

Table 1. Commercial IBV vaccines registered for use in South Africa

Vaccine/IBV Strain	Year of vaccine registration/first use	Manufacturer	Type
Nobilis IBMA5 (Mass-strain)	1996	MSD Animal Health	Live attenuated vaccine
Bioral H120 (H120-strain)	1998	Boehringer Ingelheim	Live attenuated vaccine
Gallimune 203 (Mass-strain)/407 (Mass-strain)	2001	Boehringer Ingelheim	Inactivated vaccines
Gallimune 503 (Mass-strain)	2004	Boehringer Ingelheim	Inactivated vaccine
TAbic H120 (H120-strain)	2005	Phibro	Live attenuated vaccine
TAbic VAR 223 (793/B-strain)	2013	Phibro	Live attenuated vaccine
Poulvac IB QX (QX-strain)	2013	Zoetis	Live attenuated vaccine
Nobilis IB 4-91 (4-91 (793/B)-strain)	2013	MSD Animal Health	Live attenuated vaccine
Volvac AC Plus + ND + IB + EDS KV (Mass-strain)	2013	Boehringer Ingelheim	Inactivated vaccines
Bioral H120 Neo (H120-strain)	2014	Boehringer Ingelheim	Live attenuated vaccine
TAbic IBVAR206 (Var-2-strain)	2015	Phibro	Live attenuated vaccine
Hatchpak IB H120 (H120-strain)	2015	Boehringer Ingelheim	Live attenuated vaccine
Nobilis IB Primo QX (QX-strain)	2016	MSD Animal health	Live attenuated vaccine
Poulvac IB Primer (793/B-strain)	2018	Zoetis	Live attenuated vaccine
CEVAC IBIRD (793/B-strain)	2018	Ceva	Live attenuated vaccine

Table 2. Classification system of IBV, grouped by genotype (adapted from Valastro et al., 2016).

Lineage	Period of circulation	Prototype Strain/ Strain name	Country of origin	Collection date	GenBank accession number
GI-1	1937-2013	Beaudette	USA	1937	M95169
GI-2	1954-2006	Holte	USA	1954	GU393336
GI-3	1960-2006	Gray	USA	1960	L14069
GI-4	1962-1998	Holte	USA	1962	L18988
GI-5	1962-2012	N1/62	Australia	1962	U29522
GI-6	1962-2010	VicS	Australia	1962	U29519
GI-7	1964-2012	TP/64	Taiwan	1964	AY606320
GI-8	1965-1967	L165	USA	1965	JQ964061
GI-9	1973-2011	ARK99	USA	1973	M99482
GI-10	1970s-2000s	B	New Zealand	1970s	AF151954
GI-11	1975-2009	UFMG/G	Brazil	1975	JX182775
GI-12	1978-2006	D3896	The Netherlands	1978	X52084
GI-13	1983-2013	Moroccan-G/83	Morocco	1983	EU914938
GI-14	1984-2006	B1648	Belgium	1984	X87238
GI-15	1986-2008	B4	Korea	1986	FJ807932
GI-16	1986-2011	IZO 28/86	Italy	1986	KJ941019
GI-17	1988-1999	CA/Machado/88	USA	1988	AF419315
GI-18	1993-1999	JP8127	Japan	1993	AY296744
GI-19	1993-2012	58HeN-9311	China	1993	KC577395
GI-20	1996-1999	Qu_mv	Canada	1996	AF349621
GI-21	1997-2005	Spain/97/314	Spain	1997	DQ064806
GI-22	1997-2011	40GDGZ-971	China	1997	KC577382
GI-23	1998-2012	Variant 2	Israel	1998	AF093796
GI-24	1998-2013	V13	India	1998	KF757447
GI-25	2004-2013	CA/173704	USA	2004	EU925393
GI-26	2006-2007	NGA/B401/2006	Nigeria	2006	FN182243
GI-27	2008-2013	GA08	USA	2008	GU301925
GI-28	Not available	ck/CH/LGX/11119	China	2011	KX640829
GI-29	Not available	1011/14_China	China	2014	KY407556
GI-30	Not available	18RS1461-1	Trinidad and Tobago	2014	MN696789
GI-31	Not available	92334/11/2/13/C	Cote d'Ivoire (Ivory Coast)	2013	MZ325299
GII-1	1979-1984	D1466	The Netherlands	1979	M21971
GIII-1	1988-2008	N1/88	Australia	1988	U29450
GIV-1	1992-2003	DE/072/92	USA	1991	U77298
GV-1	2002-2008	N4/02	Australia	2002	DQ059618
GVI-1	2007-2012	TC07-2	China	2007	GQ265948

GVII-1	Not available	10636/26_China	China	2016	MH924835
GVIII-1	Not available	PA/1220/98	Poland	1998	AY789942

Table 3. Samples used in this study.

Sample number	Study reference number	Lab reference number	Year isolated	Chicken type	Location
1	P4-30	147868	2010	Grand Parents	North West
2	P5-55	148576	2010	Broilers	North West
3	P5-39	149399	2010	Broilers	Western Cape
4	P4-7	152309	2010	Broilers	North West
5	P2-77	132309 H3	2010	Broilers	Gauteng
6	P4-76	151934 H6	2010	Broilers	North West
7	P5-51	151934 H6	2010	Broilers	North West
8	P4-15	167453	2011	Broilers	North West
9	P4-36	168218	2011	Broilers	Free State
10	P4-6	168355	2011	SPF	Gauteng
11	P4-17	174787	2011	Broilers	Western Cape
12	P5-37	175829	2011	Broilers	Mpumalanga
13	P3-50	175958	2011	Broilers	North West
14	P4-72	176633	2011	Broilers	Mpumalanga
15	P5-63	193307	2011	Broilers	North West
16	P5-36	152953 H9	2011	Broilers	North West
17	P3-84	154830 H6	2011	Broilers	Free State
18	P4-78	154830 H7	2011	Broilers	Free State
19	P3-61	169490 CT	2011	Broilers	North West
20	P3-68	172175 #2	2011	Broilers	North West
21	P3-92	172175 -7 #3	2011	Broilers	North West
22	P4-34	172324-2	2011	Broilers	Free State
23	P4-14	172324-4	2011	Broilers	Free State
24	P3-91	174443 TR	2011	Layers	Western Cape
25	P4-27	175744 KD H1	2011	Broilers	Free State
26	P4-12	175744 KD H1	2011	Broilers	Free State
27	P4-13	175744 KD H5	2011	Broilers	Free State
28	P4-28	175834 TR	2011	Broilers	North West
29	P1-54	193970-3	2011	broilers	Free State
30	P2-51	193970-4	2011	broilers	Free State
31	P1-8	193970-6	2011	broilers	Free State
32	P1-30	186039	2012	Broilers	Gauteng
33	P4-71	187596	2012	Broilers	North West
34	P4-84	187803	2012	Broilers	North West
35	P4-90	188172	2012	Broilers	North West
36	P2-21	188225	2012	Broilers	North West
37	P1-26	189122	2012	Broilers	North West
38	P2-62	189390	2012	Broilers	North West
39	P1-15	189628	2012	Broilers	North West
40	P2-38	189853	2012	Broilers	North West
41	P5-29	190866	2012	Broilers	Mpumalanga
42	P4-68	191145	2012	Broilers	Free State
43	P5-68	191930	2012	Broilers	North West
44	P5-11	192943	2012	Broilers	North West
45	P4-92	193013	2012	Layers	Gauteng

