

## Progress Report

During this research period, we retrieved 264 IBV field isolates (Table-1) from PA poultry during the last 30 years (1990 – 2020) and conducted both get RT-PCR and real-time RT-PCR tests to confirm positive for IBV. Of 264 isolates, 154 isolates were selected (yielded clear PCR bands and ct-values <35) and processed for S1 gene sequencing analysis.

The IBV S1-gene sequencing results shows that all the 154 IBV isolates fall into GI lineage among the five group lineages of IBV sequencing results. Within G-I lineage, there are further 27 sub-lineages. The 154 isolates belonged to six sub-lineages of 1, 3, 9, 17, 25 and 27.

Distribution of the 154 IBV isolates to the IBV sub-lineages are as 121 for GI-1, 3 for GI-3, 5 for GI-9; 19 for GI-17, 1 for GI-25, and 5 for GI-27 (Fig. 1).

For the next research period, we will conduct full genomic sequencing test. We will extract IBV viral RNAs for full genomic sequencing analysis by using the Illumina MiSeq system, conduct assembling full genome sequences, complete sequencing data analysis and document the full genomic data to Genbank.

**Table-1. Summary of test results on 264 IBV isolates by get RT-PCR and real-time RT-PCR**

Serial No.	IBV Isolate ID	S1-gene G-1 sub-lineage groups	rRT-PCR (Ct value)	OneStep rRT-PCR (1758bp)	OneStep rRT-PCR (1736bp)
1.	IBV/PA/Ck/25025/19		-	-	
2.	IBV/PA/Ck/17976/18	1	15	+	
3.	IBV/PA/Ck/16194/18	1	15.5	+	
4.	IBV/PA/Ck/14389/18	1	15	+	
5.	IBV/PA/Ck/06492/18	17	16	+	
6.	IBV/PA/Ck/04926/18	1	14.5	+	
7.	IBV/PA/Ck/04820/18	1	14.5	+	
8.	IBV/PA/Ck/04020/18	1	17	+	
9.	IBV/PA/Ck/03483/18	1	19	+	
10.	IBV/PA/Ck/02783/18	17	18	+ 2 (band)	+
11.	IBV/PA/Ck/01792/18	1	17	+	

12.	IBV/PA/Ck/20979/18	1	19	+	+
13.	IBV/PA/Ck/17970/18	17	16	+ (2 band)	+
14.	IBV/PA/Ck/15655/18	1	14	+	
15.	IBV/PA/Ck/14551/17		17	+ (2 band)	-
16.	IBV/PA/Ck/11257/17	1	19	+ (2 band)	+
17.	IBV/PA/Ck/33330/17		36	-	
18.	IBV/PA/Ck/31792/17	27	18	+	
19.	IBV/PA/Ck/32248/17	1	16	+	
20.	IBV/PA/Ck/13042/17	1	17	+	
21.	IBV/PA/Ck/14944/17	1	18	+	
22.	IBV/PA/Ck/18240/17		35	-	
23.	IBV/PA/Ck/13646/17	1	17	+	
24.	IBV/PA/Ck/12391/17		-	-	
25.	IBV/PA/Ck/27962/17	1	18	+ (2 band)	+
26.	IBV/PA/Ck/22673/17		-	-	
27.	IBV/PA/Ck/17050/17		37	Weak +	-
28.	IBV/PA/Ck/11533/17	17	14	+	
29.	IBV/PA/Ck/11660/17		-	-	
30.	IBV/PA/Ck/12623/17	17	17	+ (2 band)	+
31.	IBV/PA/Ck/12623/17		16	+ (2 band)	+
32.	IBV/PA/Ck/16327/17	1	28	+	
33.	IBV/PA/Ck/15676/17		-	-	

34.	IBV/PA/Ck/13047/17	27	32	+	
35.	IBV/PA/Ck/12793/17		-	-	
36.	IBV/PA/Ck/12614/17		-	-	
37.	IBV/PA/Ck/10488/17		-	-	
38.	IBV/PA/Ck/09647/17	1	23	+	
39.	IBV/PA/Ck/07498/17	27	25	+	
40.	IBV/PA/Ck/28732/16	1	20	+	
41.	IBV/PA/Ck/27386/16		-	-	
42.	IBV/PA/Ck/22434/16	1	29	+	
43.	IBV/PA/Ck/16433/16	17	13	+ (2 band)	+
44.	IBV/PA/Ck/13568/16	1	15	+	
45.	IBV/PA/Ck/11701/16	27	14	+	
46.	IBV/PA/Ck/10633/16	1	12	+	
47.	IBV/PA/Ck/06686/16	1	16	+	
48.	IBV/PA/Ck/04459/16	1	12	+	
49.	IBV/PA/Ck/04151/16	1	17	Not performed	+
50.	IBV/PA/Ck/03517/16	1	13	+	
51.	IBV/PA/Ck/02606/16		14	-	-
52.	IBV/PA/Ck/00100/16	25	13	Weak + (2 band)	+
53.	IBV/PA/Ck/27000/15	17	12	+	
54.	IBV/PA/Ck/26986/15	17	16	+	
55.	IBV/PA/Ck/26985/15		35	-	

