



ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

ARCHIVIO ISTITUZIONALE DELLA RICERCA

Alma Mater Studiorum Università di Bologna Archivio istituzionale della ricerca

Marek's disease viruses circulating in commercial poultry in Italy in the years 2015–2018 are closely related by their meq gene phylogeny

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Mescolini G., Lupini C., Davidson I., Massi P., Tosi G., Catelli E. (2020). Marek's disease viruses circulating in commercial poultry in Italy in the years 2015–2018 are closely related by their meq gene phylogeny. *TRANSBOUNDARY AND EMERGING DISEASES*, 67(1), 98-107 [10.1111/tbed.13327].

Availability:

This version is available at: <https://hdl.handle.net/11585/726003> since: 2020-02-14

Published:

DOI: <http://doi.org/10.1111/tbed.13327>

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).
When citing, please refer to the published version.

(Article begins on next page)

This is the final peer-reviewed accepted manuscript of:

Mescolini G, Lupini C, Davidson I, Massi P, Tosi G, Catelli E. Marek's disease viruses circulating in commercial poultry in Italy in the years 2015-2018 are closely related by their meq gene phylogeny. *Transbound Emerg Dis.* 2020 Jan;67(1):98-107.

The final published version is available online at: <https://doi.org/10.1111/tbed.13327>

Rights / License:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)

When citing, please refer to the published version.

1 **Title**

2 **Marek's disease viruses circulating in commercial poultry in Italy in the years 2015-2018 are**
3 **closely related by their *meq* gene phylogeny.**

4

5 **Running Title**

6 **Virulence of GaHV-2 strains circulating in Italian commercial chickens**

7

8 Giulia Mescolini^{1,*}, Caterina Lupini¹, Irit Davidson², Paola Massi³, Giovanni Tosi³, Elena Catelli¹

9

10 ¹ Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra 50,
11 40064, Ozzano dell'Emilia (BO), Italy

12

13 ² Division of Avian Diseases, Kimron Veterinary Institute, Bet Dagan P.O. Box 12 5025001, Israel

14

15 ³ Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Sezione
16 Diagnostica di Forlì, Via don Eugenio Servadei 3/E-3/F, 47122, Forlì (FC), Italy

17

18 *Corresponding author: Giulia Mescolini, Department of Veterinary Medical Sciences -University
19 of Bologna, Via Tolara di Sopra, 50; 40064 – Ozzano dell'Emilia (BO) Italy; Phone +39 051

20 2097560; e-mail: giulia.mescolini3@unibo.it

21

22

23

24

25 **Summary**

26 Marek's disease (MD) is a lymphoproliferative disease important to the poultry industry worldwide;
27 it is caused by Gallid alphaherpesvirus 2 (GaHV-2). The virulence of the GaHV-2 isolate has
28 shifted over the years from mild to virulent, very virulent and very virulent +. Nowadays the disease
29 is controlled by vaccination, but field strains of increased virulence are emerging worldwide.
30 Economic losses due to MD are mostly associated with its acute form, characterised by visceral
31 lymphomas. The present study aimed to molecularly classify a group of 13 GaHV-2 strains detected
32 in vaccinated Italian commercial chicken flocks during acute MD outbreaks, and to scrutinise the
33 ability of predicting GaHV-2 virulence, according to the *meq* gene sequence. The full-length *meq*
34 genes were amplified and the obtained amino acid (aa) sequences were analysed, focusing mainly
35 on the number of stretches of four proline molecules (PPPP) within the transactivation domain.
36 Phylogenetic analysis was carried out with the Maximum Likelihood method using the obtained aa
37 sequences and the sequences of Italian strains detected in backyard flocks and of selected strains
38 retrieved from GenBank. All the analysed strains showed 100% sequence identity in the *meq* gene,
39 which encodes a Meq protein of 339 aa. The Meq protein includes four PPPP motifs in the
40 transactivation domain and an interruption of a PPPP motif due to a proline-to-serine substitution at
41 position 218. These features are typically encountered in highly virulent isolates. Phylogenetic
42 analysis revealed that the analysed strains belonged to a cluster that includes high-virulence GaHV-
43 2 strains detected in Italian backyard flocks and a hypervirulent Polish strain. Our results support
44 the hypothesis that the virulence of field isolates can be suggested by *meq* aa sequence analysis.

45

46 **Keywords:** commercial chickens; Italy; GaHV-2; *meq* gene; strain virulence

47

48

49 **Introduction**

50 Marek's disease (MD) is an economically-important lymphoproliferative disease to the poultry
51 industry worldwide due to its capacity to cause clinical disease, increased mortality and reduced
52 growth, as well as sub-clinical immunosuppression, causing the exacerbation of other diseases and
53 decreased vaccinal immunity (Schat & Nair, 2013). The virus belongs to the genus *Mardivirus*,
54 subfamily *Alphaherpesvirinae*. According to the most recent nomenclature, it consists of three viral
55 species: *Gallid alphaherpesvirus 2* (GaHV-2) (aetiological agent of MD), *Gallid alphaherpesvirus*
56 *3* (GaHV-3), and *Meleagrid alphaherpesvirus 1* (MeHV-1) or Herpesvirus of turkeys (HVT)
57 (International Committee on Taxonomy of Viruses, 2017). GaHV-3 and HVT are antigenically
58 related to GaHV-2 and are widely used as vaccines, usually in association with live attenuated
59 GaHV-2 strains (e.g. CVI988/Rispens). In the field, economic losses due to MD are mostly
60 associated with the acute form of the disease, characterised by visceral lymphomas, however virus-
61 induced immunosuppression might be relevant too, even if its impact is very difficult to assess
62 (Gimeno, 2014; Gimeno & Schat, 2018).

63 The virulence of GaHV-2 isolates has shifted over the years from mild (m) to virulent (v), very
64 virulent (vv) and very virulent + (vv+) (Witter, 1997; Witter et al., 2005). The disease is controlled
65 by vaccination, but field GaHV-2 strains with increased virulence and greater fitness are emerging
66 worldwide (Trimpert et al., 2017; Nair, 2018). Complex factors might be involved in the occurrence
67 of MD outbreaks in vaccinated chicken flocks, such as: the increased virulence of GaHV-2 over
68 time (Witter, 1997); the inability of the vaccine to offer protection; non-optimal vaccine application,
69 due to its labile cell-associated form (Davidson et al., 2018; Davidson, Natour-Altory, & Shimshon,
70 2018); co-infection with immunosuppressive viruses (Schat & van Santen, 2013); and other factors.