46	P4-82	193043	2012	Broilers	North West
47	P1-49	193138	2012	Broilers	North West
48	P5-24	193304	2012	Broilers	Free State
49	P1-28	193311	2012	Broilers	North West
50	P1-13	193366	2012	Layers	Mpumalanga
51	P4-83	193368	2012	Layers	Gauteng
52	P5-72	193602	2012	Broilers	Mpumalanga
53	P2-58	193774	2012	Broilers	Mpumalanga
54	P2-55	193921	2012	Layers	Western Cape
55	P5-69	194413	2012	Broilers	North West
56	P1-10	194822	2012	Broilers	North West
57	P5-28	195324	2012	Broilers	North West
58	P2-7	195696	2012	Broilers	Mpumalanga
59	P4-96	195854	2012	Broilers	North West
60	P5-66	196674	2012	Broilers	North West
61	P5-85	196723	2012	Broilers	North West
62	P1-9	197318	2012	Broilers	North West
63	P2-14	197346	2012	Broilers	Gauteng
64	P2-10	197346	2012	Broilers	Gauteng
65	P2-46	197826	2012	Unknown	Unknown
66	P1-78	198544	2012	Rearing	Gauteng
67	P5-10	198565	2012	Broilers	Free State
68	P2-39	198573	2012	Unknown	Unknown
69	P1-20	199262	2012	Broilers	Mpumalanga
70	P1-11	199341	2012	Broilers	North West
71	P4-95	200931	2012	Broilers	Free State
72	P5-20	200957	2012	Broilers	North West
73	P5-78	201106	2012	Broilers	North West
74	P5-76	201106	2012	Broilers	North West
75	P5-12	201208	2012	Broilers	Limpopo
76	P5-9	201216	2012	Broilers	North West
77	P5-1	201216	2012	Broilers	North West
78	P5-14	201216	2012	Broilers	North West
79	P3-88	201300	2012	Broilers	Botswana
80	P2-78	201351	2012	Broilers	North West
81	P2-5	201351	2012	Broilers	North West
82	P4-86	201591	2012	Broilers	North West
83	P1-52	201602	2012	Broilers	North West
84	P5-90	202348	2012	Broilers	North West
85	P2-96	203097	2012	Broilers	Gauteng
86	P5-22	203100	2012	Broilers	Gauteng
87	P5-70	203106	2012	Broilers	Gauteng
88	P3-32	203110	2012	Broilers	Gauteng
89	P1-81	206101	2012	Broilers	Mpumalanga
90	P5-15	233607	2012	Broilers	Free State
91	P5-48	187464 KN H2 AF	2012	Broilers	Western Cape
92	P5-35	187464 KN H3	2012	Broilers	Western Cape
93	P5-49	187464 TR H1 AF	2012	Broilers	Western Cape

94	P5-40	187464 TR H2 AF	2012	Broilers	Western Cape
95	P2-56	187464 TR H2 YS	2012	Broilers	Western Cape
96	P5-47	187464 TR H3 AF	2012	Broilers	Western Cape
97	P1-4	187521 H1	2012	SPF	Gauteng
98	P5-54	187521 H2	2012	SPF	Gauteng
99	P2-41	197270 H2	2012	Broilers	North West
100	P4-94	197270 H3	2012	Broilers	North West
101	P1-27	197666 H2	2012	Broilers	Gauteng
102	P1-7	197666 H4	2012	Broilers	Gauteng
103	P2-3	197677 H1	2012	Broilers	North West
104	P1-61	197677 H2	2012	Broilers	North West
105	P5-8	198007 H2	2012	Broilers	North West
106	P5-84	198007 H3	2012	Broilers	North West
107	P5-77	198007 H4	2012	Broilers	North West
108	P1-62	198442 CE	2012	Broilers	Free State
109	P2-9	198442 SF	2012	Broilers	Free State
110	P1-44	198442 TR	2012	Broilers	Free State
111	P2-28	198999 B9	2012	Broilers	North West
112	P5-3	200656 H3	2012	Broilers	North West
113	P5-80	200656 H4	2012	Broilers	North West
114	P5-82	200656 H4	2012	Broilers	North West
115	P5-7	200677 HA	2012	Broilers	North West
116	P5-87	200677 HB	2012	Broilers	North West
117	P5-2	200963 H5	2012	Broilers	North West
118	P5-59	200963 H7	2012	Broilers	North West
119	P5-86	200963 H7	2012	Broilers	North West
120	P2-91	205069 AF H6	2012	Broilers	Free State
121	P1-87	205069 YS H6	2012	Broilers	Free State
122	P1-86	205069 YS H6	2012	Broilers	Free State
123	P3-96	208097	2013	Broilers	Free State
124	P2-89	208136	2013	Layers	Gauteng
125	P2-92	208232	2013	Broilers	North West
126	P1-85	208890	2013	Broilers	Free State
127	P2-94	208981	2013	Broilers	Free State
128	P3-74	208982	2013	Broilers	Mpumalanga
129	P3-31	212412	2013	Grand Parents	Gauteng
130	P3-33	213416	2013	Broilers	Namibia
131	P3-34	215665	2013	Broilers	North West
132	P3-75	215735	2013	Broilers	Eswatini
133	P2-83	216935	2013	Unknown	Unknown
134	P2-95	217716	2013	Layers	Gauteng
135	P1-89	218586	2013	Broilers	Mpumalanga
136	P2-84	220197	2013	Broilers	Mpumalanga
137	P1-96	220680	2013	Broilers	Mpumalanga
138	P1-91	222046	2013	Layers	Gauteng
139	P1-84	222493	2013	Broilers	North West
140	P2-87	222625	2013	Broilers	North West
141	P1-92	222760	2013	Layers	Gauteng

142	P1-82	223677	2013	Layers	Eastern Cape
143	P2-44	224387	2013	Unknown	Unknown
144	P2-86	225510	2013	Unknown	Gauteng
145	P2-66	226220	2013	Broilers	North West
146	P3-11	226600	2013	Broilers	North West
147	P5-88	226789	2013	Unknown	KwaZulu Natal
148	P3-43	227152	2013	Broilers	KwaZulu Natal
149	P1-33	227659	2013	Broilers	North West
150	P1-31	227829	2013	Broilers	North West
151	P1-22	228016	2013	Broilers	North West
152	P2-37	229015	2013	Broilers	Free State
153	P4-60	229079	2013	Broilers	Free State
154	P4-70	229081	2013	Unknown	Gauteng
155	P5-64	229507	2013	Broilers	Free State
156	P2-82	230968	2013	Broilers	North West
157	P5-30	231139	2013	Broilers	Free State
158	P5-25	231217	2013	Broilers	Gauteng
159	P4-59	231808	2013	Broilers	North West
160	P1-37	231832	2013	Broilers	Mpumalanga
161	P4-91	232718	2013	Broilers	North West
162	P5-74	232719	2013	Broilers	Gauteng
163	P2-80	233942	2013	Broilers	Mpumalanga
164	P1-29	233979	2013	Broilers	North West
165	P2-52	234109	2013	Broilers	Free State
166	P2-71	234993	2013	Broilers	North West
167	P1-6	235198	2013	Broilers	Free State
168	P1-60	235447	2013	Broilers	North West
169	P1-66	236910	2013	Broilers	North West
170	P2-27	236912	2013	Broilers	North West
171	P1-40	238153	2013	Grand Parents	KwaZulu Natal
172	P2-24	240525	2013	Broilers	Free State
173	P1-57	242100	2013	Broilers	North West
174	P2-53	243570	2013	Breeders	North West
175	P1-64	244155	2013	Broilers	Free State
176	P2-29	244663	2013	Broilers	Free State
177	P2-61	245067	2013	Broilers	Western Cape
178	P3-73	209783 CE	2013	Broilers	North West
179	P3-36	209783 TR	2013	Broilers	North West
180	P3-72	209842 H2	2013	Breeders	North West
181	P3-95	209842 H2	2013	Breeders	North West
182	P1-93	210882 H1	2013	Layers	Gauteng
183	P3-41	210882 H3	2013	Layers	Gauteng
184	P1-94	221316 - ORG	2013	Broilers	North West
185	P2-93	221316 - CE	2013	Broilers	North West
186	P2-85	221316 TR	2013	Broilers	North West
187	P1-88	222042 H1	2013	Rearing	North West
188	P2-90	222042 H2	2013	Rearing	North West
189	P1-83	225250 H1	2013	Broilers	Namibia

