Molecular characterization of a Marek's disease virus strain detected in tumour-bearing turkeys

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Abstract

Marek's disease (MD) is a lymphoproliferative disease caused by *Gallid alphaherpesvirus 2* (GaHV-2), which primarily affects chickens. However, the virus is also able to induce tumours in turkeys, albeit less frequently than in chickens. This study reports the molecular characterisation of a GaHV-2 strain detected in a flock of Italian meat-type turkeys exhibiting visceral lymphomas. Sequencing and phylogenetic analysis of the *meq* gene revealed that the turkey GaHV-2 has molecular features of high virulence and genetic similarity with GaHV-2 strains recently detected in Italian commercial and backyard chickens. GaHV-2 is ubiquitous among chickens despite the vaccination, and chicken-to-turkey transmission is hypothesised due to the presence of broilers in neighbouring pens.

Keywords: Marek's disease, turkey, *Gallid alphaherpesvirus 2*, *meq* gene, molecular characterisation, *Turkey herpesvirus*.

Research highlights

- A GaHV-2 strain from Italian turkeys was molecularly characterised;
- The turkey strain presented molecular characteristics of high virulence in its *meq* gene;
- The turkey strain was closely related to previously detected chicken strains.

Introduction

Marek's disease (MD) virus or *Gallid alphaherpesvirus 2* (GaHV-2), the causative agent of MD, is a herpesvirus belonging to the subfamily *Alphaherpesvirinae*, genus *Mardivirus*. Two other viral species are included in this genus: *Gallid alphaherpesvirus 3* (GaHV-3) and *Meleagrid alphaherpesvirus 1* (MeHV-1) or *Turkey herpesvirus* (HVT), frequently used as vaccines against MD in chickens. GaHV-2 isolates can be classified into four pathotypes: mild, virulent, very virulent and very virulent plus (Witter, 1997). GaHV-2 has been extensively studied and described in chickens, whether experimentally or naturally infected. In contrast, studies have seldom focused on GaHV-2 infections in turkeys, and scientific reports remain limited.

The first report on a Marek's disease-like condition in turkeys was from Florida, where two wild turkeys exhibited lymphoid visceral tumours resembling the MD-related tumours of the chicken (Busch & Williams, 1970). Subsequently, field cases were reported from the Netherlands (Voute & Wagenaar-Schaafsma, 1974), France (Coudert *et al*., 1995), Germany (Voeckell *et al*., 1999), Israel (Davidson *et al*., 2002) and the United Kingdom (Pennycott & Venugopal, 2002; Deuchande *et al*., 2012, Blake-Dyke & Baigent, 2013).

Susceptibility to GaHV-2 infection and tumour development has been demonstrated in experimentally infected turkeys with GaHV-2 isolates of chicken or turkey origin (Paul *et al*., 1977; Elmubarak *et al*., 1981; Powell *et al*., 1984; Davidson *et al*., 2002).

At post-mortem examination, GaHV-2-induced tumours in turkeys resemble tumours induced by either the Reticuloendotheliosis virus (REV) (Nair *et al*., 2013) or the Lymphoprolipherative disease virus (LPDV) (Biggs, 1997).

Some of these studies have primarily diagnosed MD based on histopathology, but this is not a decisive assay because even microscopically the neoplastic infiltrate can prove very similar across these lymphoproliferative diseases (Schat & Nair, 2013).

Relatively few studies have employed the PCR to confirm the GaHV-2 tumour's aetiology (Voechell *et al*., 1999, Davidson *et al.*, 2002, Deuchande *et al*., 2012; Blake-Dyke & Baigent, 2013). In our study, the *meq* gene was selected to serve for turkey GaHV-2 identification and classification, having been described as carrying virulence-specific markers (Shamblin *et al*., 2004). Indeed, Shamblin *et al.* (2004) and Renz *et al.* (2012) have observed that the number of sequences of four proline molecules (PPPP) is significantly correlated with the viral pathotype. Isolates of lower virulence present greater PPPP number than higher virulence isolates, which contain the lowest number of four-proline repeats or disrupted PPPP motifs due to point mutations. The determination of GaHV-2 virulence by molecular sequencing is only able to suggest the viral pathotype, as *in vivo* pathotyping assays (Witter *et al.*, 2005) using susceptible chickens are mandatory for an exact inclusion of GaHV-2 strains into one of the known pathotypes.