71 The GaHV-2 genome encodes more than 200 genes, including the *meq* gene, which was the first
72 discovered GaHV-2 oncogene (Jones et al., 1992). The *meq* gene encodes a protein with homology
73 to the leucine-zipper class nuclear oncogenes, which is composed of a trans-activation N-terminal

74 basic-leucine zipper (bZIP) domain and a C-terminal proline-rich trans-repression domain (Qian et
75 al., 1995; Liu et al., 1999; Ross, 1999). The oncogenic activities of the Meq protein are mediated by
76 its dimerisation, through the bZIP domain, with itself, as well as with c-Jun-like proteins, such as
77 JunB, c-Jun and c-Fos. Meq also binds to cellular transcription factors such as ATF, CREB and
78 C/EBP (Deng et al., 2010) and interacts with cellular proteins without a bZIP domain, such as the
79 cellular tumour suppressors p53, the retinoblastoma protein (pRb) and the cyclin-dependent kinase
80 2 (CDK-2) or the heat shock protein Hsp70 (Deng et al., 2010; Gennart et al., 2015). The *meq*
81 oncogene encodes a 339 amino acid unspliced open reading frame in vv and in vv+ GaHV-2
82 pathotypes and a larger form of 398 amino acids in low virulence strains, having amplifications in
83 the C-terminal proline-rich repeat region (Shamblin et al., 2004).

84 Concurrently with the stepwise evolution of the virulence of GaHV-2 (Schat & Baranowski, 2007),
85 an increased pattern of genetic polymorphism at the C-terminus domain of the *meq*-encoded
86 oncoprotein has been described (Shamblin et al., 2004). High genetic diversity has been reported for
87 the *meq* gene in spite of the relatively low evolutionary rates of change thought to commonly
88 characterise dsDNA viruses (Duffy, Shackelton, & Holmes, 2008; Firth et al., 2010). Findings by
89 Padhi & Parcels (2016) and Trimpert et al. (2017) reveal that the *meq* gene sequence evolves at a
90 much faster rate than most dsDNA viruses, and is comparable with the evolutionary rate of RNA
91 viruses. By analysing the complete *meq* gene sequence of 84 GaHV-2 strains, Padhi & Parcels
92 (2016) estimated the mean evolutionary rate, beginning from the year 1935, to be greater than other
93 dsDNA viruses, namely 1.02×10^{-4} substitutions per site per year, as compared to the range of 10^{-7}
94 to 10^{-5} for other DNA viruses. Trimpert et al. (2017) analysed 18 complete GaHV-2 genomes and
95 calculated that the GaHV-2 had a mean evolutionary rate of 1.58×10^{-5} substitutions per site per
96 year, in which the *meq* open reading frame (MDV076) was identified as one of the loci harbouring
97 the highest number of point mutations over time. The *meq* gene is evolving under positive selection,
98 most likely imposed by vaccination, reflecting viral adaptation against the host immune responses.

99 Shamblin et al. (2004) and Renz et al. (2012) demonstrated that the number of the four-proline
100 stretches (PPPP) in the *meq* gene transactivation domain are an indicative marker for the
101 pathogenicity of GaHV-2 strains isolated from chickens; the most virulent isolates showed the
102 lowest number of PPPP repeats, unlike the attenuated and the low pathogenicity isolates, which
103 showed a highest number of repeats. The determination of GaHV-2 virulence by molecular
104 sequencing could be valuable compared to the *in vivo* pathotyping assays, which require complex
105 experimental infection trials of specific genetic lines of chickens (Witter et al., 2005; Dudnikova et
106 al., 2007). At the moment *in vivo* studies are mandatory for an accurate inclusion of GaHV-2 strains
107 into one of the known pathotypes. The *meq* gene polymorphism is also useful to create
108 epidemiological molecular linkages between various GaHV-2 strains, according to the numerous
109 studies that have been recently published from various countries: China (Tian et al., 2011; Zhang et
110 al., 2011; Yu et al., 2013), Poland (Woźniakowski & Samorek-Salamonowicz, 2014), U.S.A. (Padhi
111 & Parcell, 2016), Colombia (López-Osorio et al., 2017), Egypt (Hassanin, Abdallah, & El-Araby,
112 2013; Abdallah et al., 2018), India (Gupta, Deka, & Ramneek, 2016; Suresh et al., 2017; Prathibha,
113 Sreedevi, Vinod Kumar, & Srilatha, 2018) and Japan (Abd-Ellatieff et al., 2018).

114 In the present study, we aimed to molecularly classify a group of 13 GaHV-2 strains, detected
115 during acute MD outbreaks in vaccinated Italian commercial flocks, according to the *meq* gene
116 sequence. We also aimed to scrutinise the ability of suggesting GaHV-2 virulence according to the
117 *meq* gene sequence.

118

119 **Materials and Methods**

120 **Commercial flocks and sampling**

121 MD outbreaks occurred between 2015 and 2018 in 13 commercial chicken flocks located in 6
122 different Italian regions; flock specifics are reported in Table 1. The chicken flocks were different in

123 terms of production type, genetic line and age. MD vaccination status was known for 10 out of 13
124 flocks. Flocks were vaccinated with the association of CVI988/Rispens and HVT vaccines. The
125 vaccination of broiler breeders was performed *in ovo* and repeated at 1 or 7 days of age. Cockerels
126 were vaccinated with the association of CVI988/Rispens +HVT at 1 day old. All the examined
127 flocks experienced acute MD with an increased mortality rate due to visceral lymphomas. At
128 necropsy, portions of the spleen, liver or ovary, showing gross lymphomatous lesions, were
129 sampled and stored at -20 °C until analysis.

130 **Genomic DNA extraction**

131 Genomic DNA was extracted separately from lymphomatous liver, spleen or ovary using the
132 commercial kit “NucleoSpin® Tissue” (MACHEREY-NAGEL GmbH & Co. KG, Düren,
133 Germany), following the manufacturer’s instructions.

134 **Amplification and sequencing of the *meq* gene**

135 The entire *meq* gene was amplified and sequenced as previously described (Mescolini et al., 2019).

136 **Sequence and phylogenetic analysis of the *meq* gene**

137 The obtained nucleotide (nt) sequences were edited using BioEdit Sequence Alignment Editor
138 Version 7.0.5.3 (Tom Hall, Ibis Therapeutics, Carlsbad, California, USA). Similarities between
139 Italian sequences and *meq* gene sequences available in the NCBI database were investigated
140 through the Basic Local Alignment Search Tool (BLAST).