190	P2-88	225250 H5	2013	Broilers	Namibia
191	P1-95	225518 H10	2013	Broilers	North West
192	P5-31	225518 H11	2013	Broilers	North West
193	P5-89	225518 H12	2013	Broilers	North West
194	P3-60	226668 H2	2013	Unknown	Unknown
195	P3-40	226827 HD	2013	Unknown	Unknown
196	P3-77	226827 HE	2013	Unknown	Unknown
197	P3-86	226827 HF	2013	Unknown	Unknown
198	P4-80	227831 TR	2013	Broilers	North West
199	P2-72	227831 TR	2013	Broilers	North West
200	P5-16	229207 CE	2013	Broilers	Free State
201	P5-58	229207 TR	2013	Broilers	Free State
202	P5-18	229498 H1	2013	Broilers	North West
203	P4-73	229498 H2	2013	Broilers	North West
204	P2-57	232786 H4	2013	Unknown	Unknown
205	P2-63	232786 H4	2013	Unknown	Unknown
206	P1-50	233429 CE	2013	Broilers	North West
207	P3-30	233429 CE	2013	Broilers	North West
208	P1-24	234962 H13	2013	Broilers	North West
209	P1-2	234962 H7	2013	Broilers	North West
210	P2-49	235104 TR	2013	Layers	Western Cape
211	P2-15	236104 BR	2013	Broilers	North West
212	P1-51	236104 CE	2013	Broilers	North West
213	P3-14	246538	2014	Broilers	Western Cape
214	P1-5	249371	2014	Breeders	Gauteng
215	P2-12	253792	2014	Broilers	Free State
216	P1-14	253810	2014	Broilers	Free State
217	P1-69	253811	2014	Broilers	Free State
218	P2-25	256015	2014	Broilers	Limpopo
219	P2-6	259695	2014	Broilers	North West
220	P1-35	260228	2014	Broilers	Free State
221	P1-53	260229	2014	Broilers	Free State
222	P2-32	260600	2014	Vaccine production	Gauteng
223	P1-68	260884	2014	Broilers	Free State
224	P3-64	261158	2014	Broilers	Free State
225	P3-62	263752	2014	Broilers	Free State
226	P1-63	266932	2014	Broilers	North West
227	P2-20	266968	2014	Broilers	Free State
228	P1-45	267693	2014	Broilers	Mpumalanga
229	P3-16	267739	2014	Broilers	Free State
230	P3-48	267839	2014	Broilers	Western Cape
231	P3-6	268083	2014	Broilers	Gauteng
232	P3-9	268083	2014	Broilers	Gauteng
233	P3-70	268361	2014	Broilers	Gauteng
234	P3-23	269875	2014	Broilers	Free State
235	P3-46	270254	2014	Broilers	North West
236	P1-23	274387	2014	Broilers	Free State
237	P2-60	274626	2014	Broilers	Free State

238	P3-22	275478	2014	Broilers	Free State
239	P3-1	275644	2014	Broilers	Free State
240	P1-39	276515	2014	Broilers	Mpumalanga
241	P3-47	276628	2014	Broilers	Free State
242	P4-87	276753	2014	Broilers	Free State
243	P5-61	276811	2014	Layers	Gauteng
244	P5-62	276824	2014	Broilers	Free State
245	P4-81	277553	2014	Broilers	Free State
246	P5-23	277560	2014	Broilers	North West
247	P3-15	278583	2014	Broilers	Free State
248	P3-52	278628	2014	Broilers	Free State
249	P5-26	278832	2014	Broilers	Free State
250	P3-27	280023	2014	Broilers	Free State
251	P3-25	281216	2014	Broilers	Free State
252	P3-78	281572	2014	Broilers	Free State
253	P3-44	281574	2014	Broilers	Free State
254	P5-21	281975	2014	Broilers	Free State
255	P1-41	284195	2014	Broilers	Western Cape
256	P5-65	287871	2014	Broilers	North West
257	P5-83	288325	2014	Layers	Gauteng
258	P5-5	289015	2014	Broilers	North West
259	P2-11	295637	2014	Layers	Gauteng
260	P3-20	245842 H2	2014	Broilers	Free State
261	P2-35	245842 H3	2014	Broilers	Free State
262	P1-25	259214 S1	2014	Breeders	North West
263	P2-76	259214 S2	2014	Breeders	North West
264	P2-67	264213 H1	2014	Broilers	Gauteng
265	P2-79	264213 H1	2014	Broilers	Gauteng
266	P1-38	264213 H2	2014	Broilers	Gauteng
267	P3-59	269246 AF	2014	Broilers	KwaZulu Natal
268	P3-49	269246 BR	2014	Broilers	KwaZulu Natal
269	P3-79	269246 CT	2014	Broilers	KwaZulu Natal
270	P3-17	269246 KID	2014	Broilers	KwaZulu Natal
271	P3-53	271729 H1	2014	Broilers	Free State
272	P3-3	271729 H2	2014	Broilers	Free State
273	P4-35	274025 H	2014	Broilers	Free State
274	P2-45	274025 S	2014	Broilers	Free State
275	P3-57	276453 H1	2014	Layers	Gauteng
276	P3-10	276453 H2	2014	Layers	Gauteng
277	P2-13	282562 H15	2014	Broilers	Namibia
278	P1-21	282562 H4	2014	Broilers	Namibia
279	P5-60	282913 H2	2014	Layers	Gauteng
280	P5-13	282913 H3	2014	Layers	Gauteng
281	P5-4	288392 H2	2014	Broilers	Gauteng
282	P5-67	288392 H3	2014	Broilers	Gauteng
283	P5-6	289368 H10	2014	Broilers	North West
284	P1-79	289368 H10	2014	Broilers	North West
285	P5-75	289368 H7	2014	Broilers	North West

286	P2-54	Unknown	2014	Unknown	Western Cape
287	P3-67	309400	2015	Allantoic fluid	Western Cape
288	P3-8	314462	2015	Broilers	Gauteng
289	P3-12	316661	2015	Broilers	Western Cape
290	P3-45	317372	2015	Broilers	North West
291	P3-66	318879	2015	Broilers	North West
292	P3-80	319685	2015	Broilers	Free State
293	P3-18	320002	2015	Broilers	Free State
294	P3-28	320057	2015	Broilers	North West
295	P3-29	320213	2015	Broilers	Western Cape
296	P3-4	320709	2015	Broilers	North West
297	P3-21	320844	2015	Broilers	Free State
298	P3-58	321496	2015	Broilers	Western Cape
299	P2-18	321872	2015	Breeders	Gauteng
300	P4-38	321872	2015	Breeders	Gauteng
301	P3-55	322303	2015	Broilers	Gauteng
302	P3-5	322393	2015	Broilers	Free State
303	P2-43	322394	2015	Broilers	Free State
304	P3-54	322903	2015	Broilers	Free State
305	P3-19	323131	2015	Broilers	Free State
306	P1-73	325285	2015	Broilers	Gauteng
307	P2-22	327042	2015	Broilers	Free State
308	P1-67	327066	2015	Broilers	Free State
309	P1-59	329067	2015	Broilers	Free State
310	P2-8	329169	2015	Broilers	Free State
311	P1-18	330071	2015	Broilers	Free State
312	P2-69	332216	2015	Broilers	Free State
313	P1-65	332607	2015	Broilers	Limpopo
314	P1-70	332825	2015	Broilers	Free State
315	P3-24	335734	2015	Broilers	Free State
316	P2-70	335734	2015	Broilers	Free State
317	P2-33	335997	2015	Broilers	Namibia
318	P3-76	336625	2015	Broilers	Free State
319	P1-56	337288	2015	Broilers	Free State
320	P3-42	338905	2015	Broilers	Free State
321	P2-47	339318	2015	Broilers	Free State
322	P2-16	339989	2015	Broilers	Free State
323	P5-79	340340	2015	Broilers	Gauteng
324	P5-27	340461	2015	Broilers	Gauteng
325	P1-48	340744	2015	Broilers	Gauteng
326	P5-81	341521	2015	Broilers	Gauteng
327	P4-79	342217	2015	Broilers	North West
328	P5-17	344201	2015	Breeders	North West
329	P2-74	344383	2015	Broilers	Mpumalanga
330	P1-16	344385	2015	Broilers	Free State
331	P4-67	344385	2015	Broilers	Free State
332	P2-19	345298	2015	Broilers	Gauteng
333	P1-32	345568	2015	Broilers	Free State