The aim of the present study is to report the description of GaHV-2-caused visceral tumours in Italian commercial turkeys, alongside with the first molecular characterisation of the detected GaHV-2 strain through *meq* gene sequence analysis and phylogeny.

Materials and Methods

Commercial turkeys. During the year 2016, three-to-four-month-old white meat turkeys, unvaccinated against MD and reared on a commercial free-range farm located in the Lazio region of Italy, experienced mortality. At post-mortem examination livers were enlarged and contained whitish lesions of lymphoproliferative nature. The flock had been reared indoors up to 50 days of age, before moving into outdoor pens until slaughter at five months old. On the same farm, HVT-vaccinated broiler chickens were reared outdoors in neighbouring pens.

DNA extraction. A selected tumour-bearing liver served for the genomic DNA extraction using the commercial kit NucleoSpin® Tissue (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany), according to the manufacturer's instructions.

PCRs for GaHV-2 *meq* gene amplification and HVT detection. The full-length *meq* gene of GaHV-2 was amplified with a previously described PCR protocol (Mescolini *et al*., 2019a). DNA was subjected to a further PCR protocol employing an oligonucleotide set specifically designed to amplify the US3 gene of HVT (Handberg *et al*., 2001). PCR was conducted by adding 3 µL DNA to a 22 µL reaction mixture containing 0.125 µL GoTaq G2 Flexi DNA Polymerase (Promega, Madison, WI), 5 µL 5X Colorless Go-Taq Flexi Buffer, 1.75 µL MgCl2 solution, 0.5 µL dNTPs, 13 µL H2O for molecular biology, 1 µL primer forward HVT-1 (5'-ATG GAA GTA GAT GTT GAG TCT TCG-3') and 1 µL primer reverse HVT-2 (5'-CGA TAT ACA CGC ATT GCC ATA CAC-3'). Cycling conditions were as follows: 2 min of denaturation at 95°C followed by 35 cycles, each consisting of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. A final elongation step at 72°C for 5 min completed the reaction. The PCR products were separated on agarose gel (2%), stained with ethidium bromide, and visualized under ultraviolet light after an electrophoretic run at 110 V and 400 mA for 35 min.

Sequencing and sequence analysis. PCR products were purified using ExoSAP-IT *Express* PCR Product Cleanup (Thermo Fisher Scientific, Massachusetts, USA) and sequenced by a commercial sequencing service (Macrogen Spain, Madrid, Spain).

In order to obtain the whole *meq* gene sequence, PCR primers *Eco*R-Q for (5'-GGT GAT ATA AAG ACG ATA GTC ATG-3') and *Eco*R-Q rev (5'-CTC ATA CTT CGG AAC TCC TGG AG-3') (Shamblin *et al*., 2004) as well as an additional and internal primer (meq-F, 5'-ATG TCT CAG GAG CCA GAG CCG-3') (Hassanin *et al*., 2013) were used for sequencing.

The sequence was edited and assembled using BioEdit Sequence Alignment Editor, Version 7.2.5.0 (Tom Hall, Ibis Therapeutics, Carlsbad, California, USA), before being aligned against selected complete *meq* gene sequences of 36 reference GaHV-2 strains of known pathotype and 32 GaHV-2 strains recently detected during MD outbreaks in Italian backyard (Mescolini *et al.*, 2019a) and commercial chickens (Mescolini *et al.*, 2019b) (Table 1). The number of PPPP motifs contained in the proline-rich repeats (PPRs) of the transactivation domain, the proline content (%) and the amino acid (aa) substitutions in *meq* gene-deduced amino acid sequence were evaluated.

A phylogenetic tree based on the *meq* gene aa sequences was constructed with the maximum likelihood (ML) method using MEGAX (Kumar *et al*., 2018). Nodes of the tree with bootstrap values obtained with 1,000 replicates equal to or greater than 70 were considered significant.