141 The obtained sequences were aligned against and compared with previously published complete
142 *meq* gene sequences of Italian strains detected in backyard flocks and with 57 selected complete
143 *meq* gene sequences retrieved from GenBank (Table 2), using Clustal W software (Thompson,
144 Higgins, & Gibson, 1994). Deduced amino acid (aa) sequences were analysed focusing on the
145 number of PPPPs within the proline-rich repeats (PRRs) of the transactivation domain and on the
146 presence of aa substitutions. A phylogenetic tree, based on *meq* gene aa sequences, was built using

147 the Maximum Likelihood method under the Jones–Taylor–Thornton model in MEGA version X
148 (Kumar et al., 2018). Nodal supports were estimated with 1000 bootstrap replicates and considered
149 significant when equal to or greater than 70.

150

151 **Results**

152 Thirteen GaHV-2 strains were detected by the specific PCR protocol in as many investigated
153 chicken flocks. The *meq* genes of the detected strains were genetically identical, being 1020 bp in
154 length; the obtained sequences were deposited into GenBank under the following names and
155 accession numbers: GaHV-2/Italy/Ck/456/15 - MK855054; GaHV-2/Italy/Ck/498/15 - MK855055;
156 GaHV-2/Italy/Ck/513/15 - MK855056; GaHV-2/Italy/Ck/515/15 - MK855057; GaHV-
157 2/Italy/Ck/559/15 - MK855058; GaHV-2/Italy/Ck/561/15 - MK855059; GaHV-2/Italy/Ck/565/15 -
158 MK855060; GaHV-2/Italy/Ck/567/15 - MK855061; GaHV-2/Italy/Ck/757/17 - MK855062; GaHV-
159 2/Italy/Ck/875/18 - MK855063; GaHV-2/Italy/Ck/876/18 - MK855064; GaHV-2/Italy/Ck/921/18 -
160 MK855065; GaHV-2/Italy/Ck/1083/18 - MK855066.

161 The molecular characteristics of the deduced Meq protein, compared to prototype strains CVI988,
162 CU-2, JM/102W, Md5 and 648A, are presented in Table 3. All the currently-detected-strains
163 showed a Meq protein of 339 aa that contained, in the transactivation domain, four PPPP motifs
164 and, at position 218, a proline-to-serine substitution, interrupting a hypothetical PPPP sequence at
165 the third position (PPPP → PPSP). Distinctive aa substitutions were found in the Italian GaHV-2
166 strains at positions 110 (C110S) and 218 (P218S).

167 The BLAST search showed 100% homology of the currently detected *meq* gene sequences with
168 those of the 8 Italian GaHV-2 strains (GaHV-2/Italy/Ck/625/16; GaHV-2/Italy/Ck/689/16; GaHV-
169 2/Italy/Ck/722/16; GaHV-2/Italy/Ck/801/17; GaHV-2/Italy/Ck/810/17; GaHV-2/Italy/Ck/852/17;
170 GaHV-2/Italy/Ck/853/17; GaHV-2/Italy/Ck/854/17) detected between 2016 and 2017 in Italian

171 backyard flocks that experienced acute MD, transient paralysis or sudden death, and with one of a
172 Polish strain named Polen5 (Table 2). The phylogenetic tree (Figure 1) confirmed the previously
173 reported findings, revealing that the strains detected in the present study clustered together with the
174 Italian strains detected in backyard chickens, and with the Polish strain.

175

176 **Discussion**

177 The present study reports the sequence analysis of the *meq* gene of 13 GaHV-2 strains detected in
178 several cases of MD-related visceral tumours that occurred in Italian vaccinated commercial
179 chicken flocks collected during the years 2015–2018. Surprisingly, all the *meq* gene sequences of
180 the analysed strains were identical, despite the fact that viruses were detected in flocks differing in
181 terms of production type (broiler breeders, layers or cockerels), geographical location and
182 ownership. The viruses coded for Meq proteins of 339 aa possessing features typically encountered
183 in highly virulent isolates (Shamblin et al., 2004; Renz et al., 2012) and were phylogenetically
184 related to the GaHV-2 strains currently circulating in Italian backyard flocks (Mescolini et al.,
185 2019) and with a hypervirulent strain isolated in Poland in 2010 (Trimpert et al., 2017). Common
186 trade routes may have hypothetically served as a source of dissemination for GaHV-2 between
187 European countries and between industrial and rural compartments, where biosecurity breaches may
188 have also occurred.

189 Our findings are in accordance with previous studies that reported the geographically-restricted
190 evolution of field GaHV-2 strains in China (Yu et al., 2013), India (Suresh et al., 2017), Egypt
191 (Abdallah et al., 2018) and Poland (Woźniakowski & Samorek-Salamonowicz, 2014). Recently, a
192 comprehensive time-scaled phylogeny study, performed on complete genomes, revealed evidence
193 of the geographical structuring of GaHV-2 strains, supporting the emergence of virulent viruses
194 independently in North America and Eurasia (Trimpert et al., 2017).

195 Although GaVH-2, as a dsDNA virus, was foreseen to possess high genetic stability to mutations,
196 unexpectedly, its *meq* gene sequence is mutating at a high evolutionary rate, namely 10^{-4}
197 substitutions per site per year (Padhi & Parcells, 2016). The GaHV-2 *meq* gene evolutionary rate is
198 typical for highly mutating RNA viruses, ranging between 10^{-2} to 10^{-5} substitutions per site per
199 year; the avian influenza virus, subgroup H9N2 mutate as an example, with a value of 6.1×10^{-3}
200 substitutions per site per year (Davidson et al., 2014). High evolutionary rates reflect the strong
201 positive selection that exists for GaHV-2 in commercial flocks, probably resulting from the
202 vaccination with highly effective, but imperfect vaccines that are increasing viral diversity (Padhi &
203 Parcells, 2016). The fitness and replication of highly virulent strains seems to be favoured in
204 vaccinated flocks (Read et al., 2015), in which strains able to avoid vaccine-induced protection
205 could be selected.