334	P1-42	346464	2015	Broilers	Gauteng
335	P2-2	347675	2015	Broilers	Free State
336	P3-63	348514	2015	Broilers	North West
337	P2-64	348519	2015	Broilers	North West
338	P5-19	348520	2015	Broilers	North West
339	P4-75	348521	2015	Broilers	North West
340	P1-77	349150	2015	Broilers	Gauteng
341	P2-34	349476	2015	Broilers	North West
342	P1-71	349480	2015	Broilers	North West
343	P3-13	351011	2015	Broilers	Free State
344	P3-87	351288	2015	Broilers	Free State
345	P3-7	351505	2015	Broilers	Free State
346	P1-43	305670 H2	2015	Broilers	Gauteng
347	P1-1	305670 H3	2015	Broilers	Gauteng
348	P2-48	305670 H4	2015	Broilers	Gauteng
349	P1-46	309115 H3	2015	Broilers	Gauteng
350	P2-40	309115 H8	2015	Broilers	Gauteng
351	P2-42	313190 H1	2015	Broilers	North West
352	P3-65	313190 H3	2015	Broilers	North West
353	P1-90	327985 H12	2015	Broilers	North West
354	P2-75	327985 H18	2015	Broilers	North West
355	P3-35	336344 H1	2015	Broilers	Gauteng
356	P3-71	336344 H2	2015	Broilers	Gauteng
357	P4-9	336344 H7	2015	Broilers	Gauteng
358	P3-81	336344 H8	2015	Broilers	Gauteng
359	P1-72	337109 H5	2015	Broilers	Free State
360	P1-12	337109 H7	2015	Broilers	Free State
361	P1-3	346613 H2	2015	Broilers	Free State
362	P2-36	346613 H4	2015	Broilers	Free State
363	P4-74	347477 H2	2015	Broilers	North West
364	P5-73	347477 H3	2015	Broilers	North West
365	P5-71	347626 H1	2015	Broilers	Gauteng
366	P4-69	347626 H2	2015	Broilers	Gauteng
367	P1-74	348513 AF	2015	Broilers	North West
368	P3-38	348513 CAM	2015	Broilers	North West
369	P1-58	348513 CAM	2015	Broilers	North West
370	P3-51	348517 H1	2015	Broilers	North West
371	P2-81	348517 H2	2015	Broilers	North West
372	P3-37	348517 H6	2015	Broilers	North West
373	P3-69	350676 C3	2015	Broilers	North West
374	P2-30	350676 C5	2015	Broilers	North West
375	P1-55	351290 H2	2015	Broilers	North West
376	P4-61	351290 H9	2015	Broilers	North West
377	P1-75	351290 H9	2015	Broilers	North West
378	P3-39	355673	2016	Broilers	North West
379	P2-4	363621	2016	Broilers	Gauteng
380	P4-2	372100	2016	Layers	Gauteng
381	P3-93	374409	2016	Broilers	Mpumalanga

382	P4-5	375003	2016	Broilers	Free State
383	P2-65	377455	2016	Broilers	Free State
384	P1-34	377465	2016	Broilers	Free State
385	P1-47	377467	2016	Broilers	Free State
386	P2-73	379353	2016	Broilers	Free State
387	P2-59	383258	2016	Broilers	Gauteng
388	P1-76	383258	2016	Broilers	Gauteng
389	P2-1	388559	2016	Broilers	Free State
390	P1-19	392670	2016	Broilers	Free State
391	P2-26	392673	2016	Broilers	Free State
392	P2-31	403218	2016	Broilers	Free State
393	P2-23	403218	2016	Broilers	Free State
394	P3-26	358886 AF H6	2016	Broilers	Gauteng
395	P3-2	358886 H4	2016	Broilers	Gauteng
396	P3-56	358886 YS H6	2016	Broilers	Gauteng
397	P2-50	359396 H4	2016	Broilers	Namibia
398	P1-80	359396 H5	2016	Broilers	Namibia
399	P1-36	409284	2017	Broilers	North West
400	P4-4	462411	2017	Broilers	Free State
401	P2-68	461064 H2	2017	Broilers	Free State
402	P2-17	461064 H4	2017	Broilers	Free State
403	P4-25	461064 H6	2017	Broilers	Free State
404	P4-1	526182	2018	Broilers	Gauteng
405	P3-82	526250	2018	Broilers	North West
406	P4-20	530520	2018	Broilers	North West
407	P1-17	531407	2018	Broilers	North West
408	P4-3	531796	2018	Broilers	Western Cape
409	P4-10	537307	2018	Broilers	Western Cape
410	P4-16	537309	2018	Broilers	Western Cape
411	P3-94	533511 H9	2018	Broilers	Free State
412	P3-90	533551 H6	2018	Layers	Free State
413	P4-11	538641 B4	2018	Broilers	Namibia
414	P4-8	538641 B5	2018	Broilers	Namibia
415	P4-19	537307	2019	Unknown	Unknown
416	P4-29	545467	2019	Broilers	Western Cape
417	P4-18	550819	2019	Broilers	Western Cape
418	P4-62	564876	2019	Broilers	Unknown
419	P5-43	568903	2019	Breeders	Mpumalanga
420	P5-34	579119	2019	Broilers	Eastern Cape
421	P5-41	586150	2019	Breeders	Gauteng
422	P5-57	592655	2019	Broilers	KwaZulu Natal
423	P5-42	599490	2019	Broilers	North West
424	P5-50	601368	2019	Broilers	KwaZulu Natal
425	P4-89	603902	2019	Broilers	Mpumalanga
426	P4-85	605307	2019	Unknown	Unknown
427	P5-33	606426	2019	Unknown	Unknown
428	P5-56	608301	2019	Unknown	Unknown
429	P5-45	608305	2019	Unknown	Unknown

430	P3-85	578946 N2	2019	Layers	Gauteng
431	P3-83	578946 N5	2019	Layers	Gauteng
432	P4-23	578946 N6	2019	Layers	Gauteng
433	P4-93	599479 H3	2019	Broilers	North West
434	P5-32	599479 H4	2019	Broilers	North West
435	P4-88	617999	2020	Unknown	Unknown
436	P5-46	626739	2020	Unknown	Unknown
437	P5-52	634004	2020	Unknown	Unknown
438	P4-77	634038	2020	Unknown	Unknown
439	P5-44	635300	2020	Unknown	Unknown
440	P5-53	638883	2020	Unknown	Unknown
441	P4-31	656517	2020	Unknown	Unknown
442	P4-33	658187	2020	Unknown	Unknown
443	P4-32	659486	2020	Unknown	Unknown
444	P4-24	659489	2020	Unknown	Unknown
445	P3-89	662543	2020	Unknown	Unknown
446	P5-38	638634 IV	2020	Unknown	Unknown
447	P4-22	658421 H1	2020	Unknown	Unknown

Table 4. Master mix reaction per sample

Reagent	Volume
Nuclease free water	5 μ l
Phusion Flash (Thermo Fisher Scientific, Waltham, Massachusetts)	10 μ l
mmLV RT enzyme (Thermo Fisher Scientific, Waltham, Massachusetts)	0.3 μ l
RNAse Inhibitor (Thermo Fisher Scientific, Waltham, Massachusetts)	0.2 μ l
Primers (S17F and S18R)	1 μ l
RNA template	3.5 μ l
Total reaction volume	20 μ l

Table 5. IUPAC nucleotide code

Nucleotide	Common display	Meaning	Mnemonic
A	A	A	Adenine
C	C	C	Cytosine
G	G	G	Guanine
T (or U)	T	T (or U)	Thymine (or Uracil)
R	A/G	A or G	Purine
Y	C/T	C or T (or U)	Pyrimidines
K	G/T	G or T (or U)	Bases which are ketones
M	A/C	A or C	Bases with amino groups
S	C/G	C or G	Strong interaction
W	A/T	A or T (or U)	Weak interaction
B	C/G/T	Not A (i.e., C, G, T or U)	B comes after A
D	A/G/T	Not C (i.e., A, G, T or U)	D comes after C
H	A/C/T	Not G (i.e., A, C, T or U)	H comes after G
V	A/C/G	Neither T nor U (i.e., A, C or G)	V comes after U
N	A/C/G/T	A or C or G or T (or U)	Nucleic acid
. or -	-	Gap of indeterminate length	

