

1 Detection of transmissible viral proventriculitis (TVP) and *Chicken proventricular necrosis*
2 *virus* (CPNV) in the United Kingdom

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26 **Abstract**

27 Increasing evidence suggests that a new birnavirus, named *Chicken proventricular necrosis*
28 *virus* (CPNV), is the aetiological agent of transmissible viral proventriculitis (TVP). The
29 present work aimed to explore the possible presence of both TVP and CPNV in the UK. Forty-
30 four chickens showing TVP-compatible gross lesions were classified into 3 groups based on
31 the histological lesions: i) TVP-affected chickens: lymphocytic infiltration and glandular
32 necrosis (n=15); ii) lymphocytic proventriculitis (LP)-affected chickens: lymphocytic
33 infiltration without necrosis (n=18); and iii) without proventriculitis (WP): no lymphocytic
34 infiltration or necrosis (n=11). Nine proventriculi (7 out of 15 corresponding to TVP, and 2 out
35 of 11 corresponding to LP) were positive for CPNV by RT-PCR. These results support the
36 previously suggested idea of CPNV as causative agent of TVP. Moreover, this data shows that
37 CPNV can also be detected in a number of cases with LP, which do not fulfil the histological
38 TVP criteria. Phylogenetic analysis of partial sequences of gene VP1 showed that British
39 CPNV sequences were closer to other European CPNV sequences and might constitute a
40 different lineage from the American CPNV. TVP cases with negative CPNV PCR results may
41 be due to chronic stages of the disease or to the reduced PCR sensitivity on formalin-fixed
42 paraffin embedded tissues. However, involvement of other agents in some of the cases cannot
43 totally be ruled out. As far as the authors are aware, this is the first peer-reviewed report of
44 TVP as well as of CPNV in the UK, and the first exploratory CPNV phylogenetic study.

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46 **Keywords:** Birnavirus; *Chicken proventricular necrosis virus* (CPNV); transmissible viral
47 proventriculitis (TVP); natural infection; poultry.

48

49 **Introduction**

50 Transmissible viral proventriculitis (TVP) is an infectious viral disease affecting chickens,
51 which is reported to have a significant economic impact on a global scale (Dormitorio et al.,
52 2007; Guy et al., 2005). Affected chickens display non-specific clinical signs, which may
53 include stunted growth, pallor and the presence of incompletely digested food in the faeces
54 (Goodwin et al., 1996). Flocks affected by TVP typically do not show a significant increase in
55 mortality (Hafner and Guy, 2013). TVP most commonly affects broiler chickens of four to five
56 weeks of age (Bayyari et al., 1995). However, the disease has also been identified in both
57 broiler breeders and layer hens in the age range of nine to twenty weeks (Marusak et al., 2012).
58 TVP results in enlarged, fragile proventriculi which contain characteristic microscopic lesions
59 (Goodwin et al., 1996; Hafner and Guy, 2013). Chicken flocks are diagnosed with TVP based
60 on the presence of histological lesions in the proventriculus: necrosis of oxynticopeptic cells,
61 lymphocytic infiltration and hyperplastic ductal epithelium which replaces the glandular
62 epithelium (metaplasia) (Goodwin et al., 1996; Hafner and Guy, 2013). No specific control
63 measures or treatments are currently recommended for TVP (Hafner and Guy, 2013).

64 TVP was first described in the Netherlands almost 40 years ago (Kouwenhoven et al., 1978).
65 Since then, it has been reported in several countries in North-America (Guy et al., 2011b; Noiva
66 et al., 2015), Europe (Grau-Roma et al., 2010; Marguerie et al., 2011) and Asia (Kim et al.,
67 2015). Since the emergence of the disease, there has been considerable discussion as to the
68 aetiological agent responsible. Several viruses have been suggested including: an adenovirus
69 (Kouwenhoven et al., 1978), a reovirus (Jones, 2000), *Infectious bronchitis virus* (IBV) (Yu et
70 al., 2001), *Infectious bursal disease virus* (IBDV) (Huff et al., 2001), and a picornavirus (Kim
71 et al., 2015). Few years ago, a new birnavirus, named *Chicken proventricular necrosis virus*
72 (CPNV), was detected in naturally and experimentally reproduced TVP-affected cases in USA
73 and proposed to be its cause (Guy et al., 2011a; Guy et al., 2011b). CPNV has subsequently

74 been detected in a few other studies in TVP-affected broiler chickens in France and USA
75 (Marguerie et al., 2011; Noiva et al., 2015).

76 Despite few cases with proventricular lesions compatible to TVP have previously been reported
77 in the UK¹ (Randall & Reece, 1996), as far as the authors are aware, there is no peer-reviewed
78 report indicating the presence of neither TVP nor CPNV in the United Kingdom (UK). Based
79 on the recent description of both TVP (Grau-Roma et al., 2010; Marguerie et al., 2011) and
80 CPNV (Marguerie et al., 2011) in Europe, the aims of this study were to determine whether
81 TVP and CPNV are present in chickens in the UK, as well as to evaluate their association
82 suggested previously.

83 **Materials and methods**

84 **Study design.** At the end of 2014, a prospective study was designed based on the collection
85 of samples from chicken post-mortems performed by poultry clinicians in different locations
86 of the UK. Clinicians were asked to send proventricular samples fixed in 10% formalin to the
87 School of Veterinary Medicine and Science (SVMS) at the University of Nottingham when
88 TVP-compatible gross lesions were observed. These lesions included thickening of the
89 proventricular wall, dilation of proventriculus and/or evidence of white spots visible through
90 the proventricular serosa. Clinicians were also asked to report any other relevant
91 abnormalities observed during the post-mortem examination.