206 There is a growing need for new MD vaccines that are efficacious against currently circulating
207 viruses, due to the occurrence of breaks in vaccine immunity. Since vaccine failures are occurring
208 worldwide (López-Osorio et al., 2017; Sun et al., 2017; Abdallah et al., 2018; Abd-Elattieff et al.,
209 2018), more studies must be conducted to evaluate the protection conferred by Rispens-type
210 vaccines against more recent, and not yet pathotyped, field strains with a history of high virulence.
211 Naturally circulating low virulence strains could represent a solution if they offer improved
212 protection over CVI988/Rispens vaccine.

213 The rapid increase in the sequencing activities of the *meq* gene of field GaHV-2 strains all over the
214 world has made possible to epidemiologically correlate a significant number of molecular data. The
215 *meq* gene aa sequence has been correlated to GaHV-2 strains virulence (Shamblin et al., 2004; Renz
216 et al., 2012). Our data support this last finding, having our strains molecular features of high
217 virulence and having been detected during severe MD outbreaks in vaccinated chickens.

218 *Meq* gene sequencing alone is known to be an insufficient method to include field strains into
219 defined pathotypes, therefore, recently, the research is focusing on finding other genetic predictors

220 of GaHV-2 virulence using complete or targeted DNA sequencing (Dunn et al., 2019). This will
221 provide a highly advantageous alternative to the classical “gold standard” method of *in vivo*
222 pathotyping (Witter et al., 2005), which requires the experimental infection of a large number of a
223 specific type of chickens with standard GaHV-2 prototype strains. As that pathotype classification
224 assay is difficult and not feasible worldwide, Dudnikova et al. (2007) developed an alternative “best
225 fit” pathotyping assay. Although the “best fit” pathotyping assay is simplified, it also employs long-
226 term trials using specific pathogen free chicks. *In vivo* pathotyping assays are not generally
227 accessible, as those experimental infections require the use of chicks vaccinated at 1 day of age,
228 challenged with virulent isolates and housed in poultry isolation units for 56 days post challenge.
229 As *in vivo* pathotyping is not easily achievable, the *meq* gene molecular characterisation would be
230 the most rapid and accessible way to suggest virulence of field strains. However, the molecular
231 findings should be supported by clinical observations, necropsy findings and vaccination status.

232

233 **Conflict of Interest Statement**

234 The authors declare no conflict of interest.

235

236 **Ethical Statement**

237 Ethical statement is not applicable.

238

239 **References**

240 Abd-Ellatieff, H. A., Abou Rawash, A. A., Ellakany, H. F., Goda, W. M., Suzuki, T., & Yanai, T.
241 (2018). Molecular characterization and phylogenetic analysis of a virulent Marek’s disease
242 virus field strain in broiler chickens in Japan. *Avian Pathology*, 47(1), 47–57.

243 <https://doi.org/10.1080/03079457.2017.1362497>

244 Abdallah, F., Hassanin, O., Attar, E., Ali, H., Megahed, M., & Nair, V. (2018). Marek's Disease
245 Virus in Egypt: Historical Overview and Current Research Based on the Major MDV-Encoded
246 Oncogene Meq. *Hosts and Viruses*, 5, 35–43.
247 <http://dx.doi.org/10.17582/journal.hv/2018/5.3.35.43>

248 Davidson, I., Fusaro, A., Heidari, A., Monne, I., & Cattoli, G. (2014). Molecular evolution of H9N2
249 avian influenza viruses in Israel. *Virus Genes*, 48(3), 457–463. [https://doi.org/10.1007/s11262-](https://doi.org/10.1007/s11262-014-1037-0)
250 [014-1037-0](https://doi.org/10.1007/s11262-014-1037-0)

251 Davidson, I., Natour-Altory, A., Raibstein, I., Kin, E., Dahan, Y., Krispin, H., & Elkin, N. (2018).
252 Monitoring the uptake of live avian vaccines by their detection in feathers. *Vaccine*, 36, 637–
253 643. <https://doi.org/10.1016/j.vaccine.2017.12.052>

254 Davidson, I., Natour-Altory, N., & Shimshon Y. (2018). Evaluation of Live Vaccine Application in
255 Commercial Poultry Flocks Using Feathers – In Practice. *Israel Journal of Veterinary*
256 *Medicine*, 73, 8–13.

257 Deng, X., Li, X., Shen, Y., Qiu, Y., Shi, Z., Shao, D., Jin, Y., Chen, H., Ding, C., LiLi, Chen, P., &
258 Ma, Z. (2010). The Meq oncoprotein of Marek's disease virus interacts with p53 and inhibits
259 its transcriptional and apoptotic activities. *Virology Journal*, 7: 348.
260 <https://doi.org/10.1186/1743-422X-7-348>

261 Dudnikova, E., Norkina, S., Vlasov, A., Slobodchuk, A., Lee, L. F., & Witter, R. L. (2007).
262 Evaluation of Marek's disease field isolates by the “best fit” pathotyping assay. *Avian*
263 *Pathology*, 36(2), 135–143. <https://doi.org/10.1080/03079450701209857>

264 Duffy, S., Shackelton, L. A., & Holmes, E. C. (2008). Rates of evolutionary change in viruses:
265 Patterns and determinants. *Nature Reviews Genetics*, 9, 267–276.
266 <https://doi.org/10.1038/nrg2323>

267 Dunn, J.R., Black Pyrkosz, A., Steep, A. & Cheng, H.H. (2019). Identification of Marek's disease
268 virus genes associated with virulence of US strains. *Journal of General Virology*, 100, 1132-
269 1139. <https://doi.org/10.1099/jgv.0.001288>

270 Firth, C., Kitchen, A., Shapiro, B., Suchard, M. A., Holmes, E. C., & Rambaut, A. (2010). Using
271 time-structured data to estimate evolutionary rates of double-stranded DNA viruses. *Molecular*
272 *Biology and Evolution*, 27(9), 2038–2051. <https://doi.org/10.1093/molbev/msq088>

273 Gennart, I., Coupeau, D., Pejaković, S., Laurent, S., Rasschaert, D., & Muylkens, B. (2015).
274 Marek's disease: Genetic regulation of gallid herpesvirus 2 infection and latency. *Veterinary*
275 *Journal*, 205, 339–348. <https://doi.org/10.1016/j.tvjl.2015.04.038>

276 Gimeno, I. M. (2014). Marek's Disease and Differential Diagnosis with Other Tumor Viral
277 Diseases of Poultry. In *Encyclopedia of Agriculture and Food Systems* (pp. 156–171).
278 <https://doi.org/https://doi.org/10.1016/B978-0-444-52512-3.00193-5>