The HVT US3 gene amplicon was sequenced in both directions using the PCR primers and was submitted to the basic local alignment search tool (BLAST) for a similarity search.

Accession numbers. Sequences were submitted to the GenBank database and are available under the following accession numbers: MN017102 (*meq* gene of GaHV-2) and MN017103 (US3 gene of MeHV-1).

Results

The analysed sample was positive at PCR for the GaHV-2 *meq* gene, producing an amplicon of the expected size. The detected strain was named GaHV-2/Italy/Turkey/601/16. Sequence analysis revealed a *meq* gene encoding for a 339 aa-long Meq protein isoform with a proline content of 21.18% and a 100% nucleotide sequence identity with Italian GaHV-2 strains

recently detected in commercial (Mescolini *et al.*, 2019b) and rural chicken flocks (Mescolini *et al.*, 2019a).

Four PPPPs were identified in the transactivation domain together with a PPSP sequence, in which a serine replaced a proline at position 218 (P218S). The overall molecular characteristics of the detected strain are reported in Table 2. GaHV-2/Italy/Turkey/601/16 showed an aa substitution (S71A) that is typically found in all *in vivo* pathotyped vv+ strains and other three aa substitutions (D80Y, C110S and P218S) found in field strains from Italy (Mescolini *et al.*, 2019a, b) and Poland (Woźniakowski *et al*., 2010; Woźniakowski & Samorek-Salamonowicz, 2014; Trimpert *et al.*, 2017) with an history of elevated virulence in the field. The phylogenetic analysis (Figure 1) confirmed the close relationship of the turkey strain with GaHV-2s recently detected in Italy from MD outbreaks in chickens, as they belong to the same cluster.

Finally, an amplicon of the expected size (505 bp) was obtained when the specific PCR for the US3 gene of HVT was applied. The BLAST search confirmed the detection of an HVT strain (MeHV-1/Italy/Turkey/601/16), presenting a 100% sequence identity with the US3 gene of the HVT strain FC126 (GenBank accession number AF291866), commonly used as MD vaccine in chickens.

Discussion

The present report, which molecularly identifies a GaHV-2 strain in free-range commercial turkeys, builds upon the few existing studies of turkeys infected by GaHV-2, which is primarily a chicken's pathogen. The *meq* gene, the main GaHV-2 viral oncogene, was selected for the molecular characterisation of the GaHV-2/Italy/Turkey/601/16 strain owing to its molecular variability, which correlates with the level of virulence of the strain (Lee *et al*., 2000; Shamblin *et al*., 2004). The GaHV-2 strain showed molecular features suggestive of

high virulence due to the presence, in the transactivation domain of the Meq protein, of a low number of four-proline repeats, of a disrupted PPPP motif and of aa substitutions typically found in all vv+strains (S71A) and in Italian and Polish strains (D80Y, C110S and P218S) with an history of high virulence in the field (Woźniakowski *et al*., 2010; Woźniakowski & Samorek-Salamonowicz, 2014; Trimpert *et al.*, 2017; Mescolini *et al.*, 2019a, b). In order to report the original turkey GaHV-2 sequence without any possible molecular changes that may have occurred during tissue culture propagation (Shamblin *et al*., 2004), this study employed the original turkey tissue for amplification and sequencing, as advocated by Davidson *et al.* (1995) and Davidson and Silva (2008).

For the first time a turkey GaHV-2 *meq* gene sequence was obtained and compared with *meq* gene sequence GaHV-2 strains of known pathotype and GaHV-2 strains recently detected during MD outbreaks in Italian chickens.

A resemblance of the turkey GaHV-2 to chicken GaHV-2 strains with molecular characteristics suggestive of high virulence previously detected in Italy was evident from the *meq* gene sequence characterisation and phylogenetic analysis.