92 In addition, chicken cases received at the Veterinary Pathology Service (VPS) of the SVMS
93 between January 2014 and June 2015 having a diagnosis of 'lymphocytic and/or necrotizing
94 proventriculitis' were included in the study as suspected TVP-affected cases.

¹ VLA (currently APHA) quarterly surveillance report: January-March, Volume 14, No. 1, page 14 (<http://www.thepoultrysite.com/articles/1765/uk-poultry-disease-quarterly-surveillance-report-january-march-2010>).

95 **Histopathology.** A complete cross-section on the central area of the proventriculus was
96 performed from each chicken, routinely processed for histology and stained with haematoxylin
97 and eosin. Proventriculi were assessed for the three key histopathological findings which are
98 characteristic of TVP: (i) glandular lymphocytic infiltration; (ii) hyperplastic and metaplastic
99 changes of ductal epithelial cells; and (iii) necrosis of oxynticopeptic cells. These parameters
100 were semi-quantified as follows: - (absence), + (>0 to 10% of the glands affected), ++ (>10 to
101 50% of the glands affected), +++ (>50% of the glands affected). In addition, the presence of
102 necrosis was also assessed as the percentage of glandular parenchyma affected in the most
103 affected gland, following the same percentages as described above. A mean of the 2 necrosis
104 scores was then calculated, and mean scores between severity levels (e.g. +/++) were rounded
105 up to the higher level. The presence of inflammatory infiltrate within the lamina propria was
106 not taken into consideration, as it is reported to be a frequent finding in healthy birds (Kadhim
107 *et al.*, 2011).

108 Based on the histopathological results, chickens were allocated a case status as follows: (i)
109 transmissible viral proventriculitis (TVP)-affected chickens: lymphocytic infiltration and
110 necrosis present in the proventriculus; (ii) lymphocytic proventriculitis (LP)-affected chickens:
111 lymphocytic infiltration without necrosis present in the proventriculus; (iii) chickens without
112 proventriculitis (WP): no lymphocytic infiltration or necrosis present in the proventriculus.

113 **RNA extraction and RT-PCR.** RNA was extracted from all formalin-fixed paraffin embedded
114 (FFPE) proventriculi and tested subsequently by RT-PCR for CPNV. RNA extraction was done
115 using four 25 µm-sections of each sample. Briefly, the extraction method used incubation with
116 xylol (twice) followed by centrifugation. Pellet re-suspension was performed first with ethanol
117 and second with a digestion buffer containing Proteinase K (Roche, Mannheim, Germany).
118 After overnight incubation at 56°C and centrifugation, supernatant was mixed with TRIzol®
119 Reagent (Invitrogen, 15596-018). Samples were then homogenized with chloroform (Sigma,

120 C2432) and centrifuged. The transparent phase was discharged and the pellet was mixed with
121 isopropanol (Sigma, I9516). Two more centrifugations followed by addition of cold ethanol
122 were performed. Finally, the pellet was left to dry and received 25 µl of warm RNase-free
123 water.

124 A RT-PCR procedure was performed to amplify a 171 nucleotide (nt) sequence within the VP1
125 gene of CPNV using primers and protocols described previously (Guy et al., 2011b). FTA cards
126 with proventricular imprints from positive CPNV cases, kindly provided by Dr. Guerin
127 (National Veterinary School of Toulouse, France), were used as positive controls.

128 **Sequencing of RT-PCR product and phylogenetic studies.** The amplified products from the
129 positive CPNV RT-PCR cases were purified using Mini Elute Gel Extraction Kit (Qiagen,
130 Valencia, CA). Sequencing reactions were performed with ABI Prism BigDye Terminator
131 Cycle Sequencing v.31 Ready Reaction (Applied Biosystems, Foster City, CA), and analysed
132 using an ABI Prism model 3730 automated sequencer (Applied Biosystems, Foster City, CA).
133 Positive and negative controls of extraction and amplification were added to each batch of
134 samples tested.

135 Partial VP1 CPNV sequences obtained from British cases were compared with the sequence of
136 the American CPNV isolate R11/3 (Guy *et al.*, 2011a) available in the Genbank
137 (<http://www.ncbi.nlm.nih.gov>, accession number HM038436.1), partial sequences obtained
138 from Spanish cases (Costa et al., submitted for publication) and the positive control (FTA card)
139 using MEGA6 (Molecular Evolutionary Genetics Analysis version 6.0) software (Tamura et
140 al., 2013). Sequences were aligned using ClustalW method. A nucleotide distance matrix
141 between sequences was computed to infer phylogenies and a Neighbor-joining (NJ)
142 phylogenetical tree was generated. The partial VP1 CPNV sequences reported in this work
143 have been deposited at GenBank under accession numbers KU933595 to KU933603.

144 **Statistical analyses.** Minitab version 17 was used for statistical analyses. Values -, +, ++ and
145 +++ from the histological assessment were converted to 0, 1, 2 and 3, respectively, prior to the
146 analyses. The distribution of variables was assessed using the Ryan-Joiner test. Kruskal-Wallis
147 and Mann-Whitney U tests were used to assess for differences in variables between case
148 statuses for nonparametric data.