279 Gimeno, I.M., & Schat K.A. (2018). Virus-Induced Immunosuppression in Chickens. *Avian*
280 *Diseases*, 62, 272–285. <https://doi.org/10.1637/11841-041318-Review.1>

281 Gupta, M., Deka, D., & Ramneek. (2016). Sequence analysis of Meq oncogene among Indian
282 isolates of Marek's disease herpesvirus. *Meta Gene*, 9, 230–236.
283 <https://doi.org/10.1016/j.mgene.2016.07.009>

284 Hassanin, O., Abdallah, F., & El-Araby, I. E. (2013). Molecular characterization and phylogenetic
285 analysis of Marek's disease virus from clinical cases of Marek's disease in Egypt. *Avian*
286 *Diseases*, 57(Suppl 2), 555–561. <https://doi.org/10.1637/10337-082912-Reg.1>

287 International Committee on Taxonomy of Viruses, 2017. Master Species List 2017 [WWW
288 Document]. ICTV. Retrieved from <https://talk.ictvonline.org/files/master-species->
289 [lists/m/msl/7185](https://talk.ictvonline.org/files/master-species-lists/m/msl/7185)

290 Jones, D., Lee, L., Liu, J.L., Kung H. J., & Tillotson, J. K. (1992). Marek's disease virus encodes a

291 basic-leucine zipper gene resembling the fos/jun oncogenes that is highly expressed in
292 lymphoblastoid tumors. *Proceedings of the National Academy of Sciences*, 89, 4042–4046.
293 <https://doi.org/10.1073/pnas.89.9.4042>

294 Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary
295 genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35(6), 1547–
296 1549. <https://doi.org/10.1093/molbev/msy096>

297 Liu, J. L., Lin, S. F., Xia, L., Brunovskis, P., Li, D., Davidson, I., Lee, L.F., & Kung, H. J. (1999).
298 Meq and V-IL8: Cellular genes in disguise? *Acta Virologica*, 43, 94–101.

299 López-Osorio, S., Piedrahita, D., Espinal-Restrepo, M. A., Ramírez-Nieto, G. C., Nair, V.,
300 Williams, S. M., Baigent, S., Ventura-Polite, C., Aranzazu-Taborda, D.A., & Chaparro-
301 Gutiérrez, J. J. (2017). Molecular characterization of Marek's disease virus in a poultry layer
302 farm from Colombia. *Poultry Science*, 96(6), 1598–1608.
303 <https://doi.org/https://doi.org/10.3382/ps/pew464>

304 Mescolini, G., Lupini, C., Felice, V., Guerrini, A., Silveira, F., Cecchinato, M., & Catelli, E. (2019).
305 Molecular characterization of the meq gene of Marek's disease viruses detected in
306 unvaccinated backyard chickens reveals the circulation of low- and high-virulence strains.
307 *Poultry Science*, pii: pez095. doi: 10.3382/ps/pez095. [Epub ahead of print]

308 Nair, V. (2018). Spotlight on avian pathology: Marek's disease. *Avian Pathology*, 47, 440–442.
309 <https://doi.org/10.1080/03079457.2018.1484073>

310 Padhi, A., & Parcels, M. S. (2016). Positive selection drives rapid evolution of the meq oncogene
311 of Marek's disease virus. *PLoS ONE*, 11(9). <https://doi.org/10.1371/journal.pone.0162180>

312 Prathibha, Y., Sreedevi, B., Vinod Kumar, N., & Srilatha, C. (2018). Molecular characterization and
313 phylogenetic analysis of oncogenes from virulent serotype-1 Marek's disease virus in India.
314 *Acta Virologica*, 62(3), 277–286. https://doi.org/10.4149/av_2018_221

- 315 Qian, Z., Brunovskis, P., Rauscher 3rd, F., Lee, L., & Kung, H. J. (1995). Transactivation activity
316 of Meq, a Marek's disease herpesvirus bZIP protein persistently expressed in latently infected
317 transformed T cells. *Journal of Virology*, 69(7), 4037–4044.
318 <http://www.ncbi.nlm.nih.gov/pubmed/7769661>
- 319 Read, A. F., Baigent, S. J., Powers, C., Kgosana, L. B., Blackwell, L., Smith, L. P., Kennedy, D.A.,
320 Walkden-Brown, S.W., & Nair, V. K. (2015). Imperfect Vaccination Can Enhance the
321 Transmission of Highly Virulent Pathogens. *PLOS Biology*, 13(7), e1002198.
322 <https://doi.org/10.1371/journal.pbio.1002198>
- 323 Renz, K. G., Cooke, J., Clarke, N., Cheetham, B. F., Hussain, Z., Fakhru Islam, A. F. M., Tannock,
324 G.A., & Walkden-Brown, S. W. (2012). Pathotyping of Australian isolates of Marek's disease
325 virus and association of pathogenicity with meq gene polymorphism. *Avian Pathology*, 41(2),
326 161–176. <https://doi.org/10.1080/03079457.2012.656077>
- 327 Ross, N. L. J. (1999). T-cell transformation by Marek's disease virus. *Trends in Microbiology*, 7,
328 22–29. [https://doi.org/10.1016/S0966-842X\(98\)01427-9](https://doi.org/10.1016/S0966-842X(98)01427-9)
- 329 Schat, K. A., & Baranowski, E. (2007). Animal vaccination and the evolution of viral pathogens.
330 *Revue Scientifique Et Technique-Office International Des Epizooties*, 26(2), 327–338.
- 331 Schat, K.A., & Nair V. (2013). Neoplastic diseases: Marek's disease. In D. E. Swayne, J. R.
332 Glisson, L. R. McDougald, L. K. Nolan, D. L. Suarez, V. L. Nair, (Eds.) *Diseases of Poultry*
333 (13th ed., pp. 515–552). Ames, Iowa, USA: Wiley-Blackwell Hoboken.
- 334 Schat, K.A., & van Santen, V.L. (2013). Chicken infectious anemia. In D. E. Swayne, J. R. Glisson,
335 L. R. McDougald, L. K. Nolan, D. L. Suarez, V. L. Nair (Eds.) *Diseases of Poultry* (13th ed.,
336 pp. 248–275). Ames, Iowa, USA: Wiley-Blackwell Hoboken.
- 337 Shamblin, C. E., Greene, N., Arumugaswami, V., Dienglewicz, R. L., & Parcels, M. S. (2004).
338 Comparative analysis of Marek's disease virus (MDV) glycoprotein-, lytic antigen pp38- and