Table 6. Reference sequences

Lineage	GenBank accession number
GI-1	FJ888351, AY561711, M95169
GI-2	GU393336, DQ070840
GI-3	L14069, L14070
GI-4	L18988, AY251816
GI-5	DQ490215, U29522
GI-6	U29519, DQ515802
GI-7	AY606320, DQ646405
GI-8	M99484
GI-9	AF006624, M99482, DQ912831
GI-10	AF15159, AF15160, AF15154
GI-11	GU393339, JX182783
GI-12	X52084, X15832
GI-13	Z83975, EU914938
GI-14	X87238, FN182277
GI-15	FJ807932, FJ807944
GI-16	AF286302, KJ941019
GI-17	AF510656, AF027509, AF419315
GI-18	AY296744, KC577391
GI-19	AF193423, KC577395, AY189157
GI-20	AF349620, AF349621
GI-21	AJ457137, DQ064808
GI-22	GQ265940, DQ167147, KC577382
GI-23	KY805846, AF093796
GI-24	KF757447, KF809796
GI-25	KM660636, KP085595, EU925393
GI-26	MZ325299_D2334, FN182268, FN182270
GI-27	GU301925, KM660634
GI-28	JX291989, KX640829
GI-29	KY407556, KY407558
GII-1	M21971, M21968
GIII-1	U29450, U29521
GIV-1	U77298, AF274436
GV-1	FJ235194, JX018208, DQ059618
GVI-1	KF007209, GQ265948
GVII-1	MH924835, KM365468
GVIII	AY789942

Table 7. DNA concentrations and RRT - PCR Results

Sample number	Study reference number	Repeat reference number	Lab reference number	Band present	Repeat band present	DNA Conc. (ng/μl)	Repeat Conc. (ng/μl)
1	P4-30		147868	Yes		16.2	
2	P5-55		148576	Yes		14.7	
3	P5-39		149399	Yes		12	
4	P4-7		152309	Yes		8.1	
5	P4-15		167453	Yes		12.7	
6	P4-36	P8-27	168218	No	Yes		10.8
7	P4-6		168355	Yes		8.3	
8	P4-17		174787	Yes		10.2	
9	P5-37		175829	Yes		13.4	
10	P3-50		175958	Yes		16.6	
11	P4-72		176633	Yes		12.6	
12	P1-30		186039	Yes		20.7	
13	P4-71		187596	Yes		30.1	
14	P4-84	P8-32	187803	Yes	Yes	13.5	15.3
15	P4-90		188172	Yes		13.9	
16	P2-21		188225	Yes		8.3	
17	P1-26		189122	Yes		10.7	
18	P2-62		189390	Yes		16.4	
19	P1-15		189628	Yes		10.2	
20	P2-38		189853	Yes		13.1	
21	P5-29		190866	Yes		15	
22	P4-68		191145	Yes		11.9	
23	P5-68		191930	Yes		17	
24	P5-11		192943	Yes		26.1	
25	P4-92		193013	Yes		22.6	
26	P4-82		193043	Yes		8.7	
27	P1-49		193138	Yes		14.9	
28	P5-24		193304	Yes		21.2	
29	P5-63		193307	Yes		17.3	
30	P1-28		193311	Yes		12.9	
31	P1-13		193366	Yes		12.8	
32	P4-83	P8-31	193368	Yes	Yes	6.5	12.3
33	P5-72		193602	Yes		8.4	
34	P2-58		193774	Yes		16.2	
35	P2-55	P9-29	193921	Yes	Yes	22.6	4.9
36	P5-69		194413	Yes		15.8	
37	P1-10		194822	Yes		13.6	
38	P5-28		195324	Yes		14.4	
39	P2-7		195696	Yes		8.1	
40	P4-96		195854	Yes		16.2	
41	P5-66		196674	Yes		14.5	
42	P5-85	P8-52	196723	No	No		
43	P1-9	P9-2	197318	No	Yes		8.5
44	P2-14		197346	Yes		6.5	
45	P2-10	P9-13	197346	Yes	Yes	0.4	8.1
46	P2-46	P9-20	197826	Yes	Yes	7.5	4.1
47	P1-78		198544	Yes		9.4	

48	P5-10	P8-34	198565	Yes	Yes	41.4	9.5
49	P2-39		198573	Yes		13.1	
50	P1-20		199262	Yes		5.8	
51	P1-11		199341	Yes		13.1	
52	P4-95		200931	Yes		15	
53	P5-20		200957	Yes		19.1	
54	P5-78	P8-50	201106	Yes	Yes	8.5	8.2
55	P5-76	P8-49	201106	Yes	Yes	15.7	29.9
56	P5-12	P8-35	201208	Yes	No	4.5	
57	P5-9		201216	Yes		13.6	
58	P5-1		201216	Yes		26.1	
59	P5-14	P8-37	201216	Yes	Yes	12.8	5.7
60	P3-88	P8-19	201300	Yes	Yes	6	8.8
61	P2-78	P9-43	201351	Yes	Yes	13.7	5.1
62	P2-5	P8-4	201351	Yes	No	1.2	
63	P4-86		201591	Yes		6.2	
64	P1-52		201602	Yes		8.3	
65	P5-90		202348	Yes		13.7	
66	P2-96	P9-52	203097	Yes	Yes	17.4	11
67	P5-22		203100	Yes		10.5	
68	P5-70		203106	Yes		13.7	
69	P3-32	P9-69	203110	Yes	Yes	31.3	7
70	P1-81		206101	Yes		9.8	
71	P3-96	P8-22	208097	Yes	Yes	14.6	3.7
72	P2-89	P8-8	208136	Yes	Yes	16.2	4.5
73	P2-92	P8-9	208232	Yes	No	17.8	
74	P1-85		208890	Yes		16.2	
75	P2-94		208981	Yes		16.5	
76	P3-74	P9-83	208982	Yes	Yes	17.8	14.1
77	P3-31		212412	Yes		20.4	
78	P3-33	P9-70	213416	Yes	Yes	19.2	11.5
79	P3-34		215665	Yes		20.2	
80	P3-75	P8-13	215735	Yes	Yes	10.6	9.2
81	P2-83	P9-48	216935	Yes	Yes	8.2	9.5
82	P2-95		217716	Yes		27.3	
83	P1-89		218586	Yes		13.8	
84	P2-84	P9-49	220197	Yes	Yes	13.4	12.4
85	P1-96	P9-9	220680	Yes	Yes	0.4	21.8
86	P1-91		222046	Yes		20.6	
87	P1-84	P9-6	222493	No	Yes		7.8
88	P2-87	P8-7	222625	Yes	Yes	13.1	4.7
89	P1-92	P9-7	222760	No	Yes		9.1
90	P1-82		223677	Yes		2.8	
91	P2-44	P9-18	224387	Yes	Yes	9	11.1
92	P2-86	P9-51	225510	Yes	Yes	13.9	13.1
93	P2-66	P9-34	226220	No	No		
94	P3-11		226600	Yes		25.8	
95	P5-88		226789	Yes		5.8	
96	P3-43		227152	Yes		12.9	
97	P1-33		227659	Yes		9.6	
98	P1-31		227829	Yes		22.2	

99	P1-22		228016	Yes		17.9	
100	P2-37		229015	Yes		13.5	
101	P4-60		229079	Yes		13.8	
102	P4-70	P8-29	229081	No	Yes		13.5
103	P5-64		229507	Yes		33.4	
104	P2-82	P9-47	230968	Yes	Yes	25.3	14.8
105	P5-30	P8-39	231139	No	Yes		10.2
106	P5-25		231217	Yes		22	
107	P4-59		231808	Yes		15.9	
108	P1-37		231832	Yes		14.7	
109	P4-91		232718	Yes		13	
110	P5-74		232719	Yes		34.8	
111	P5-15	P8-38	233607	No	No		
112	P2-80	P9-45	233942	Yes	Yes	27.1	21.3
113	P1-29		233979	Yes		12.4	
114	P2-52	P9-26	234109	Yes	Yes	19.1	5.5
115	P2-71	P9-38	234993	Yes	Yes	25.1	8
116	P1-6		235198	Yes		5.8	
117	P1-60		235447	No			
118	P1-66		236910	Yes		7.8	
119	P2-27		236912	Yes		21.4	
120	P1-40		238153	Yes		25.1	
121	P2-24		240525	Yes		18.1	
122	P1-57		242100	Yes		11.4	
123	P2-53	P9-27	243570	Yes	Yes	12.3	7.3
124	P1-64		244155	Yes		9.3	
125	P2-29		244663	Yes		12.8	
126	P2-61		245067	Yes		21.8	
127	P3-14	P9-63	246538	Yes	Yes	23.7	11.8
128	P1-5		249371	Yes		6.7	
129	P2-12		253792	Yes		3.1	
130	P1-14		253810	Yes		11.7	
131	P1-69		253811	Yes		10.6	
132	P2-25		256015	Yes		18	
133	P2-6	P8-5	259695	No	Yes		7.5
134	P1-35		260228	Yes		13.1	
135	P1-53		260229	Yes		14.1	
136	P2-32		260600	Yes		10.5	
137	P1-68	P9-4	260884	No	Yes		11.9
138	P3-64		261158	Yes		27.6	
139	P3-62		263752	Yes		25	
140	P1-63		266932	Yes		16	
141	P2-20		266968	Yes		17.2	
142	P1-45		267693	Yes		27.1	
143	P3-16		267739	Yes		23.2	
144	P3-48	P9-74	267839	Yes	No	7.9	
145	P3-6	P9-58	268083	Yes	Yes	18.2	13.5
146	P3-9	P9-60	268083	Yes	Yes	25.1	9.7
147	P3-70		268361	Yes		15.1	
148	P3-23	P9-67	269875	Yes	Yes	40.7	10.1
149	P3-46		270254	Yes		8.6	