149 **Results**

150 **Epidemiological data and case status allocation.** Forty-four chickens were included in this
151 study (Table 1). Thirty-nine chickens came from the prospective study (chickens with TVP-
152 compatible gross lesions, chickens 1 to 39) and five chickens were received in the VPS and
153 were selected due to the presence of lymphocytic infiltration and/or necrosis of oxynticopeptic
154 cells (suspected TVP-affected cases, chickens 40 to 44). All the chickens were received
155 between April 2014 and June 2015. The farm postcode was provided in 40 out of the 44
156 chickens, showing that the chickens came from 17 different farms located in England (n=16)
157 and Wales (n=1). The number of chickens received per farm ranged between 1 and 7. All the
158 received chickens were reported to have thickened and/or dilated proventriculus (Figure 1).
159 Most of the received cases corresponded to broiler chickens (42 out of 44), 1 corresponded to
160 a layer hen (chicken 44), while the age of the chicken was not indicated in the remaining case.
161 Out of the 44 chickens studied, a total of 15 (34%) were classified as TVP-affected chickens,
162 18 (41%) as LP-affected chickens, and 11 (25%) as chickens without proventriculitis (WP)
163 (Table 1). Excluding the layer hen, the mean±SD age for each case status was: TVP=36±12,
164 LP=24±6 and WP=20±6 days. The only studied layer hen was 38 weeks-old, and was classified
165 within the TVP-affected chickens. In 8 out of the 15 TVP-affected chickens, submitted
166 veterinarians reported abnormal intestinal contents (including orange jejunal contents,
167 intestinal dilation and loose caecal contents).

168 The TVP-affected chickens came from 9 different farms, all of them located in England. In
169 these farms, the mean \pm SD number of chickens on a farm was 64,417 \pm 56,781, with a range of
170 20,000 to 203,000 chickens. The vaccination status was available for 5 out of these 9 farms
171 (from where 10 TVP-affected chickens came from, specifically cases 2, 3, 15, 17, 20 to 24 and
172 41). All of them were vaccinated with a live attenuated IBV vaccine variant strain 4-91, which
173 was combined with the virus strain IB Ma5 in 4 of the farms. In addition, the latter 4 farms also
174 used a live IBDV vaccine containing IBDV strain 228E. No other vaccines were used in these
175 farms. Data on monthly percentage mortality of flocks was available from 4 farms, which
176 ranged from 3.03% to 4.49%.

177 **Histopathology.** The histopathological results of each chicken are detailed in Table 1.

178 The mean necrosis of oxynticopeptic cells score was mild (+) in 8 out of the 15 TVP-affected
179 cases (53%), and moderate (++) in the remaining 7 (47%). Necrotic cells showed
180 hypereosinophilia, fragmentation and karyorrhexis, karyolysis and/or pyknosis, and were
181 usually seen as small clusters within the lumen of dilated proventricular alveoli, often within
182 the edge of the lobule (Figure 2). Collecting ducts (secondary ducts) were often dilated and
183 filled with necrotic debris and sloughed cells. No inclusion bodies were observed in any of the
184 cases.

185 Thirteen out of the 15 TVP-affected chickens (87%) had severe lymphocytic infiltration, while
186 in the remaining 2 chickens (13%) the lesion was moderate. Lymphocytic infiltration was
187 usually multifocal, located within the interstitium of the proventricular glands (Figure 3). In
188 some cases, lymphocytic cells formed nodular aggregates. Unaffected glands were usually
189 intermingled with affected ones. The median of the lymphocytic infiltration score in the TVP-
190 affected group (3) was significantly higher than in the LP-affected group (1.5) ($p=0.002$).

191 Although the inflammation in TVP-affected cases was predominantly lymphocytic, few plasma
192 cells, macrophages and occasional heterophils were also present.

193 Finally, all the 15 TVP-affected chickens (100%) had severe hyperplasia and metaplasia of
194 ductal epithelial cells (Figure 2 and 3), whereas it was present in 11 out of 18 (61%) LP-affected
195 cases and in 2 out of the 11 (18%) chickens within the WP group. The median score for TVP-
196 affected group (3.0) was significantly higher than for LP-affected group (1.5) ($p < 0.001$) and
197 for WP (0.0) ($p < 0.001$). However, trend only was observed between the median score for LP
198 and WP ($p = 0.053$).

199 **CPNV RT-PCR and phylogenetic studies.** Nine chickens gave positive results for CPNV
200 RT-PCR in the proventriculus (Table 1). Seven out of these 9 chickens (78%) belonged to the
201 TVP-affected group and the remaining 2 (22%) to the LP-affected group. When looking at
202 each group, 7 out of the 15 TVP-affected cases (47%) and 2 out of the 18 LP-affected
203 chickens (11%) were positive for CPNV RT-PCR. None of the proventriculi from chickens
204 belonging to the WP group gave positive results.

205 All RT-PCR CPNV positive cases corresponded to broiler chickens. The only analysed layer
206 hen, which was histologically classified as TVP, gave a negative CPNV RT-PCR results.

207 The VP1 gene of the 9 positive CPNV cases was partially sequenced, including a fragment of
208 171 nucleotides (nt). Figure 4 shows a phylogenetical tree including these 9 sequences, 1
209 sequences obtained from the French CPNV RT-PCR positive control, 2 Spanish CPNV
210 sequences and 1 American case (Genbank accession number: HM038436.1). All the 9 British
211 CPNV sequences were closer to each other than to sequences obtained from other countries.
212 Their percentage of similarity was below 90% only when compared with the American and
213 one of the Spanish sequences. Specifically, the cases from the UK showed 99.4-100%
214 nucleotide similarity between the sequences. When compared with the sequences retrieved

215 from the other countries, the % similarity was 92.4- 92.8%, 88.3-94.2% and 89.5-90.1%, with
216 the French, Spanish and American sequences, respectively (Table 2).

