3192/17
aMPV/B/Spain/Ck/1955-1/17
aMPV/B/Spain/Ck/10283-2/16
aMPV/B/Spain/Ty/1255/16
aMPV/B/Spain/Ty/2598/15
aMPV/B/Spain/Ck/1080-1/15
aMPV/B/Spain/Ty/11776/14
aMPV/B/Italy/Ck/86/14
aMPV/B/Italy/Ck/185/15
aMPV/B/Italy/Ck/248/15
aMPV/B/Italy/Ck/478/16
aMPV/B/Italy/Ty/626-10/16
aMPV/B/Italy/Ck/41/14
aMPV/B/Italy/Ck/1298/19
aMPV/B/Italy/Ck/376/15
aMPV/B/Italy/Ck/336/15
aMPV/B/Italy/Ty/1997/12
aMPV/B/Italy/GuineaFowl/1818/12
aMPV/B/Italy/Ty/2009/13
aMPV/B/Italy/Ck/569-03/15
aMPV/B/Italy/Ty/629-04/16
10aMPV Chicken Greece 2016
aMPV/B/Italy/Ck/500/16
aMPV/B/Italy/Ck/164/13
aMPV/B/Italy/Ty/1213/10
IT/Ty/531/10
31aMPV Chicken Greece 2016
33aMPV Chicken Greece 2016
IT/Ty/2a/01
IT/Ck/33a/02
IT/Ty/129-18/04
IT/Ck/1348-01/07
aMPV/B/Italy/Ty/628-19/16
IT/Ty/53/09
aMPV/B/Italy/Ty/629-16/16
IT/Ty/1077-02/09
Algeria/26/aMPVB/turkey

Vaccine (strain PL21), vaccine-derived strains, and older strains

Vaccines (strains VCO3, 11/94, 1062), vaccine-derived strains, and older strains

Isr/1708/02 (israel Turkey)
aMPV/B/Spain/Ty/470/15
aMPV/B/Spain/Ck/6881/15
aMPV/B/Spain/Ck/9615/14
aMPV/Chicken/IR/PCRLAB/HG/2017
PCRLAB/HG2013 (Iran Chicken 2013)
54/2011 (Iran Chicken 2011)
aMPV/Chicken/PCRLAB/HG/2010 (Iran)
aMPV/Chicken/IR/H1248-41-15R/2018
aMPV/Chicken/IR/H1248-41-15/2018
31/2011 (Iran Turkey 2011)
59/2011 (Iran Chicken 2011)
61/2011 (Iran Chicken 2011)
aMPV/Chicken/IR/H1248-9-15R/2016
aMPV/Chicken/IR/H1248-9-15/2016
aMPV/B/Russia/chicken/10/2008
2302 24 Chicken Turkey 2018
2311 3 Chicken Turkey 2018
2401 7 Chicken Turkey 2018
2302 13 Chicken Turkey 2018
aMPV/B/Ukraine/chicken/05/2009
aMPV/B/Russia/chicken/22/2010
aMPV/B/Russia/chicken/14/2009
aMPV/B/Russia/chicken/12/2010
GB 716/08
aMPV/B/France/Ck/1289-19/18
aMPV/B/France/Ty/1275/19
aMPV/B/France/Ty/1269-19/18
aMPV/B/France/Ty/1286-19/17
aMPV/B/France/Ty/1281-19/17
aMPV/B/France/GuineaFowl/1265-19/17
aMPV/B/France/Ty/839/17
aMPV/B/France/Ck/775/17
chicken/B/Brazil/25A-07/2007
chicken/B/Brazil/27A-07/2007
aMPV/B/Brazil-06/USP-07
aMPV/B/Brazil-05/USP-03
aMPV/B/Brazil-05/USP-04
aMPV/B/Brazil-05/USP-02
aMPV/B/Brazil-07/USP-05
aMPV/B/Brazil-07/USP-06
aMPV/B/Brazil-07/USP-08

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