150	P1-23		274387	Yes		22.3	
151	P2-60	P9-33	274626	Yes	Yes	21.9	5.9
152	P3-22	P9-66	275478	Yes	Yes	21.2	9.1
153	P3-1	P9-53	275644	Yes	Yes	26.8	13.5
154	P1-39		276515	Yes		12.8	
155	P3-47		276628	Yes		20.6	
156	P4-87		276753	Yes		13.1	
157	P5-61		276811	Yes		20.8	
158	P5-62		276824	Yes		16.9	
159	P4-81		277553	Yes		6.3	
160	P5-23		277560	Yes		18.5	
161	P3-15	P9-64	278583	Yes	Yes	17.4	10.5
162	P3-52		278628	Yes		25.7	
163	P5-26		278832	Yes		21.1	
164	P3-27		280023	Yes		30	
165	P3-25		281216	Yes		28.1	
166	P3-78	P8-16	281572	Yes	No	17.9	
167	P3-44		281574	Yes		21.6	
168	P5-21		281975	Yes		21.2	
169	P1-41		284195	Yes		17.2	
170	P5-65		287871	Yes		17.7	
171	P5-83		288325	Yes		28.5	
172	P5-5		289015	Yes		14.5	
173	P2-11		295637	Yes		5.3	
174	P3-67	P9-78	309400	Yes	Yes	16.2	8.2
175	P3-8		314462	Yes		31.4	
176	P3-12	P9-62	316661	Yes	Yes	17.4	14
177	P3-45	P9-73	317372	Yes	Yes	12.3	13.8
178	P3-66		318879	Yes		18.5	
179	P3-80	P8-18	319685	Yes	Yes	17.7	12.3
180	P3-18	P9-65	320002	Yes	Yes	14.8	10.5
181	P3-28		320057	Yes		26.9	
182	P3-29		320213	Yes		35.6	
183	P3-4	P9-56	320709	Yes	Yes	16.4	13.2
184	P3-21		320844	Yes		20.2	
185	P3-58		321496	Yes		27	
186	P2-18		321872	Yes		13.9	
187	P4-38	P8-28	321872	Yes	Yes	23.4	10
188	P3-55	P9-75	322303	No	No		
189	P3-5	P9-57	322393	Yes	Yes	15.1	12.4
190	P2-43		322394	Yes		21	
191	P3-54		322903	Yes		22	
192	P3-19		323131	Yes		18.8	
193	P1-73		325285	Yes		14	
194	P2-22		327042	Yes		15.9	
195	P1-67		327066	Yes		12.4	
196	P1-59		329067	Yes		7.3	
197	P2-8	P8-6	329169	Yes	Yes	8.2	4
198	P1-18		330071	Yes		13.2	
199	P2-69	P9-36	332216	No	No		
200	P1-65		332607	Yes		6.1	

201	P1-70		332825	Yes		9.2	
202	P3-24		335734	Yes		29.9	
203	P2-70	P9-37	335734	Yes	No	9.2	
204	P2-33		335997	Yes		19.8	
205	P3-76	P8-14	336625	Yes	Yes	21.1	8.9
206	P1-56		337288	Yes		14	
207	P3-42	P9-72	338905	No	No		
208	P2-47	P9-21	339318	Yes	Yes	19.6	12.1
209	P2-16		339989	Yes		23.1	
210	P5-79		340340	Yes		25.1	
211	P5-27		340461	Yes		13.4	
212	P1-48		340744	Yes		12.9	
213	P5-81		341521	Yes		34.8	
214	P4-79		342217	Yes		16.5	
215	P5-17		344201	Yes		16.8	
216	P2-74		344383	No			
217	P1-16		344385	Yes		21.3	
218	P4-67		344385	Yes		7.1	
219	P2-19		345298	Yes		17.4	
220	P1-32		345568	Yes		28.3	
221	P1-42		346464	Yes		13.9	
222	P2-2		347675	Yes		8.8	
223	P3-63		348514	Yes		30.9	
224	P2-64		348519	Yes		28.7	
225	P5-19		348520	Yes		22.5	
226	P4-75		348521	Yes		15.4	
227	P1-77		349150	Yes		7.3	
228	P2-34		349476	Yes		10.7	
229	P1-71		349480	Yes		13.1	
230	P3-13		351011	Yes		11.3	
231	P3-87		351288	Yes		22.9	
232	P3-7	P9-59	351505	No	Yes		11.1
233	P3-39		355673	Yes		29.3	
234	P2-4	P9-11	363621	Yes	Yes	11.6	10.1
235	P4-2		372100	Yes		6	
236	P3-93		374409	Yes		25.8	
237	P4-5		375003	Yes		12.9	
238	P2-65		377455	Yes		21.3	
239	P1-34		377465	Yes		11.4	
240	P1-47		377467	Yes		21.2	
241	P2-73	P9-39	379353	Yes	Yes	10.5	6.3
242	P2-59	P9-32	383258	Yes	Yes	14.8	13.5
243	P1-76	P9-5	383258	No	Yes		14.2
244	P2-1		388559	Yes		11.4	
245	P1-19		392670	Yes		23.3	
246	P2-26		392673	Yes		13.9	
247	P2-31		403218	Yes		13.4	
248	P2-23		403218	Yes		18.7	
249	P1-36		409284	Yes		15.2	
250	P4-4		462411	Yes		15.3	
251	P4-1		526182	Yes		9.9	

252	P3-82		526250	Yes		19.3	
253	P4-20		530520	Yes		20.2	
254	P1-17		531407	Yes		22.7	
255	P4-3		531796	Yes		20.8	
256	P4-10		537307	Yes		10.7	
257	P4-19	P8-23	537307	No	Yes		6.3
258	P4-16		537309	Yes		15.5	
259	P4-29		545467	Yes		12.3	
260	P4-18		550819	Yes		11.3	
261	P4-62		564876	Yes		7.9	
262	P5-43		568903	Yes		8.6	
263	P5-34		579119	Yes		16.9	
264	P5-41		586150	Yes		5.1	
265	P5-57		592655	Yes		11.8	
266	P5-42		599490	Yes		14.4	
267	P5-50	P8-43	601368	No	Yes		14
268	P4-89		603902	Yes		9.8	
269	P4-85	P8-33	605307	Yes	Yes	18.9	11.1
270	P5-33	P8-40	606426	No	Yes		14
271	P5-56		608301	Yes		16.2	
272	P5-45		608305	Yes			
273	P4-88		617999	Yes		14.2	
274	P5-46	P8-42	626739	No	Yes		12.9
275	P5-52		634004	Yes		13.4	
276	P4-77		634038	Yes		12.5	
277	P5-44		635300	Yes		25.1	
278	P5-53		638883	Yes		11.1	
279	P4-31		656517	Yes		8.9	
280	P4-33		658187	Yes		17.9	
281	P4-32		659486	Yes		10.7	
282	P4-24	P8-25	659489	Yes	Yes	14.7	12.9
283	P3-89		662543	Yes		26.2	
284	P2-77	P9-42	132309 H3	Yes	Yes	21.4	5.7
285	P4-76		151934 H6	Yes		12	
286	P5-51		151934 H6	Yes		14	
287	P5-36		152953 H9	Yes		29.1	
288	P3-84		154830 H6	Yes		24.5	
289	P4-78		154830 H7	Yes		16.4	
290	P3-61	P9-77	169490 CT	Yes	Yes	4.3	8.4
291	P3-68		172175 #2	Yes		17.8	
292	P3-92		172175 -7 #3	Yes		17.8	
293	P4-34		172324-2	Yes		19.4	
294	P4-14		172324-4	Yes		6.8	
295	P3-91		174443 TR	Yes		21.9	
296	P4-27		175744 KD H1	Yes		24.9	
297	P4-12		175744 KD H1	Yes		8.3	
298	P4-13		175744 KD H5	Yes		10.6	
299	P4-28		175834 TR	Yes		11.2	
300	P5-48		187464 KN H2 AF	Yes		16.1	
301	P5-35		187464 KN H3	Yes		15.7	
302	P5-49		187464 TR H1 AF	Yes		18.6	