217 **Discussion**

218 Cases of chickens with lymphocytic and necrotising proventriculitis, consistent with TVP, have
219 been reported in the USA (Guy et al., 2011b), South Korea (Kim et al., 2015) and several
220 countries in Europe (Grau-Roma et al., 2010; Kouwenhoven et al., 1978; Marguerie et al.,
221 2011). However, as far as the authors are aware, this is the first peer-reviewed description of
222 TVP as well as of CPNV in the UK.

223 The mean mortality of broiler flocks in the UK has previously been reported at 4.1% (Dawkins
224 et al., 2004). This percentage is close to the mortality recorded in the TVP-affected farms in
225 this study. These results are therefore in line with earlier studies that state there is no significant
226 increase in mortality in flocks affected by TVP (Hafner and Guy, 2013). All the TVP-affected
227 chickens came from farms located in counties across England. TVP-affected broilers were in
228 the age range of 21 to 49 days old, which is consistent with previous reports of the disease
229 (Bayyari et al., 1995; Hafner and Guy, 2013). The majority of the chickens submitted to the
230 study were Ross 308 broiler chickens, with only 1 case corresponding to a layer hen. There
231 may be 2 reasons for this: (i) TVP affects mainly broiler chickens; (ii) broilers chickens equate
232 to 80% of the chicken post-mortems carried out by the poultry clinicians submitting the
233 samples. The studied layer hen studied was histologically classified as TVP, becoming the
234 second report of this disease in layer hens in peer-reviewed literature (Marusak et al., 2012).
235 Marusak et al. (2012) diagnosed TVP in broiler breeder and commercial layer hens ranging
236 from 9 to 20 weeks of age. Therefore, the hen included here is the oldest chicken reported to
237 be affected by TVP, although it was negative by CPNV RT-PCR.

238 The detection of CPNV by RT-PCR in almost 50% (7 out of 15) of TVP-affected chickens
239 together with the negative results in all the chickens within the WP group supports the idea that
240 CPNV is the cause of TVP (Guy et al., 2011a; Guy et al., 2011b). All the TVP-affected cases
241 showed moderate to severe lymphocytic infiltrates and severe tubular hyperplasia and
242 metaplasia, which are features of chronicity as demonstrated in experimentally reproduced
243 TVP-affected cases (Guy et al., 2011b). This experimental infection also showed that CPNV
244 was only detectable by RT-PCR from 1 to 14 days post exposure (PE), while the microscopic
245 lesions were present from 5 to 35 days PE (Guy et al., 2011b). Therefore, the TVP cases with
246 negative RT-PCR CPNV in the present study may correspond to chronically CPNV infected
247 chickens, where the virus is not detectable further within the lesions. In addition, the well-
248 known reduced sensitivity of RT-PCR on FFPE tissues compared to fresh tissues might account
249 for a number of these negative RT-PCR results (Lewis et al., 2001). Finally, it can not however
250 be ruled out that other infectious or non-infectious agents, alone or together with CPNV, may
251 be involved in some of the TVP-affected chickens presented here (Huff et al., 2001; Dormitorio
252 et al., 2007; Kim et al., 2015).

253 It has been reported that proventriculitis can be caused by a number of different factors,
254 including infectious (viruses, bacteria, fungi or parasites), mycotoxins and nutritional factors
255 (Dormitorio et al., 2007). It seems likely that, in this study, the group of LP-affected chickens
256 correspond to a mixture of cases with different aetiologies. Amongst them, a number of LP-
257 affected chickens with negative CPNV RT-PCR results may correspond to chronically affected
258 TVP cases, where the virus is not detectable (Guy et al., 2011b). Interestingly, 11% of LP-
259 affected chickens gave positive results by CPNV RT-PCR. These chickens likely correspond
260 to chronic TVP-affected chickens, where necrosis of oxynticopeptic cells is not observed, but
261 where the virus is still detectable. This finding indicates that TVP should still be suspected in
262 cases of LP without glandular necrosis and that the CPNV RT-PCR can have diagnostic value

263 in these cases. It is worth mentioning that areas of necrosis may have been present within other
264 non-examined areas of the proventriculi, since this study was performed on a single complete
265 cross-section of each proventriculus.

266 The % of nucleotide similarities showed the highest value similarity when comparing the
267 sequences obtained from within the UK. Moreover, the phylogenetical tree of nucleotide
268 sequences suggested that the UK CPNV sequences may be more similar to the other European
269 sequences than to the American sequence. These geographical variations may be due to
270 mutations as a result of different selection pressures between countries and continents, as
271 previously suggested for other virus such as IBDV (Jackwood and Sommer-Wagner, 2007).
272 Although the current study included short nucleotide sequences (171 nt) within the VP1 gene,
273 it must be acknowledged that this gene encodes for the RNA-dependent RNA-polymerase,
274 which is known to be a well-conserved gene in cellular organisms as well as in viruses (Pan et
275 al., 2007). For this reason, based on the results obtained, it could be hypothesized that two
276 distinct lineages of CPNV, i.e. European and American, are present in the two continents. It
277 would, however, be necessary to perform larger studies, increasing the number of sequences
278 and their length, to try to confirm these initial observations

279 In conclusion, this study identified the presence of TVP amongst chicken populations in the
280 UK. Results indicate that CPNV can often be detected within the proventriculus of TVP-
281 affected chickens, and in a number of chickens with LP. Moreover, preliminary phylogenetic
282 studies on partial CPNV sequences indicate that European and American chicken populations
283 may have 2 different CPNV genetic lineages. Additional investigations, encompassing larger
284 sample sizes, are needed in order to determine the incidence and prevalence of TVP in the UK
285 and globally.