56.	IBV/PA/Ck/26183/15	1	16	+	
57.	IBV/PA/Ck/26184/15	17	14	+ (2 band)	+
58.	IBV/PA/Ck/23318/15	1	13	+	
59.	IBV/PA/Ck/16506/15	17	13	+(2 band)	+
60.	IBV/PA/Ck/12893/15	1	15	+	
61.	IBV/PA/Ck/12340/15	1	16	+	
62.	IBV/PA/Ck/12036/15	1	16	+	
63.	IBV/PA/Ck/11382/15		20	NSB	-
64.	IBV/PA/Ck/09789/15	1	15	+	
65.	IBV/PA/Ck/09537/15		31	-	
66.	IBV/PA/Ck/06455/15	17	15	+	+
67.	IBV/PA/Ck/04191/15	1	13	+	
68.	IBV/PA/Ck/03510/15	1	22	+	
69.	IBV/PA/Ck/02561/15	1	29	+	
70.	IBV/PA/Ck/00937/15	1	27	+	
71.	IBV/PA/Ck/00625/15	1	26	+	
72.	IBV/PA/Ck/00215/15	1	26	+	
73.	IBV/PA/Ck/27318/14	1	28	+	
74.	IBV/PA/Ck/27313/14	1	18	+	
75.	IBV/PA/Ck/22997/14	1	26	+	
76.	IBV/PA/Ck/20264/14	1	14	+	
77.	IBV/PA/Ck/16232/14	1	18	+	

78.	IBV/PA/Ck/06608/14	1	13	+	
79.	IBV/PA/Ck/06564/14	1	14	Weak +	+
80.	IBV/PA/Ck/01384/14	1	14	+	
81.	IBV/PA/Ck/26698/13	1	15	+	
82.	IBV/PA/Ck/25345/13	1	13	+	
83.	IBV/PA/Ck/21515/13	1	32	+	
84.	IBV/PA/Ck/20901/13		33	-	
85.	IBV/PA/Ck/08722/13	1	29	+	
86.	IBV/PA/Ck/06983/13	17	14	+	
87.	IBV/PA/Ck/04458/13	1	12	+	
88.	IBV/PA/Ck/04457/13	1	12	+	
89.	IBV/PA/Ck/04455/13	1	12	+	
90.	IBV/PA/Ck/24559/12	1	12	+	
91.	IBV/PA/Ck/10441/12	1	11	+	
92.	IBV/PA/Ck/10028/12	1	16	+	
93.	IBV/PA/Ck/09600/12	1	14	+	
94.	IBV/PA/Ck/03974/12	1	15	+	+
95.	IBV/PA/Ck/00851/12		19	NSB	-
96.	IBV/PA/Ck/32667/11	1	17	+	
97.	IBV/PA/Ck/28591/11		32	-	
98.	IBV/PA/Ck/23853/11	9	16	+	
99.	IBV/PA/Ck/16089/11		33	-	

100.	IBV/PA/Ck/14510/11		34	-	
101.	IBV/PA/Ck/32350/10		18	-	-
102.	IBV/PA/Ck/18882/10		14	-	-
103.	IBV/PA/Ck/08326/08		13	+ (two bands)	+ (two bands)
104.	IBV/PA/Ck/08101/08		15	NSB	+ (two bands)
105.	IBV/PA/Ck/07497/08		22	NSB	+ (two bands)
106.	IBV/PA/Ck/10094/08		15	NSB	+ (two bands)
107.	IBV/PA/Ck/16132/05		30	-	-
108.	IBV/PA/Ck/05890/05		19	NSB	-
109.	IBV/PA/Ck/05809/05		16	-	-
110.	IBV/PA/Ck/32559/04	1	15	NSB	+
111.	IBV/PA/Ck/05142/04	17	17	+ (2 band)	+
112.	IBV/PA/Ck/03830/04		14	NSB	-
113.	IBV/PA/Ck/02799/04		17	NSB	NSB
114.	IBV/PA/Ck/02797/04		14	NSB	NSB
115.	IBV/PA/Ck/02795/04		19	NSB	NSB
116.	IBV/PA/Ck/02794/04	1	18	+	+
117.	IBV/PA/Ck/02793/04		20	+ (2 band)	+ (2 band)
118.	IBV/PA/Ck/02703/04	1	34	-	+
119.	IBV/PA/Ck/02764/04	17	27	-	+
120.	IBV/PA/Ck/02699/04		18	NSB	NSB
121.	IBV/PA/Ck/02698/04		17	NSB	NSB