303	P5-40		187464 TR H2 AF	Yes		18.7	
304	P2-56	P9-30	187464 TR H2 YS	No	No		
305	P5-47		187464 TR H3 AF	Yes		35.1	
306	P1-4		187521 H1	Yes		6.3	
307	P5-54	P8-44	187521 H2	Yes	Yes	13.4	14.8
308	P1-54		193970-3	Yes		19	
309	P2-51	P9-25	193970-4	Yes	Yes	15.9	7.6
310	P1-8		193970-6	Yes		8.7	
311	P2-41	P9-17	197270 H2	Yes	Yes	5.8	7.8
312	P4-94		197270 H3	Yes		14.8	
313	P1-27		197666 H2	Yes		17.6	
314	P1-7		197666 H4	Yes		11.2	
315	P2-3	P9-10	197677 H1	Yes	Yes	9.3	9.4
316	P1-61	P9-3	197677 H2	Yes	Yes	16.1	9.6
317	P5-8		198007 H2	Yes		22.9	
318	P5-84		198007 H3	Yes		17	
319	P5-77		198007 H4	Yes		2.9	
320	P1-62		198442 CE	Yes		3.4	
321	P2-9	P9-12	198442 SF	No	No		
322	P1-44		198442 TR	Yes		18.1	
323	P2-28	P9-15	198999 B9	Yes	Yes	21	10.8
324	P5-3		200656 H3	Yes		12.8	
325	P5-80		200656 H4	Yes		6.2	
326	P5-82	P8-51	200656 H4	No	Yes		18.3
327	P5-7		200677 HA	Yes		11.8	
328	P5-87		200677 HB	Yes		5.2	
329	P5-2		200963 H5	Yes		14.4	
330	P5-59	P8-46	200963 H7	Yes	Yes	4.3	9.9
331	P5-86		200963 H7	Yes		4.4	
332	P2-91		205069 AF H6	Yes			
333	P1-87		205069 YS H6	Yes		15.4	
334	P1-86		205069 YS H6	No			
335	P3-73	P9-82	209783 CE	Yes	Yes	8.8	7.4
336	P3-36	P9-71	209783 TR	No	No		
337	P3-72	P9-81	209842 H2	Yes	Yes	25.3	17
338	P3-95	P8-21	209842 H2	Yes	No	24.7	
339	P1-93		210882 H1	Yes		18.4	
340	P3-41	P8-12	210882 H3	Yes	Yes	30.1	12.6
341	P1-94	P9-8	221316 - ORG	No	Yes		13.1
342	P2-93		221316 - CE	Yes		19.4	
343	P2-85	P9-50	221316 TR	Yes	Yes	19.7	13.4
344	P1-88		222042 H1	Yes		17.5	
345	P2-90		222042 H2	Yes		13.1	
346	P1-83		225250 H1	Yes		15.2	
347	P2-88		225250 H5	Yes		15.2	
348	P1-95		225518 H10	Yes		6.2	
349	P5-31		225518 H11	Yes		19.3	
350	P5-89		225518 H12	Yes		25.5	
351	P3-60		226668 H2	Yes		17.4	
352	P3-40		226827 HD	Yes		21.3	
353	P3-77	P8-15	226827 HE	Yes	Yes	10	5.2

354	P3-86		226827 HF	Yes		23.8	
355	P4-80	P8-30	227831 TR	No	Yes		5.2
356	P2-72		227831 TR	Yes		11.1	
357	P5-16		229207 CE	Yes		6.5	
358	P5-58	P8-45	229207 TR	Yes	Yes	15.8	9.4
359	P5-18		229498 H1	Yes		16.3	
360	P4-73		229498 H2	Yes		10.5	
361	P2-57	P9-31	232786 H4	Yes	Yes	13.1	4.7
362	P2-63		232786 H4	Yes		18.6	
363	P1-50	P8-2	233429 CE	No	No		
364	P3-30	P9-68	233429 CE	No	No		
365	P1-24		234962 H13	Yes		8.8	
366	P1-2		234962 H7	Yes		9.4	
367	P2-49	P9-23	235104 TR	Yes	Yes	12.4	12.5
368	P2-15		236104 BR	Yes		23.5	
369	P1-51		236104 CE	Yes		19.6	
370	P3-20		245842 H2	Yes		39.9	
371	P2-35		245842 H3	Yes		16.3	
372	P1-25		259214 S1	Yes		16.8	
373	P2-76	P9-41	259214 S2	Yes	Yes	17.7	6.8
374	P2-67		264213 H1	Yes		15.3	
375	P2-79	P9-44	264213 H1	Yes	Yes	13.9	8.5
376	P1-38		264213 H2	Yes		25.8	
377	P3-59	P9-76	269246 AF	No	Yes		11.4
378	P3-49		269246 BR	Yes		22	
379	P3-79	P8-17	269246 CT	Yes	No	18.4	
380	P3-17		269246 KID	Yes		37	
381	P3-53		271729 H1	Yes		45	
382	P3-3	P9-55	271729 H2	No	Yes		12.1
383	P4-35	P9-19	274025 H	Yes	Yes	33.3	10.1
384	P2-45		274025 S	Yes		9.8	
385	P3-57		276453 H1	Yes		27.1	
386	P3-10	P9-61	276453 H2	Yes	Yes	16.3	10.1
387	P2-13		282562 H15	Yes		26.7	
388	P1-21		282562 H4	Yes		22.1	
389	P5-60	P8-47	282913 H2	No	Yes		17.8
390	P5-13	P8-36	282913 H3	No	Yes		12.1
391	P5-4		288392 H2	Yes		18.6	
392	P5-67		288392 H3	Yes		13	
393	P5-6		289368 H10	Yes		18.1	
394	P1-79		289368 H10	Yes		13.9	
395	P5-75		289368 H7	Yes		14.5	
396	P1-43		305670 H2	Yes		13.7	
397	P1-1	P9-1	305670 H3	No	Yes		13.4
398	P2-48	P9-22	305670 H4	Yes	Yes	15.7	13.7
399	P1-46		309115 H3	Yes		20.3	
400	P2-40	P9-16	309115 H8	Yes	Yes	17.8	10.9
401	P2-42		313190 H1	Yes		20.4	
402	P3-65		313190 H3	Yes		36.8	
403	P1-90		327985 H12	Yes		17.8	
404	P2-75	P9-40	327985 H18	No	Yes		6.8