This report strengthens the previously sporadic observation of the potentially detrimental effects of virulent GaHV-2 strains infecting the turkey. In particular, turkeys reared with the possibility of contact with GaHV-2-affected chickens are prone to infection by circulating GaHV-2 strains. Whereas Davidson *et al.* (2002) reported MD in commercial turkey flocks reared in poultry houses previously occupied by MD affected chickens, the present report describes free-range birds of both species located in neighbouring pens. Due to the high and efficient horizontal environmental spread of GaHV-2 by means of desquamated feather follicle epithelial cells, which harbour infectious viral particles, it can be assumed that the affected turkey flock has been subjected to considerable risk of infection due to the continuous and close presence of broilers. Unfortunately, the neighbouring broiler flock was

not tested for GaHV-2 presence, but the virus is ubiquitous in chickens and might infect vaccinated chickens asymptomatically.

Although the susceptibility of turkeys to GaHV-2 infection has been recognised, reports on MD in this species are rare. This can be attributed to a lack of awareness, to different degrees of MD genetic resistance, or to the widespread presence of HVT in this species, which as hypothesised by Witter and Solomon (1971) may confer a certain degree of protection against the disease.

Nevertheless, the latter possibility has been contested by Elmubarak *et al.* (1981), who have found that HVT vaccination is ineffective in protecting turkeys against MD under experimental conditions, and Blake-Dyke and Baigent (2013), who report that an early infection with HVT may prove unable to provide adequate immunity and protect turkeys from the challenge with a field GaHV-2 strain. The moment at which the birds in our investigation became infected with HVT is unknown, because the virus was detected simultaneously with the MD outbreak, and so the role of HVT in protecting turkeys from MD remains unclear.

The genetic similarity of the detected HVT strain with the FC126 vaccine strain suggests that the virus probably came from the neighbouring broilers, but it cannot be excluded that the examined turkey flock naturally harboured the detected HVT strain.

The protection of turkeys against MD is at present heavily reliant on management procedures, namely the effective separation from GaHV-2-affected chickens. Further studies are required to understand whether the associations of currently available vaccines are able to prevent MD in turkeys.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Table 1. Chicken GaHV-2 strains included in the analysis.

GAHV-2 strain	Country	Year	Pathotype	Size of Meq (aa)	PPPPs (N°)	GenBank Accession N°	Reference
CVI988	The Netherlands	1969	att[a]	398	7	DQ530348	Spatz *et al*., 2007
814	China	1986	att	398	7	JF742597	Zhang *et al*., 2012
3004	Russia	NA[b]	att	398	7	EU032468	NA
CU-2	USA	1970s	m[c]	398	7	AY362708	Shamblin *et al*., 2004
MD70/13	Hungary	1970	v[d]	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[e]	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+[f]	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
GaHV-2/Italy/Ck/487/15	Italy	2015	NA	339	5	MK139660	Mescolini *et al*., 2019a
GaHV-2/Italy/Ck/507/15	Italy	2015	NA	418	9	MK139661	Mescolini *et al*., 2019a
GaHV-2/Italy/Ck/509/15	Italy	2015	NA	418	9	MK139662	Mescolini *et al*., 2019a
GaHV-2/Italy/Ck/510/15	Italy	2015	NA	418	9	MK139663	Mescolini *et al*., 2019a
GaHV-2/Italy/Ck/562/15	Italy	2015	NA	418	9	MK139664	Mescolini *et al*., 2019a
GaHV-2/Italy/Ck/599/16	Italy	2016	NA	418	9	MK139665	Mescolini *et al*., 2019a
GaHV-2/Italy/Ck/625/16	Italy	2016	NA	339	4	MK139666	Mescolini *et al*., 2019a

GaHV-2/Italy/Ck/674/16	Italy	2016	NA	339	4	MK139667	Mescolini *et al*., 2019a
GaHV-2/Italy/Ck/689/16	Italy	2016	NA	339	4	MK139668	Mescolini *et al*., 2019a
GaHV-2/Italy/Ck/722/16	Italy	2016	NA	339	4	MK139669	Mescolini *et al*., 2019a
GaHV-2/Italy/Ck/801/17	Italy	2017	NA	339	4	MK139670	Mescolini *et al*., 2019a
GaHV-2/Italy/Ck/810/17	Italy	2017	NA	339	4	MK139671	Mescolini *et al*., 2019a
GaHV-2/Italy/Ck/847/17	Italy	2017	NA	418	10	MK139672	Mescolini *et al*., 2019a
GaHV-2/Italy/Ck/848/17	Italy	2017	NA	418	9	MK139673	Mescolini *et al*., 2019a
GaHV-2/Italy/Ck/850/17	Italy	2017	NA	339	5	MK139674	Mescolini *et al*., 2019a
GaHV-2/Italy/Ck/852/17	Italy	2017	NA	339	4	MK139675	Mescolini *et al*., 2019a
GaHV-2/Italy/Ck/853/17	Italy	2017	NA	339	4	MK139676	Mescolini *et al*., 2019a
GaHV-2/Italy/Ck/854/17	Italy	2017	NA	339	4	MK139677	Mescolini *et al*., 2019a
GaHV-2/Italy/Ck/855/17	Italy	2017	NA	298	2	MK139678	Mescolini *et al*., 2019a