122.	IBV/PA/Ck/02687/04		16	NSB	NSB
123.	IBV/PA/Ck/02696/04	1	15	+	+
124.	IBV/PA/Ck/02693/04	1	15	+	+
125.	IBV/PA/Ck/02763/04		32	-	-
126.	IBV/PA/Ck/02926/04		30	-	-
127.	IBV/PA/Ck/02760/04		15	NSB	NSB
128.	IBV/PA/Ck/32385/03	1	14	+	
129.	IBV/PA/Ck/32690/03	1	17	+	
130.	IBV/PA/Ck/35483/03	1	15	+	
131.	IBV/PA/Ck/34785/03	17	15	+ (2 band)	+
132.	IBV/PA/Ck/35359/03	1	13	+	
133.	IBV/PA/Ck/33027/02		15	NSB	-
134.	IBV/PA/Ck/32940/02	1	16	+	
135.	IBV/PA/Ck/30692/02	1	15	+	
136.	IBV/PA/Ck/31899/02		30	NSB	-
137.	IBV/PA/Ck/26197/02		12	NSB	Weak+
138.	IBV/PA/Ck/36199/02		13	NSB	Weak+
139.	IBV/PA/Ck/37536/02	1	20	+	
140.	IBV/PA/Ck/33935/01		23	NSB	-
141.	IBV/PA/Ck/38184/01		18	NSB	NSB
142.	IBV/PA/Ck/33185/01		17	NSB	Weak+
143.	IBV/PA/Ck/33184/01	3	16	+	

144.	IBV/PA/Ck/37674/00		35	-	
145.	IBV/PA/Ck/37457/00		17	NSB	NSB
146.	IBV/PA/Ck/35852/00		19	NSB	-
147.	IBV/PA/Ck/35662/00		16	NSB	-
148.	IBV/PA/Ck/35865/00	17	12	+ (2 band)	+
149.	IBV/PA/Ck/37597/00	9	19	+	
150.	IBV/PA/Ck/34131/00	1	13	+	
151.	IBV/PA/Ck/35270/00		15	-	-
152.	IBV/PA/Ck/33316/00	1	13	+	
153.	IBV/PA/Ck/32876/00	1	16	+	
154.	IBV/PA/Ck/32698/00	17	17	+ (2 band)	+
155.	IBV/PA/Ck/32099/00		15	NSB	-
156.	IBV/PA/Ck/32862/00	1	15	+	
157.	IBV/PA/Ck/36095/00	1	18	+	
158.	IBV/PA/Ck/36664/00	1	14	+	
159.	IBV/PA/Ck/30265/00	1	15	+ (2 band)	+
160.	IBV/PA/Ck/31098/00	1	20	+	
161.	IBV/PA/Ck/32790/00		35	-	
162.	IBV/PA/Ck/32791/00	1	19	+	
163.	IBV/PA/Ck/32861/00	1	17	+	
164.	IBV/PA/Ck/31416/00	1	16	+	
165.	IBV/PA/Ck/36461/00		35	-	