286 **Acknowledgements**

287 The authors would like to thank A. McDaniel, W. Garton, C. Blake-Dyke, M. Phelps and C.
288 Lopez from Minster Veterinary Practice who kindly provided the proventricular samples. We
289 also thank Dr. Guerin, from the National Veterinary School of Toulouse (France) for kindly
290 providing CPNV positive controls and Rosa Maria Valle and Mónica Pérez (CReSA, IRTA-
291 UAB) and Alan Lasslett (UoN) for technical support. The research leading to these results has
292 received funding from the People Programme (Marie Curie Actions) of the European Union's
293 Seventh Framework Programme (FP7/2007-2013) under REA grant agreement No
294 PCOFUND-GA-2012-600181.

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376 **Tables**

377 **Table 1.** Microscopic proventricular lesion scores^a, RT-PCR results and case status^b for each
 378 chicken. Genbank accession numbers for positive *Chicken proventricular necrosis virus*
 379 (CPNV) RT-PCR cases are detailed.

Chicken	Microscopic proventricular lesion scores			Case Status	CPNV RT-PCR	Identifier	Accession number
	Mean necrosis score ^c	Interstitial lymphocytic infiltration	Ductal epithelial hyperplasia and metaplasia				
1	-	-	-	WP	Negative		
2	+	+++	+++	TVP	Negative		
3	+	+++	+++	TVP	Negative		
4	-	-	-	WP	Negative		
5	-	-	-	WP	Negative		
6	-	+	-	LP	Negative		
7	-	+++	+++	LP	Negative		
8	-	+	-	LP	Negative		
9	-	++	++	LP	Negative		
10	-	+	-	LP	Negative		
11	-	-	-	WP	Negative		
12	-	-	-	WP	Negative		
13	-	+	-	LP	Negative		
14	-	+++	++	LP	Negative		
15	++	+++	+++	TVP	Positive	CPNV-UK-1	KU933597
16	-	++	++	LP	Positive	CPNV-UK-2	KU933598
17	++	+++	+++	TVP	Negative		
18	-	+	++	LP	Negative		
19	-	-	++	WP	Negative		
20	++	+++	+++	TVP	Negative		
21	+	+++	+++	TVP	Positive	CPNV-UK-3	KU933599
22	++	++	+++	TVP	Positive	CPNV-UK-4	KU933600
23	++	+++	+++	TVP	Positive	CPNV-UK-5	KU933601
24	++	+++	+++	TVP	Positive	CPNV-UK-6	KU933602
25	+	++	+++	TVP	Negative		
26	+	+++	+++	TVP	Negative		
27	+	+++	+++	TVP	Negative		
28	-	++	-	LP	Negative		
29	-	+++	++	LP	Negative		
30	-	-	-	WP	Negative		
31	-	+	++	LP	Negative		
32	-	-	-	WP	Negative		
33	-	+	+	LP	Negative		
34	-	-	++	WP	Negative		
35	-	+	-	LP	Negative		
36	-	-	-	WP	Negative		
37	-	+	+	LP	Negative		
38	-	-	-	WP	Negative		
39	-	++	-	LP	Negative		
40	-	+++	+++	LP	Negative		

41	+	+++	+++	TVP	Positive	CPNV-UK-7	KU933603
42	-	+++	+++	LP	Positive	CPNV-UK-8	KU933595
43	++	+++	+++	TVP	Positive	CPNV-UK-9	KU933596
44	+	+++	+++	TVP	Negative		

380 ^a -: absence; +: >0 to 10% of the glands affected; ++: >10 to 50% of the glands affected, +++:
381 >50% of the glands affected

382 ^bTVP: transmissible viral proventriculitis; LP: lymphocytic proventriculitis; WP: without
383 proventriculitis.

384 ^cMean necrosis score is the mean combined score of necrosis of oxynticopeptic cells in all
385 glands and necrosis of glandular parenchyma in the most affected gland.

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397 **Table 2.** Percentage of homology between the studied *Chicken proventricular necrosis virus* (CPNV) partial VP1 sequences. Sequences
 398 included are American (CPNV-USA, n=1), French (CPNV-Fr, n=1), Spanish (CPNV-Sp, n=2) and British (CPNV-UK, n=9).