339 transformation antigen Meq-encoding genes: Association of meq mutations with MDVs of
340 high virulence. *Veterinary Microbiology*, 102(3–4), 147–167.
341 <https://doi.org/10.1016/j.vetmic.2004.06.007>

342 Spatz, S. J., Petherbridge, L., Zhao, Y., & Nair, V. (2007). Comparative full-length sequence
343 analysis of oncogenic and vaccine (Rispens) strains of Marek’s disease virus. *Journal of*
344 *General Virology*, 88(4), 1080–1096. <https://doi.org/10.1099/vir.0.82600-0>

345 Spatz, S.J., & Silva, R.F. (2007). Polymorphisms in the repeat long regions of oncogenic and
346 attenuated pathotypes of Marek’s disease virus 1. *Virus Genes*, 35, 41–53.
347 [doi:10.1007/s11262-006-0024-5](https://doi.org/10.1007/s11262-006-0024-5)

348 Spatz, S.J., Smith, L.P., Baigent, S.J., Petherbridge, L., & Nair, V. (2011). Genotypic
349 characterization of two bacterial artificial chromosome clones derived from a single DNA
350 source of the very virulent gallid herpesvirus-2 strain C12/130. *Journal of General Virology*,
351 92, 1500–1507. [doi:10.1099/vir.0.027706-0](https://doi.org/10.1099/vir.0.027706-0)

352 Sun, G. R., Zhang, Y. P., Lv, H. C., Zhou, L. Y., Cui, H. Y., Gao, Y. L., Qi, X. Le, Wang, Y. Q., Li,
353 K., Gao, L., Pan, Q., Wang, X. M., & Liu, C. J. (2017). A Chinese variant Marek’s disease
354 virus strain with divergence between virulence and vaccine resistance. *Viruses* 9, 71.
355 <https://doi.org/10.3390/v9040071>

356 Suresh, P., Johnson Rajeswar, J., Sukumar, K., Harikrishnan, T. J., & Srinivasan, P. (2017).
357 Complete nucleotide sequence analysis of the oncogene “Meq” from serotype 1 Marek’s
358 disease virus isolates from India. *British Poultry Science*, 58, 111–115.
359 <https://doi.org/10.1080/00071668.2016.1257780>

360 Teng, L. Q., Wei, P., Song, Z. B., He, J. J., & Cui, Z. Z. (2011). Molecular epidemiological
361 investigation of Marek’s disease virus from Guangxi, China. *Archives of Virology*, 156, 203–
362 206. [doi:10.1007/s00705-010-0840-8](https://doi.org/10.1007/s00705-010-0840-8)

- 363 Thompson, J.D., Higgins, D.G., & Gibson, T.J. (1994). CLUSTAL W: improving the sensitivity of
364 progressive multiple sequence alignment through sequence weighting, position-specific gap
365 penalties and weight matrix choice. *Nucleic Acids Research*, 22, 4673–4680. doi:
366 10.1093/nar/22.22.4673
- 367 Tian, M., Zhao, Y., Lin, Y., Zou, N., Liu, C., Liu, P., Cao, S., Wen, X., & Huang, Y. (2011).
368 Comparative analysis of oncogenic genes revealed unique evolutionary features of field
369 Marek's disease virus prevalent in recent years in China. *Virology Journal*, 8, 121.
370 <https://doi.org/10.1186/1743-422X-8-121>
- 371 Trimpert, J., Groenke, N., Jenckel, M., He, S., Kunec, D., Szpara, M. L., Spatz, S. J., Osterrieder,
372 N., & McMahon, D. P. (2017). A phylogenomic analysis of Marek's disease virus reveals
373 independent paths to virulence in Eurasia and North America. *Evolutionary Applications*,
374 10(10), 1091–1101. <https://doi.org/10.1111/eva.12515>
- 375 Tulman, E. R., Afonso, C. L., Lu, Z., Zsak, L., Rock, D. L., & Kutish, G. F. (2000). The Genome of
376 a Very Virulent Marek's Disease Virus. *Journal of Virology*, 74, 7980–7988.
377 doi:10.1128/jvi.74.17.7980-7988.2000
- 378 Witter, R. L. (1997). Increased Virulence of Marek's Disease Virus Field Isolates. *Avian Diseases*,
379 41(1), 149. <https://doi.org/10.2307/1592455>
- 380 Witter, R. L., Calnek, B. W., Buscaglia, C., Gimeno, I. M., & Schat, K. A. (2005). Classification of
381 Marek's disease viruses according to pathotype: Philosophy and methodology. *Avian*
382 *Pathology*, 34, 75–90. <https://doi.org/10.1080/03079450500059255>
- 383 Woźniakowski, G., & Samorek-Salamonowicz, E. (2014). Molecular Evolution of Marek's Disease
384 Virus (MDV) Field Strains in a 40-Year Time Period. *Avian Diseases*, 58(4), 550–557.
385 <https://doi.org/10.1637/10812-030614-Reg.1>
- 386 Yu, Z.-H., Man, T., Lou, J., Wang, X.-W., Ding, K., Yu, L.-L., Su, J.-W., Chi, J.-Q., Zhao, P., Hu, B.,

- 387 Zhang, G.-P., & Liu, J.-X. (2013). Molecular characteristics and evolutionary analysis of field
388 Marek's disease virus prevalent in vaccinated chicken flocks in recent years in China. *Virus*
389 *Genes*, 47, 282–291. doi: 10.1007/s11262-013-0942-y
- 390 Zhang, F., Liu, C. J., Zhang, Y. P., Li, Z. J., Liu, A. L., Yan, F. H., Cong, F., & Cheng, Y. (2012).
391 Comparative full-length sequence analysis of Marek's disease virus vaccine strain 814.
392 *Archives of Virology*, 157, 177–183. doi:10.1007/s00705-011-1131-8
- 393 Zhang, Y. P., Liu, C. J., Zhang, F., Shi, W., & Li, J. (2011). Sequence analysis of the meq gene in
394 the predominant Marek's disease virus strains isolated in China during 2006-2008. *Virus*
395 *Genes*, 43(3), 353–357. <https://doi.org/10.1007/s11262-011-0645-1>

Table 1. Description of Italian commercial flocks affected by acute Marek's Disease and their vaccination status.