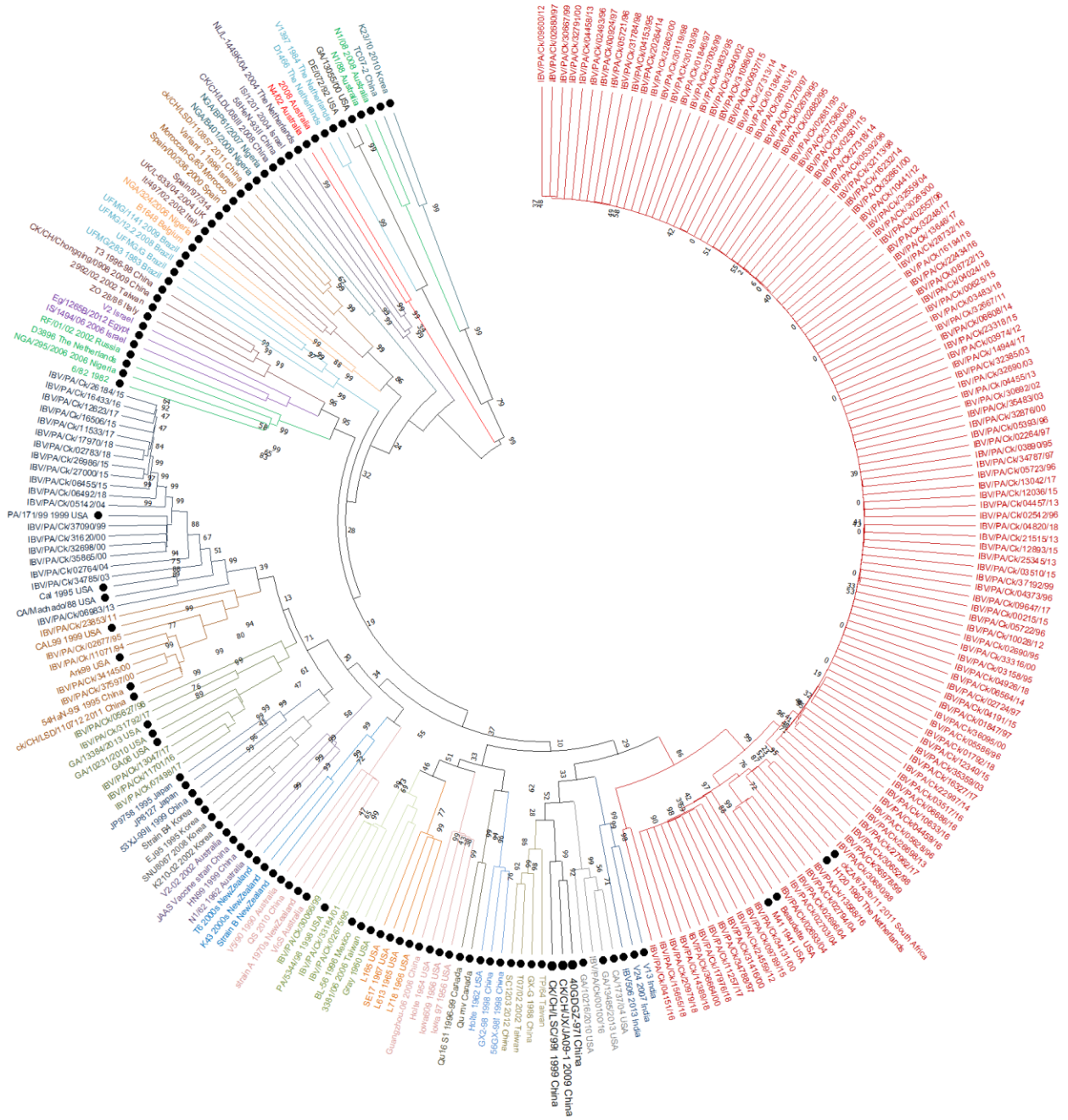
166.	IBV/PA/Ck/31620/00	17	16	+ (2 band)	+
167.	IBV/PA/Ck/31714/00		21	-	-
168.	IBV/PA/Ck/34248/00		17	NSB	Weak+
169.	IBV/PA/Ck/34145/00	9	20	+	
170.	IBV/PA/Ck/34316/00		14	NSB	Weak+
171.	IBV/PA/Ck/34882/00		35	-	
172.	IBV/PA/Ck/34895/00		14	NSB	-
173.	IBV/PA/Ck/35288/00		14	Weak +	-
174.	IBV/PA/Ck/30193/99	1	16	+	
175.	IBV/PA/Ck/30066/99	3	21	+	
176.	IBV/PA/Ck/37755/99		35	-	
177.	IBV/PA/Ck/37192/99	1	17	+	
178.	IBV/PA/Ck/37090/99	17	14	NSB	+
179.	IBV/PA/Ck/37005/99	1	14	+	
180.	IBV/PA/Ck/36978/99	1	15	Weak+	+
181.	IBV/PA/Ck/36836/99		35	-	
182.	IBV/PA/Ck/38003/99		37	-	
183.	IBV/PA/Ck/37600/99	1	20	-	+
184.	IBV/PA/Ck/30667/99	1	13	+	
185.	IBV/PA/Ck/32933/99		31	-	
186.	IBV/PA/Ck/31784/98	1	14	+	
187.	IBV/PA/Ck/36222/98		21	NSB	-