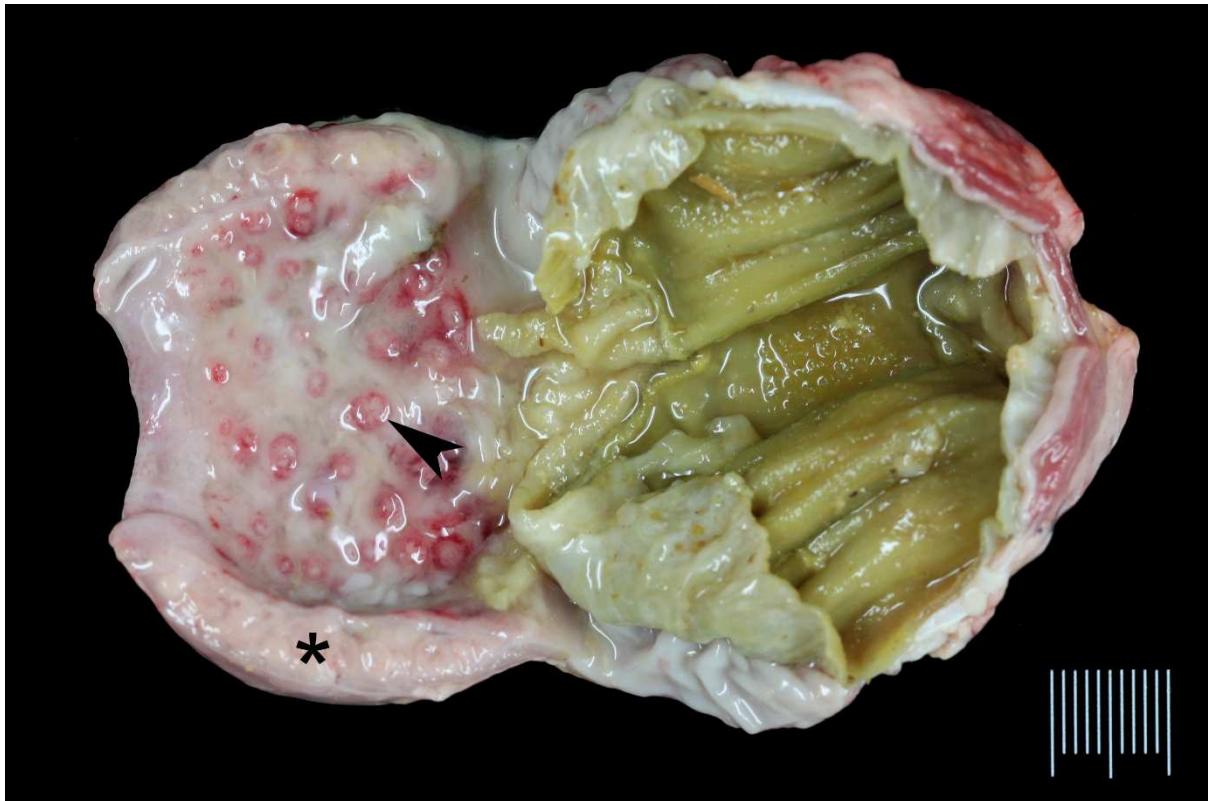
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	CPNV- USA-1	CPNV- Fr-1	CPNV- Sp-2	CPNV- Sp-1	CPNV- UK-8	CPNV- UK-9	CPNV- UK-1	CPNV- UK-2	CPNV- UK-3	CPNV- UK-4	CPNV- UK-5	CPNV- UK-6	CPNV- UK-7
CPNV-USA-1	100.00	90.06	89.47	91.81	89.47	89.47	90.06	90.06	90.06	90.06	90.06	90.06	90.06
CPNV-Fr-1	90.06	100.00	94.15	92.40	92.40	92.40	92.98	92.98	92.98	92.98	92.98	92.98	92.98
CPNV-Sp-2	89.47	94.15	100.00	94.74	93.57	93.57	94.15	94.15	94.15	94.15	94.15	94.15	94.15
CPNV-Sp-1	91.81	92.40	94.74	100.00	88.30	88.30	88.89	88.89	88.89	88.89	88.89	88.89	88.89
CPNV-UK-8	89.47	92.40	93.57	88.30	100.00	100.00	99.42	99.42	99.42	99.42	99.42	99.42	99.42
CPNV-UK-9	89.47	92.40	93.57	88.30	100.00	100.00	99.42	99.42	99.42	99.42	99.42	99.42	99.42
CPNV-UK-1	90.06	92.98	94.15	88.89	99.42	99.42	100.00	100.00	100.00	100.00	100.00	100.00	100.00
CPNV-UK-2	90.06	92.98	94.15	88.89	99.42	99.42	100.00	100.00	100.00	100.00	100.00	100.00	100.00
CPNV-UK-3	90.06	92.98	94.15	88.89	99.42	99.42	100.00	100.00	100.00	100.00	100.00	100.00	100.00
CPNV-UK-4	90.06	92.98	94.15	88.89	99.42	99.42	100.00	100.00	100.00	100.00	100.00	100.00	100.00
CPNV-UK-5	90.06	92.98	94.15	88.89	99.42	99.42	100.00	100.00	100.00	100.00	100.00	100.00	100.00
CPNV-UK-6	90.06	92.98	94.15	88.89	99.42	99.42	100.00	100.00	100.00	100.00	100.00	100.00	100.00
CPNV-UK-7	90.06	92.98	94.15	88.89	99.42	99.42	100.00	100.00	100.00	100.00	100.00	100.00	100.00