Flock ID	Region of origin	Year	Production type	Genetic line	Age (weeks)	Vaccine strain	1 st vaccination	2 nd vaccination	MD form
456/15	Emilia-Romagna	2015	Broiler breeders	Ross 708	NA [†]	CVI988+HVT	<i>In ovo</i>	1 day	Acute
498/15	Emilia-Romagna	2015	Broiler breeders	NA	NA	NA	NA	NA	Acute
513/15	Emilia-Romagna	2015	Layers	Hy-Line	31	NA	NA	NA	Acute
515/15	Emilia-Romagna	2015	Layers	NA	NA	NA	NA	NA	Acute
559/15	Veneto	2015	Broiler breeders	Ross 308	41	CVI988+HVT	<i>In ovo</i>	1 day	Acute
561/15	Friuli-Venezia Giulia	2015	Broiler breeders	Ross 708	56	CVI988+HVT	<i>In ovo</i>	1 day	Acute
565/15	Veneto	2015	Broiler breeders	Ross 708	36	CVI988+HVT	<i>In ovo</i>	1 day	Acute
567/15	Marche	2015	Cockerels	Hy-Line	11	CVI988+HVT	1 day	- [‡]	Acute
757/17	Emilia-Romagna	2017	Broiler breeders	Ross 308	51	CVI988+HVT	<i>In ovo</i>	1 day	Acute
875/18	Emilia-Romagna	2018	Broiler breeders	Ross 308	40	CVI988+HVT	<i>In ovo</i>	7 days	Acute
876/18	Emilia-Romagna	2018	Broiler breeders	Ross 308	31	CVI988+HVT	<i>In ovo</i>	7 days	Acute
921/18	Abruzzo	2018	Broiler breeders	Ross 308	27	CVI988+HVT	<i>In ovo</i>	1 day	Acute
1083/18	Tuscany	2018	Cockerels	Hy-Line	10	CVI988+HVT	1 day	-	Acute

[†] Not available[‡] Not performed

396

397

398

399

400

Table 2. Details of the GaHV-2 strains, retrieved from GenBank, which were used for the phylogenetic analysis.

GAHV-2 strain	Country	Year	Pathotype	Size of Meq (aa)	PPPPs (N°)	GenBank Accession N°	Reference
CVI988	Netherlands	1969	att	398	7	DQ530348	Spatz et al., 2007
814	China	1986	att	398	7	JF742597	Zhang et al., 2012
3004	Russia	NA [†]	att	398	7	EU032468	NA
CU-2	USA	1970s	m	398	7	AY362708	Shamblin et al., 2004
MD70/13	Hungary	1970	v	339	5	MF431495	Trimpert et al., 2017
571	USA	1989	v	339	3	AY362710	Shamblin et al., 2004
617A	USA	1993	v	339	4	AY362712	Shamblin et al., 2004
MPF57	Australia	1994	v	398	5	EF523774	Renz et al., 2012
04CRE	Australia	2004	v	398	5	EF523773	Renz et al., 2012
573	USA	NA	v	339	4	AY362711	Shamblin et al., 2004
567	USA	NA	v	339	4	AY362709	Shamblin et al., 2004
637	USA	NA	v	339	4	AY362713	Shamblin et al., 2004
BC-1	USA	NA	v	398	7	AY362707	Shamblin et al., 2004
JM	USA	NA	v	398	7	AY243331	Shamblin et al., 2004
JM/102W	USA	NA	v	399	7	DQ534539	Spatz & Silva, 2007
Md5	USA	1977	vv	339	4	AF243438	Tulman et al., 2000
549	USA	1987	vv	339	2	AY362714	Shamblin et al., 2004
595	USA	1991	vv	339	2	AY362715	Shamblin et al., 2004
C12/130	UK	1992	vv	339	5	FJ436096	Spatz et al., 2011
Woodlands1	Australia	1992	vv	399	5	EF523775	Renz et al., 2012
643P	USA	1994	vv	339	2	AY362716	Shamblin et al., 2004
02LAR	Australia	2002	vv	398	5	EF523772	Renz et al., 2012
FT158	Australia	2002	vv	398	5	EF523771	Renz et al., 2012
RB1B	USA	NA	vv	339	5	AY243332	Shamblin et al., 2004
648A	USA	1994	vv+	339	2	AY362725	Shamblin et al., 2004
New	USA	1999	vv+	339	2	AY362719	Shamblin et al., 2004

W	USA	1999	vv+	339	4	AY362723	Shamblin et al., 2004
ATE2539	Hungary	2000	vv+	339	5	MF431493	Trimpert et al., 2017
660-A	USA	NA	vv+	339	2	AY362726	Shamblin et al., 2004
686	USA	NA	vv+	339	2	AY362727	Shamblin et al., 2004
L	USA	NA	vv+	339	2	AY362717	Shamblin et al., 2004
N	USA	NA	vv+	339	2	AY362718	Shamblin et al., 2004
RL	USA	NA	vv+	339	2	AY362720	Shamblin et al., 2004
TK	USA	NA	vv+	339	2	AY362721	Shamblin et al., 2004
U	USA	NA	vv+	339	2	AY362722	Shamblin et al., 2004
X	USA	NA	vv+	339	2	AY362724	Shamblin et al., 2004
EU-1	Italy	1992	NA	339	5	MF431494	Trimpert et al., 2017
0093	China	2002	NA	339	3	AF493550	NA
0095	China	2002	NA	339	3	AF493552	NA
0297	China	2002	NA	339	3	AF493553	NA
0304	China	2002	NA	339	2	AF493554	NA
G2	China	2002	NA	339	4	AF493556	NA
YLO40920	China	2005	NA	339	3	DQ174459	Teng et al., 2011
GXY2	China	2007	NA	339	3	EF546430	Teng et al., 2011
GX070060	China	2008	NA	339	3	EU427303	Teng et al., 2011
GX070079	China	2008	NA	339	3	EU427304	Teng et al., 2011
Polen5	Poland	2010	NA	339	4	MF431496	Trimpert et al., 2017
tn-n1	India	2010	NA	339	5	HM749324	NA
tn-n2	India	2010	NA	339	4	HM749325	NA
UDEACO-04/13	Colombia	2013	NA	339	2	KU058701	López-Osorio et al., 2017
UDEACO-06/13	Colombia	2013	NA	339	2	KU058696	López-Osorio et al., 2017
UDEACO-07/13	Colombia	2013	NA	339	3	KU058697	López-Osorio et al., 2017
bd2	USA	2015	NA	339	2	KU173119	Trimpert et al., 2017
bf1	USA	2015	NA	339	2	KU173117	Trimpert et al., 2017
bf2	USA	2015	NA	339	2	KU173118	Trimpert et al., 2017
sd1	USA	2015	NA	339	2	KU173116	Trimpert et al., 2017
sd2	USA	2015	NA	339	2	KU173115	Trimpert et al., 2017