188.	IBV/PA/Ck/31786/98		15	NSB	
189.	IBV/PA/Ck/35153/98		18	NSB	-
190.	IBV/PA/Ck/30119/98	1	20	+	
191.	IBV/PA/Ck/30118/98		22	NSB	-
192.	IBV/PA/Ck/32113/98	1	26	+	
193.	IBV/PA/Ck/30652/98	1	16	+	
194.	IBV/PA/Ck/00924/97	1	17	+	
195.	IBV/PA/Ck/02264/97	1	17	+	
196.	IBV/PA/Ck/01561/97		22	-	-
197.	IBV/PA/Ck/01846/97	1	15	+	
198.	IBV/PA/Ck/01847/97	1	15	+	
199.	IBV/PA/Ck/00056/97		12	NSB	-
200.	IBV/PA/Ck/00924/97	1	12	+	
201.	IBV/PA/Ck/01111/97		30	-	
202.	IBV/PA/Ck/01561/97		17	NSB	NSB
203.	IBV/PA/Ck/01482/97		21	NSB	-
204.	IBV/PA/Ck/01270/97	1	17	+	
205.	IBV/PA/Ck/02680/97	1	16	+	
206.	IBV/PA/Ck/02982/97		16	NSB	-
207.	IBV/PA/Ck/02724/97	1	16	+	
208.	IBV/PA/Ck/34787/97	1	16	+	
209.	IBV/PA/Ck/34886/97		13	NSB	NSB

210.	IBV/PA/Ck/34788/97	1	14	+	
211.	IBV/PA/Ck/30680/98	1	14	NSB	+
212.	IBV/PA/Ck/36228/97			NSB	NSB
213.	IBV/PA/Ck/36277/97			NSB	NSB
214.	IBV/PA/Ck/05627/96	27		+	
215.	IBV/PA/Ck/01876/96			NSB	NSB
216.	IBV/PA/Ck/01006/96			+ (2 band)	-
217.	IBV/PA/Ck/02493/96	1		+	
218.	IBV/PA/Ck/02542/96	1		+	
219.	IBV/PA/Ck/02557/96	1		+	
220.	IBV/PA/Ck/05392/96	1		+	
221.	IBV/PA/Ck/05393/96	1		+	
222.	IBV/PA/Ck/05586/96	1		+	
223.	IBV/PA/Ck/05721/96	1			+
224.	IBV/PA/Ck/05722/96	1		+	
225.	IBV/PA/Ck/05723/96	1		+	
226.	IBV/PA/Ck/05628/96	1		+	
227.	IBV/PA/Ck/05292/96			NSB	-
228.	IBV/PA/Ck/00971/96			NSB	-
229.	IBV/PA/Ck/00340/96			NSB	-
230.	IBV/PA/Ck/04373/96	1		+	
231.	IBV/PA/Ck/04074/96			-	-

232.	IBV/PA/Ck/02675/95	3		+	
233.	IBV/PA/Ck/02676/95			-	-
234.	IBV/PA/Ck/02677/95	9		+	
235.	IBV/PA/Ck/02679/95	1			+
236.	IBV/PA/Ck/02680/95			-	Weak + (2 band)
237.	IBV/PA/Ck/02681/95	1			+
238.	IBV/PA/Ck/02682/95	1			+
239.	IBV/PA/Ck/02690/95	1			+
240.	IBV/PA/Ck/02893/95			-	-
241.	IBV/PA/Ck/03157/95			NSB	-
242.	IBV/PA/Ck/03890/95	1			+
243.	IBV/PA/Ck/03900/95			-	-
244.	IBV/PA/Ck/03158/95	1			+
245.	IBV/PA/Ck/01343/95			-	-
246.	IBV/PA/Ck/00051/95			-	-
247.	IBV/PA/Ck/04838/95			-	-
248.	IBV/PA/Ck/04832/95	1			+
249.	IBV/PA/Ck/04263/95			-	-
250.	IBV/PA/Ck/04153/95	1			+
251.	IBV/PA/Ck/04699/95			-	-
252.	IBV/PA/Ck/05514/95			NSB	Weak +
253.	IBV/PA/Ck/11071/94	9			+

254.	IBV/PA/Ck/79009/94			-	NSB
255.	IBV/PA/Ck/79008/94			-	NSB
256.	IBV/PA/Ck/79005/94			-	NSB
257.	IBV/PA/Ck/79007/94			-	-
258.	IBV/PA/Ck/79200/94			-	NSB
259.	IBV/PA/Ck/79013/94			-	NSB
260.	IBV/PA/Ck/79199/94			-	NSB
261.	IBV/PA/Ck/79300/94			-	Weak+
262.	IBV/PA/Ck/07753/93			-	-
263.	IBV/PA/Ck/07752/93			-	-
264.	IBV/PA/Ck/79299/93			-	-



**Fig 1.** IBV phylogeny of sub-lineages for the 154 IBV field isolates from PA poultry, 1990 - 2000.