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401 **Figures**

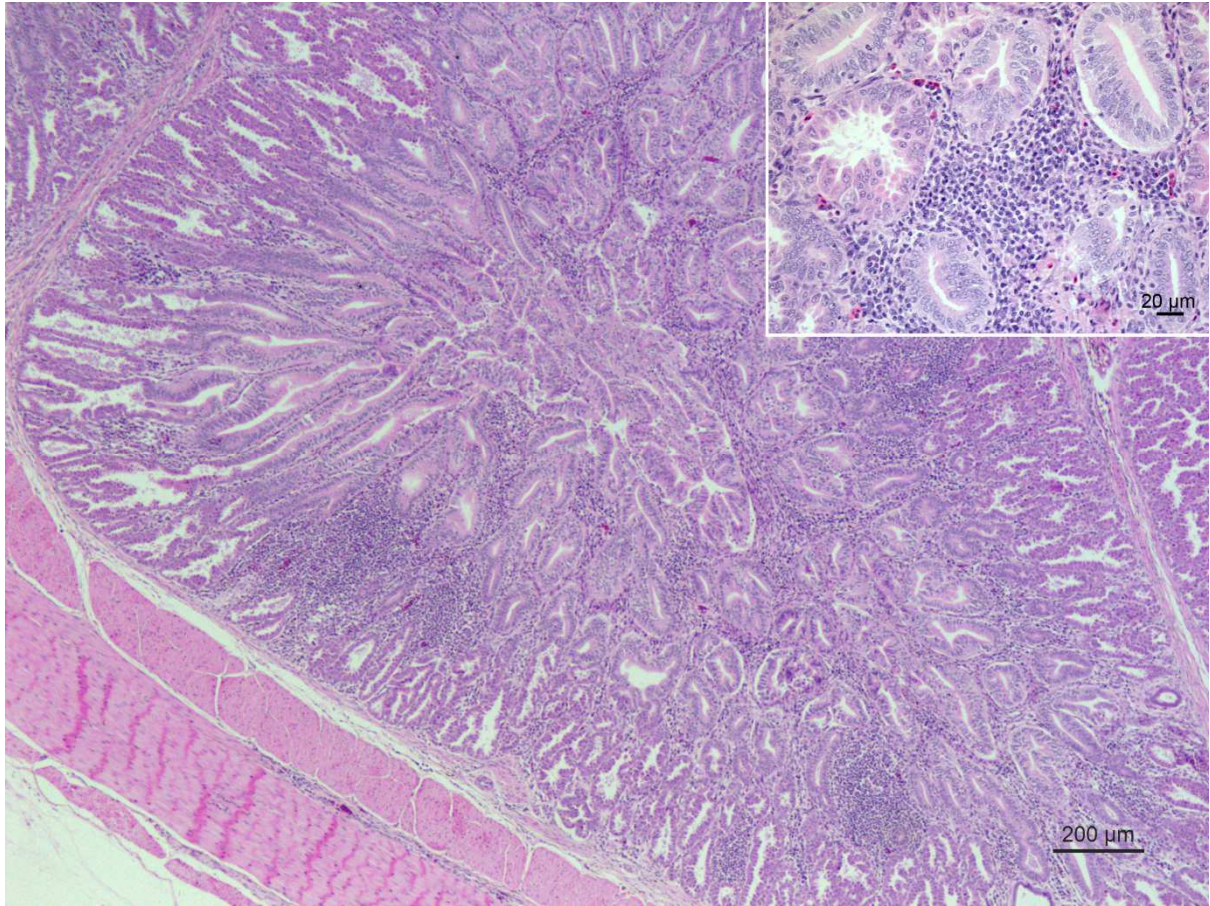
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404 **Figure 1.** Proventriculus and gizzard, broiler chicken, chicken 22, Transmissible viral
405 proventriculitis (TVP)-affected case. The proventricular wall is severely and diffusely
406 thickened. Multifocal and small (up to 0.5 cm) circular areas of congestion and haemorrhage
407 are present within the proventricular mucosa generating a pattern that highlights the
408 proventricular mucosal papillae (arrowhead). The proventricular wall shows prominent
409 glandular lobules (asterisk). Gizzard shows no gross lesions.

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412 **Figure 2.** Proventriculus, broiler chicken, chicken 17. Transmissible viral proventriculitis
413 (TVP)-affected case. Photomicrograph showing severe proventricular interstitial lymphocytic
414 infiltration and moderate replacement of glandular epithelium by hyperplastic ductal
415 epithelium (ductal epithelial metaplasia). Inset: Higher magnification of the same
416 proventriculus, showing the predominantly lymphocytic interstitial infiltration as well as the
417 tubular epithelial hyperplasia and metaplasia. Haematoxylin and eosin.

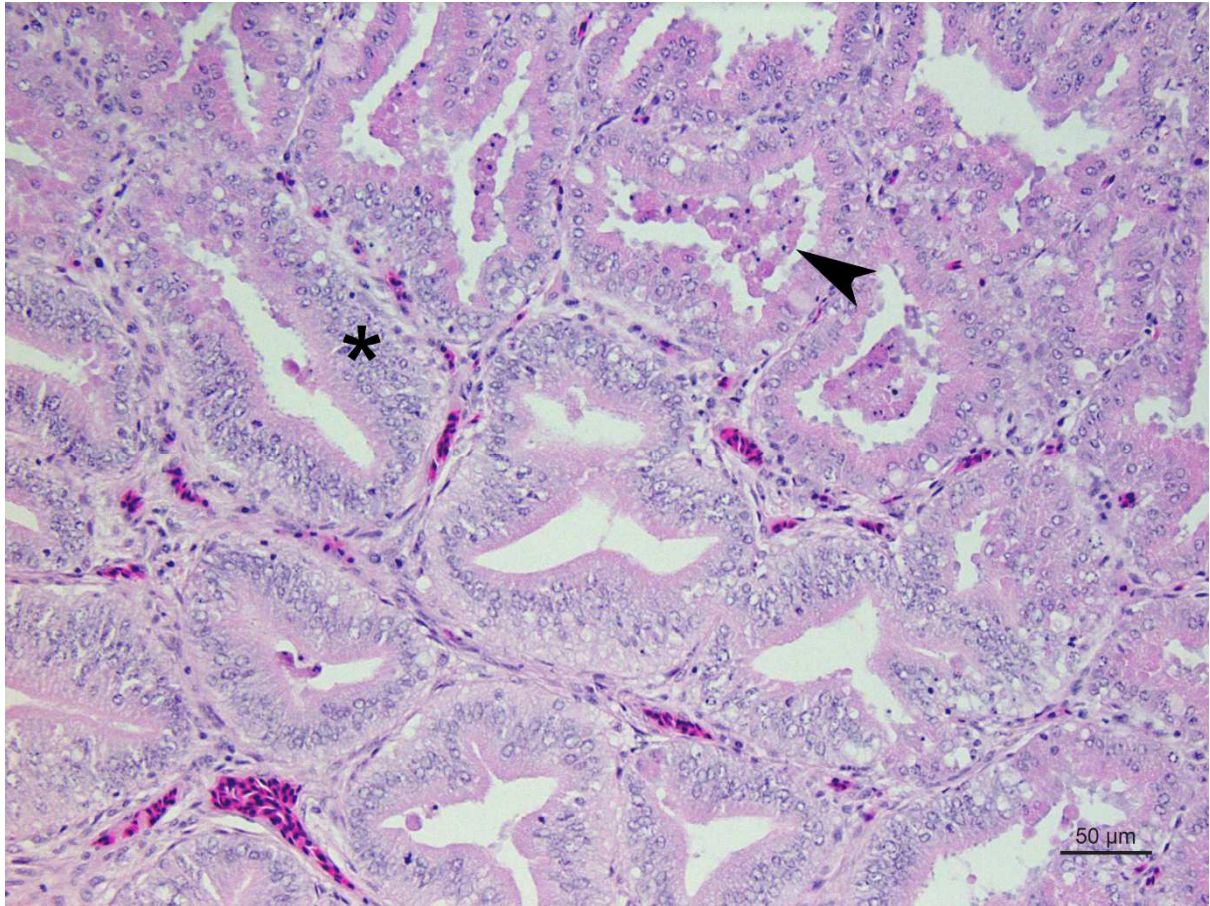
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424 **Figure 3.** Proventriculus, broiler chicken, chicken 20. Transmissible viral proventriculitis
425 (TVP)-affected case. Photomicrograph showing multifocal aggregates of necrotic cells
426 (arrowhead) within the lumen of proventricular alveoli located at the periphery of a
427 proventricular lobule. Areas with replacement of glandular epithelium by hyperplastic ductal
428 epithelium (ductal epithelial metaplasia) are also present (asterisk). Haematoxylin and eosin.