GaHV-2/Italy/Ck/456/15	Italy	2015	NA	339	4	MK855054	Mescolini *et al*., 2019b
GaHV-2/Italy/Ck/498/15	Italy	2015	NA	339	4	MK855055	Mescolini *et al*., 2019b
GaHV-2/Italy/Ck/513/15	Italy	2015	NA	339	4	MK855056	Mescolini *et al*., 2019b
GaHV-2/Italy/Ck/515/15	Italy	2015	NA	339	4	MK855057	Mescolini *et al*., 2019b
GaHV-2/Italy/Ck/559/15	Italy	2015	NA	339	4	MK855058	Mescolini *et al*., 2019b
GaHV-2/Italy/Ck/561/15	Italy	2015	NA	339	4	MK855059	Mescolini *et al*., 2019b
GaHV-2/Italy/Ck/565/15	Italy	2015	NA	339	4	MK855060	Mescolini *et al*., 2019b
GaHV-2/Italy/Ck/567/15	Italy	2015	NA	339	4	MK855061	Mescolini *et al*., 2019b
GaHV-2/Italy/Ck/757/17	Italy	2017	NA	339	4	MK855062	Mescolini *et al*., 2019b
GaHV-2/Italy/Ck/875/18	Italy	2018	NA	339	4	MK855063	Mescolini *et al*., 2019b
GaHV-2/Italy/Ck/876/18	Italy	2018	NA	339	4	MK855064	Mescolini *et al*., 2019b
GaHV-2/Italy/Ck/921/18	Italy	2018	NA	339	4	MK855065	Mescolini *et al*., 2019b

GaHV-2/Italy/Ck/1083/18	Italy	2018	NA	339	4	MK855066	Mescolini *et al*., 2019b

[a] Attenuated

[b] Not available, the strain has not been subjected to the *in vivo* pathotyping test.

[c] Mild

[d] Virulent

[e] Very virulent

[f] Very virulent plus

Table 2. Molecular characteristics of the *meq* protein aa sequence of GaHV-2/Italy/Turkey/601/16 strain, compared to prototype strains. Amino acid substitutions interrupting PPPPs are underlined.

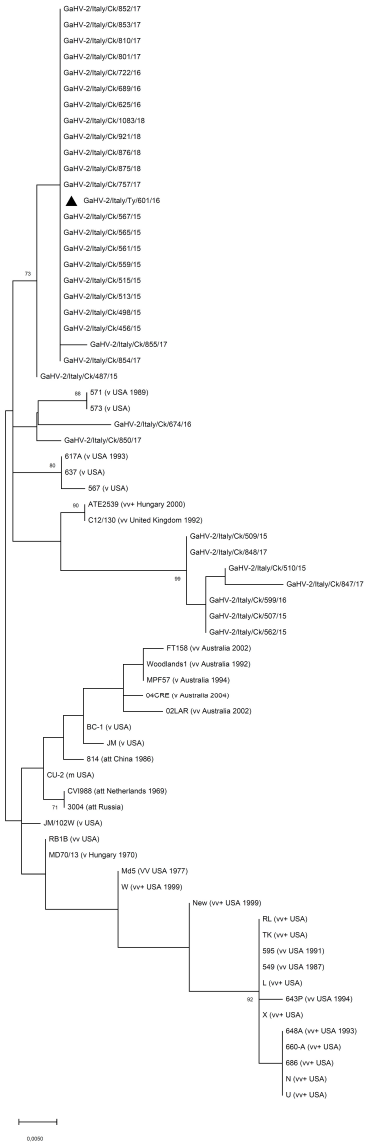
Strain	Meq length (aa)	PPPPs (n°)	Amino acid substitutions														
			71	77	80	110	119	153	176	180	216[a]	217	218	277	283	320	326
CVI988	398	7	S	E	D	C	C	P	P	T	P	P	P	L	A	I	I
CU-2	398	7	S	E	D	C	C	P	P	T	P	P	P	L	A	I	T
JM/102W	399	7	A	E	D	C	C	P	P	T	S	P	P	L	A	I	T
Md5	339	4	A	K	D	C	C	P	P	T	P	A	P	L	V	T	T
648A	339	2	A	K	D	C	R	Q	A	A	P	A	P	P	A	I	T
GaHV-2/Italy/Turkey/601/16	339	4	A	E	Y	S	C	P	P	T	P	P	S	L	A	I	T