405	P3-35		336344 H1	Yes		27.9	
406	P3-71	P9-80	336344 H2	Yes	Yes	16.9	13.2
407	P4-9		336344 H7	Yes		24.8	
408	P3-81		336344 H8	Yes		22.6	
409	P1-72		337109 H5	Yes		11.2	
410	P1-12		337109 H7	Yes		4.8	
411	P1-3		346613 H2	Yes		14.6	
412	P2-36		346613 H4	Yes		10.6	
413	P4-74		347477 H2	Yes		10.3	
414	P5-73		347477 H3	Yes		14.4	
415	P5-71	P8-48	347626 H1	No	Yes		14.6
416	P4-69		347626 H2	Yes		12	
417	P1-74		348513 AF	Yes		8.8	
418	P3-38	P8-11	348513 CAM	No	Yes		3.8
419	P1-58	P8-3	348513 CAM	No	No		
420	P3-51		348517 H1	Yes			
421	P2-81	P9-46	348517 H2	Yes	Yes	15.8	19.7
422	P3-37	P8-10	348517 H6	Yes	No	33.4	
423	P3-69	P9-79	350676 C3	Yes	Yes	24.2	10.9
424	P2-30		350676 C5	Yes		8.5	
425	P1-55		351290 H2	Yes		21.6	
426	P4-61		351290 H9	Yes		45.3	
427	P1-75		351290 H9	Yes		10.9	
428	P3-26		358886 AF H6	Yes		37.4	
429	P3-2	P9-54	358886 H4	Yes	Yes	15.9	12.5
430	P3-56		358886 YS H6	Yes		28.1	
431	P2-50	P9-24	359396 H4	Yes	Yes	5.5	10.4
432	P1-80		359396 H5	Yes		17.2	
433	P2-68	P9-35	461064 H2	No	Yes		5.7
434	P2-17	P9-14	461064 H4	Yes	Yes	13.2	10.3
435	P4-25	P8-26	461064 H6	Yes	No	16.9	
436	P3-94	P8-20	533511 H9	Yes	Yes	22.6	8.1
437	P3-90		533551 H6	Yes		25.2	
438	P4-11		538641 B4	Yes		11.6	
439	P4-8		538641 B5	Yes		21.2	
440	P3-85		578946 N2	Yes		21.6	
441	P3-83		578946 N5	Yes		3.2	
442	P4-23		578946 N6	Yes		17.6	
443	P4-93		599479 H3	Yes		16.6	
444	P5-32		599479 H4	Yes		12.7	
445	P5-38	P8-41	638634 IV	No	Yes		16.9
446	P4-22	P8-24	658421 H1	Yes	Yes	18.5	12.3
447	P2-54	P9-28	Unknown	Yes	Yes	29.4	7.1

Table 8. Mixed sequences excluded from the study

Study reference code	Lab reference number	Analysis
P1-20	199262	Mixed
P5-20	200957	Mixed
P2-96	203097	Mixed
P5-22	203100	Mixed
P1-91	222046	Mixed
P2-86	225510	Mixed
P1-66	236910	Mixed
P3-47	276628	Mixed
P3-8	314462	Mixed
P1-18	330071	Mixed
P1-65	332607	Mixed
P2-16	339989	Mixed
P1-16	344385	Mixed
P2-64	348519	Mixed
P1-71	349480	Mixed
P3-7	351505	Mixed
P5-45	608305	Mixed
P1-61	197677 H2	Mixed
P5-86	200963 H7	Mixed
P5-58	229207 TR	Mixed
P1-79	289368 H10	Mixed
P1-1	305670 H3	Mixed
P2-75	327985 H18	Mixed
P1-75	351290 H9	Mixed

Table 9. Samples with previous sequencing data available versus current study data

Sample Number	Study Reference Code	Lab Reference Number	Lineage as presented in this study	Previous sequencing results
1	P1-28	193311	GI-19	GI-19
2	P1-10	194822	GI-19	GI-19
3	P5-20	200957	Could not be determined	4-91 Path
4	P3-32	203110	GI-19	GI-19
5	P2-84	220197	GI-1	Mixed: GVI-1, GI-19
6	P1-84	222493	GI-19	GI-19
7	P1-92	222760	GI-11	GI-19
8	P2-52	234109	GI-19	GI-19
9	P2-71	234993	GI-1	GI-1
10	P1-5	249371	GI-19	GI-11
11	P3-16	267739	GI-19	GI-1
12	P3-6	268083	GVI-1	GI-19
13	P3-9	268083	Could not be determined	GI-11
14	P3-22	275478	GI-19	GI-1
15	P3-15	278583	GI-19	GI-19
16	P5-26	278832	GI-19	Mixed unable to type
17	P5-83	288325	GI_11	GI-19
18	P3-8	314462	Could not be determined	GI-1
19	P3-12	316661	GI-1	GI-19
20	P3-80	319685	GI-19	GI-19
21	P3-28	320057	GI-13	GI-19
22	P3-4	320709	GI-13	GI-19
23	P3-58	321496	GI-1	GI-19
24	P2-18	321872	GVI-1	GI-19
25	P4-38	321872	GI-19	GI-19
26	P3-19	323131	GI-19	GI-11
27	P1-59	329067	GI-1	GI-1
28	P2-8	329169	GI-19	GI-1
29	P1-70	332825	GI-19	GI-19
30	P2-70	335734	Could not be determined	GI-1
31	P2-33	335997	GI-13	GI-1
32	P1-56	337288	GI-13	GI-19
33	P2-16	339989	Could not be determined	GI-1
34	P5-81	341521	GI-13	GI-19
35	P4-79	342217	GI-19	GI-19
36	P1-16	344385	Could not be determined	GI-1
37	P4-67	344385	GI-19	GI-19
38	P1-42	346464	GI-13	GI-19
39	P3-63	348514	GI-19	GI-19
40	P2-64	348519	Could not be determined	GI-19
41	P4-75	348521	GI-1	Mixed: GI-1, GI-13
42	P3-87	351288	GI-13	GI-19
43	P2-4	363621	GI-13	GI-19
44	P2-73	379353	GI-13	GI-19
45	P4-31	656517	GI-23	GI-19
46	P3-36	209783 TR	Could not be determined	GI-1
47	P2-75	327985 H18	Could not be determined	GI-19

48

P3-83

578946 N5

GI-23

GI-19

Table 10. Number of samples isolated per year, according to lineage

Lineage	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
GI-1			19	16	16	22	3		2		
GI-11		1	1	5	4		1				1
GI-13		1	2	7	8	15	2		3	1	
GI-19	4	18	44	37	32	31	5	4	1	6	2
GI-23	1					1				8	9
GIV-1		1		1							
GVI-1	1	1	2	4	3	2	7		5	3	1

Table 11. Number of samples isolated per province, according to lineage

	South Africa								Botswana	Eswatini	Namibia	Unknown
Lineage	Gauteng	North West	Eastern Cape	Western Cape	Free State	KwaZulu-Natal	Limpopo	Mpumalanga				
GI-1	12	32		4	23		1	5				1
GI-11	4	2		1		2		1				3
GI-13	10	8		1	12	1		2			3	2
GI-19	30	67	1	10	56	4		6	1	1		8
GI-23	3	2		2	1	1						10
GIV-1		1						1				
GVI-1	5	4	1	3	4			2			6	4

Table 12. The distribution of lineages across the different classifications of poultry

Classification	GI-1	G-1	GI-13	GI-19	GI-23	GIV-1	GVI-1
Breeders	1		1	6			2
Broilers	70	6	30	159	6	2	20
Layers	3	4	3	6	3		2
Grandparent			2				1
Rearing	2						
SPF	1			2			
Unknown	1	3	3	11	10		4

Isolate descriptions and Genbank accession numbers are listed in Annexure 1.

Isolate description	Genbank Accession number
201216_2012	OR727107
235198_2013	OR727111
347477_H2_2015	OR727108
377455_2016	OR727109
526250_H2_2018	OR727110
175829_H3_2011	OR742726
210882_H1_2013	OR742727
222760_2013	OR742730
226668_H2_2013	OR742731
226827_HD_2013	OR742729
269246_AF_2014	OR742732
269246_KID_2014	OR742733
538641_B5_2019	OR742734
568903_2019	OR742735
244155_2014	OR742736
174443_2011	OR742737
260600_2014	OR742738
626739_2020	OR742728
168355_2011	OR742765
187521_H1_2012	OR742763
193311_2012	OR742753
201106_2012	OR742758
201602_2012	OR742754
231139_2013	OR742755
236104_2013	OR742766
249371_2014	OR742762
259214_S2_2014	OR742757
276811_2014	OR742761
347675_2015	OR742759
403218_2016	OR742760
461064_H2_2017	OR742756
579119_2019	OR742764
149399_2010	OR742739
336625_2015	OR742740
617999_2020	OR742741
638883_2020	OR742742
169490_2011	OR704347
206101_2013	OR704348
147868_2010	OR742743
172324_4_2011	OR742744
537307_D_2018	OR742747
197826_H6_2012	OR742748
213416_2013	OR742750
225250_H1_2013	OR742751

233942_H5_2013	OR742749
282562_H15_2014	OR742752
374409_2016	OR742745
530520_C1_2018	OR742746