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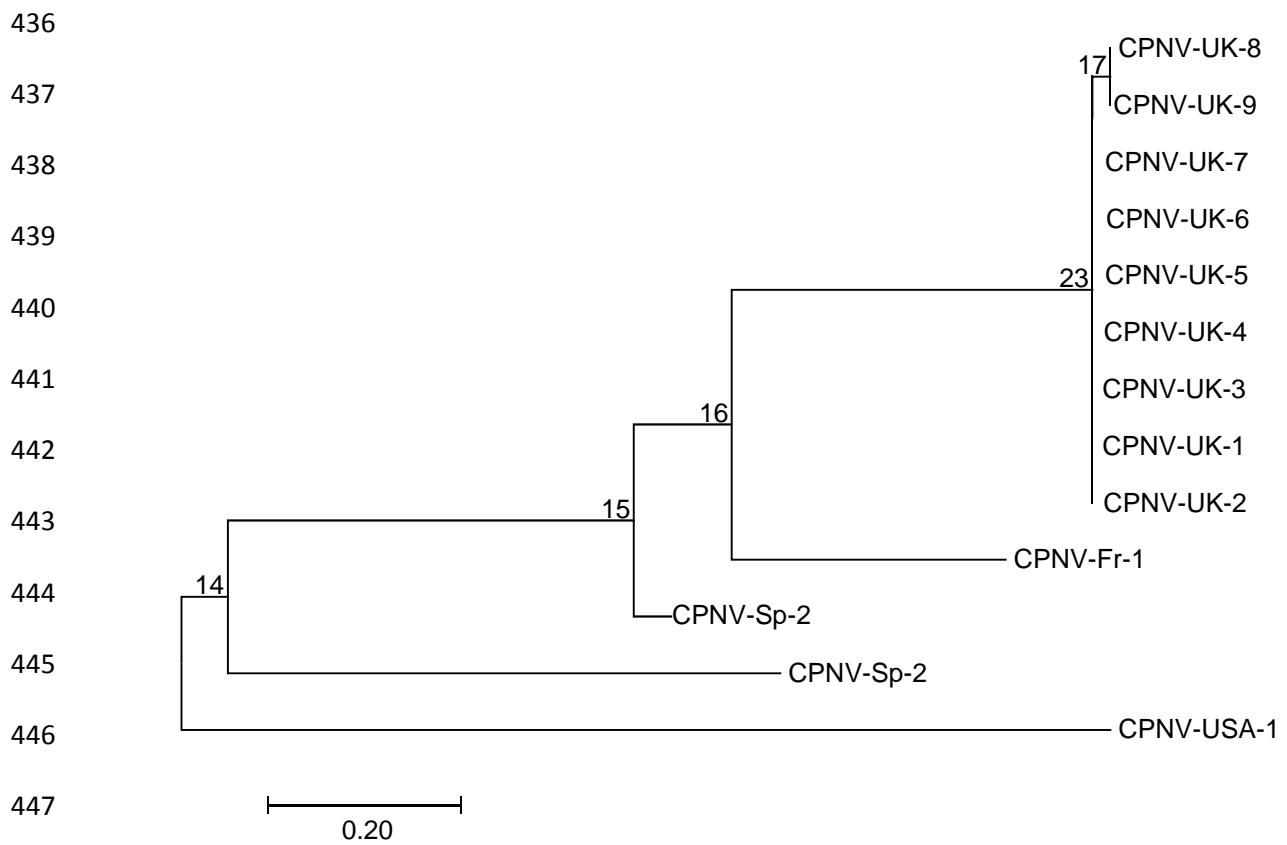
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449 **Figure 4.** Phylogenetic tree based on the NJ method for 13 partial (171 nucleotides) VP1
 450 CPNV sequences. Sequences originate from 4 different countries: USA (CPNV-USA-1),
 451 France (CPNV-Fr-1), Spain (CPNV-Sp-1 and 2), and UK (CPNV-UK-1 to 9). Numbers
 452 along the branches refer to the percentages of confidence.