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

337 **Legend to the Figure**

338 **Figure 1.** Phylogenetic tree based on *meq* aa sequences of GaHV-2/Italy/Turkey/601/16 (marked with a black triangle), reference GaHV-2

339 retrieved from GenBank, Italian GaHV-2 and three vaccine strains (CVI988, 814 and 3004).


GaHV-2/Italy/Ck/852/17
GaHV-2/Italy/Ck/853/17
GaHV-2/Italy/Ck/810/17
GaHV-2/Italy/Ck/801/17
GaHV-2/Italy/Ck/722/16
GaHV-2/Italy/Ck/689/16
GaHV-2/Italy/Ck/625/16
GaHV-2/Italy/Ck/1083/18
GaHV-2/Italy/Ck/921/18
GaHV-2/Italy/Ck/876/18
GaHV-2/Italy/Ck/875/18
GaHV-2/Italy/Ck/757/17
GaHV-2/Italy/Ty/801/16
GaHV-2/Italy/Ck/567/15
GaHV-2/Italy/Ck/565/15
GaHV-2/Italy/Ck/561/15
GaHV-2/Italy/Ck/559/15
GaHV-2/Italy/Ck/515/15
GaHV-2/Italy/Ck/513/15
GaHV-2/Italy/Ck/498/15
GaHV-2/Italy/Ck/456/15
GaHV-2/Italy/Ck/855/17
GaHV-2/Italy/Ck/854/17
GaHV-2/Italy/Ck/487/15
73
66
571 (v USA 1989)
573 (v USA)
GaHV-2/Italy/Ck/674/16
GaHV-2/Italy/Ck/850/17
617A (v USA 1993)
80
637 (v USA)
567 (v USA)
60
ATE2539 (vv+ Hungary 2000)
C12/130 (vv United Kingdom 1992)
GaHV-2/Italy/Ck/509/15
GaHV-2/Italy/Ck/848/17
GaHV-2/Italy/Ck/510/15
99
GaHV-2/Italy/Ck/847/17
GaHV-2/Italy/Ck/599/16
GaHV-2/Italy/Ck/507/15
GaHV-2/Italy/Ck/562/15
FT158 (vv Australia 2002)
Woodlands1 (vv Australia 1992)
MPF57 (v Australia 1994)
04CRE (v Australia 2004)
02LAR (vv Australia 2002)
BC-1 (v USA)
JM (v USA)
814 (att China 1986)
CU-2 (m USA)
CVI988 (att Netherlands 1969)
71
3004 (att Russia)
JM/102W (v USA)
RB1B (vv USA)
MD70/13 (v Hungary 1970)
Md5 (VV USA 1977)
W (vv+ USA 1999)
New (vv+ USA 1999)
RL (vv+ USA)
TK (vv+ USA)
595 (vv USA 1991)
549 (vv USA 1987)
L (vv+ USA)
92
643P (vv USA 1994)
X (vv+ USA)
648A (vv+ USA 1993)
660-A (vv+ USA)
686 (vv+ USA)
N (vv+ USA)
U (vv+ USA)
0,0050

340