GaHV-2/Italy/Ck/487/15	Italy	2015	NA	339	5	MK139660	Mescolini et al., 2019
GaHV-2/Italy/Ck/507/15	Italy	2015	NA	418	9	MK139661	Mescolini et al., 2019
GaHV-2/Italy/Ck/509/15	Italy	2015	NA	418	9	MK139662	Mescolini et al., 2019
GaHV-2/Italy/Ck/510/15	Italy	2015	NA	418	9	MK139663	Mescolini et al., 2019
GaHV-2/Italy/Ck/562/15	Italy	2015	NA	418	9	MK139664	Mescolini et al., 2019
GaHV-2/Italy/Ck/599/16	Italy	2016	NA	418	9	MK139665	Mescolini et al., 2019
GaHV-2/Italy/Ck/625/16	Italy	2016	NA	339	4	MK139666	Mescolini et al., 2019
GaHV-2/Italy/Ck/674/16	Italy	2016	NA	339	4	MK139667	Mescolini et al., 2019
GaHV-2/Italy/Ck/689/16	Italy	2016	NA	339	4	MK139668	Mescolini et al., 2019
GaHV-2/Italy/Ck/722/16	Italy	2016	NA	339	4	MK139669	Mescolini et al., 2019
GaHV-2/Italy/Ck/801/17	Italy	2017	NA	339	4	MK139670	Mescolini et al., 2019
GaHV-2/Italy/Ck/810/17	Italy	2017	NA	339	4	MK139671	Mescolini et al., 2019
GaHV-2/Italy/Ck/847/17	Italy	2017	NA	418	10	MK139672	Mescolini et al., 2019
GaHV-2/Italy/Ck/848/17	Italy	2017	NA	418	9	MK139673	Mescolini et al., 2019
GaHV-2/Italy/Ck/850/17	Italy	2017	NA	339	5	MK139674	Mescolini et al., 2019
GaHV-2/Italy/Ck/852/17	Italy	2017	NA	339	4	MK139675	Mescolini et al., 2019
GaHV-2/Italy/Ck/853/17	Italy	2017	NA	339	4	MK139676	Mescolini et al., 2019
GaHV-2/Italy/Ck/854/17	Italy	2017	NA	339	4	MK139677	Mescolini et al., 2019
GaHV-2/Italy/Ck/855/17	Italy	2017	NA	298	2	MK139678	Mescolini et al., 2019

† Not available

401

402

403

404

Table 3. Molecular characteristics of the deduced Meq protein, compared to prototype strains. Amino acid substitutions interrupting PPPPs are underlined. Distinctive aa substitutions of Italian GaHV-2 strains are framed.

Strain	Meq length (aa)	PPPPs (n°)	Amino acid substitutions														
			71	77	80	110	119	153	176	180	216 [†]	217	218	277	283	320	326
CVI988	398	7	S	E	D	C	C	P	P	T	P	P	P	L	A	I	I
CU-2	398	7	S	E	D	C	C	P	P	T	P	P	P	L	A	I	T
JM/102W	399	7	A	E	D	C	C	P	P	T	<u>S</u>	P	P	L	A	I	T
Md5	339	4	A	K	D	C	C	P	P	T	P	<u>A</u>	P	L	V	T	T
648A	339	2	A	K	D	C	R	<u>Q</u>	<u>A</u>	A	P	<u>A</u>	P	P	A	I	T
GaHV-2/Italy/Ck/456/15	339	4	A	E	Y	<u>S</u>	C	P	P	T	P	P	<u>S</u>	L	A	I	T
GaHV-2/Italy/Ck/498/15	339	4	A	E	Y	<u>S</u>	C	P	P	T	P	P	<u>S</u>	L	A	I	T
GaHV-2/Italy/Ck/513/15	339	4	A	E	Y	<u>S</u>	C	P	P	T	P	P	<u>S</u>	L	A	I	T
GaHV-2/Italy/Ck/515/15	339	4	A	E	Y	<u>S</u>	C	P	P	T	P	P	<u>S</u>	L	A	I	T
GaHV-2/Italy/Ck/559/15	339	4	A	E	Y	<u>S</u>	C	P	P	T	P	P	<u>S</u>	L	A	I	T
GaHV-2/Italy/Ck/561/15	339	4	A	E	Y	<u>S</u>	C	P	P	T	P	P	<u>S</u>	L	A	I	T
GaHV-2/Italy/Ck/565/15	339	4	A	E	Y	<u>S</u>	C	P	P	T	P	P	<u>S</u>	L	A	I	T
GaHV-2/Italy/Ck/567/15	339	4	A	E	Y	<u>S</u>	C	P	P	T	P	P	<u>S</u>	L	A	I	T
GaHV-2/Italy/Ck/757/17	339	4	A	E	Y	<u>S</u>	C	P	P	T	P	P	<u>S</u>	L	A	I	T
GaHV-2/Italy/Ck/875/18	339	4	A	E	Y	<u>S</u>	C	P	P	T	P	P	<u>S</u>	L	A	I	T
GaHV-2/Italy/Ck/876/18	339	4	A	E	Y	<u>S</u>	C	P	P	T	P	P	<u>S</u>	L	A	I	T
GaHV-2/Italy/Ck/921/18	339	4	A	E	Y	<u>S</u>	C	P	P	T	P	P	<u>S</u>	L	A	I	T
GaHV-2/Italy/Ck/1083/18	339	4	A	E	Y	<u>S</u>	C	P	P	T	P	P	<u>S</u>	L	A	I	T

[†] Amino acid position according to the 339 aa-long Meq isoform

405 **Figure legend**

406 **Figure 1.** Phylogenetic tree based on *meq* gene complete amino acid sequences of the 13 GaHV-2
407 strains detected in Italian commercial flocks (marked with a black dot, ●) and of the 76 strains
408 retrieved from GenBank.