Bagaza virus and Israel turkey meningoencephalomyelitis virus are a single virus species

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Bagaza virus (BAGV) and Israel turkey meningoencephalomyelitis virus (ITV) are classified in the genus Flavivirus of the family Flaviviridae. Serologically, they are closely related, belonging to the Ntaya serocomplex. Nucleotide sequences available to date consist of several complete sequences of BAGV isolates, but only partial sequences of ITV isolates. Sequence comparisons of partial envelope (E) and NS5 regions reveal a close genetic relationship between these viruses. Despite this, BAGV and ITV are considered as separate virus species in the database of the International Committee on Taxonomy of Viruses. In this work, complete nucleotide sequences for five ITV isolates are provided, thereby permitting a phylogenetic comparison with other complete sequences of flaviviruses in the Ntaya serogroup. We conclude that BAGV and ITV are the same virus species and propose that both viruses be designated by a new unified name: Avian meningoencephalomyelitis virus.

The past decade has witnessed an upsurge in the incidence and geographical spread of a variety of mosquito-borne flavivirus infections that have wild birds as reservoirs. Remarkable examples include members of the Japanese encephalitis serogroup such as West Nile virus, Usutu virus and others (Weissenböck et al., 2010). Likewise, although less known, the emergence of flaviviruses from the Ntaya serogroup, such as Bagaza virus (BAGV) in Europe (Aguero et al., 2011), and Tembusu virus and Tembusu-related Baiyangdian virus in China (Su et al., 2011; Tang et al., 2012) are also of concern.

BAGV was initially isolated in 1966 from mosquitoes in Bagaza, Central African Republic (Digoutte, 1978) and it has been isolated from mosquitoes in other Western African countries (Diallo et al., 2005; Gordon et al., 1992; Traore-Lamizana et al., 1994) as well as in India (BONDRE et al., 2009). In this latter country, serological evidence suggests that it is able to infect humans (BONDRE et al., 2009), although its pathogenicity is still uncertain. Until September 2010, when BAGV was first isolated in Spain from sick game birds (partridges and pheasants) (Aguero et al., 2011), this virus had not previously been isolated from vertebrates and little was known about its reservoir species. This outbreak corresponds to the first detection of BAGV in Europe and allowed for its first isolation from a vertebrate host.

It is not surprising that BAGV was able to infect avian species since available partial nucleotide sequences corresponding to the envelope (E) and NS5-coding regions of the genome related BAGV to Israel turkey meningoencephalomyelitis virus (ITV) (BONDRE et al., 2009; Kuno & Chang, 2007). ITV was first isolated in Israel in 1958 from domesticated turkeys, Meleagris gallapavo (hence its name) (Komarov & Kalmar, 1960), and was classified in the mosquito-borne cluster, clade XI (KUNO et al., 1998), within the Ntaya serocomplex (Calisher et al., 1989). Apart from Israel, ITV had only previously been reported in South Africa, also in domesticated turkeys (Barnard et al., 1980).

ITV has been detected in a number of different species of Culicidae mosquitoes and Culicoides midges, and shown to
Fig. 1. (a) Phylogenetic relationships between five full-length sequences of ITV obtained in this study (black dots) and 42 flaviviral full-length sequences including three BAGV isolates from GenBank. The phylogenetic tree was inferred using the maximum-likelihood method. The tree with the highest log-likelihood (−269972.3995) is shown. Percentages of successful bootstrap replicates over 70% (n = 1000) are indicated at nodes. The evolutionary distances were computed using the optimal GTR + G + I model. The tree is drawn to scale, with branch lengths measured by the number of substitutions per site. (b) Similar phylogenetic trees for景区 serogroup Ntaya serogroup.
be capable of infecting Culex pipiens and Phlebotomus papatasi experimentally (Braverman et al., 2003), thereby suggesting that these arthropods could act as transmission vectors. ITV causes a severe neuroparalytic disease in turkeys, leading to paresis, a lack of coordination, drooping wings and mortality rates of over 15–30 %, while morbidity can affect up to 80 % of the flock. In Israel, attenuated vaccines have been developed (Ianconescu et al., 1975) and vaccination campaigns have been conducted in this country as a routine control strategy for decades.

Several partial sequence analyses of the NS5 and E protein-coding regions from the genomes of both BAGV and ITV have been made available (Davidson & Weissman, 1999; Davidson et al., 1998; Gaunt et al., 2001; Kuno et al., 1998), thereby enabling the establishment of genetic relatedness between the two viruses. According to Kuno et al. (1998), a similarity of >84 % in the flavivirus gene sequences in conjunction with virus cross-neutralization activity serves as a criterion for species identification. BLAST analysis of the three available ITV partial nucleotide sequences (GenBank accession nos AF098456 and AF013377 of the NS5 gene and AF372415 of the E gene) reveal a sequence homology of 93, 94 and 95 %, respectively, with the homologous regions of the BAGV-type sequence (GenBank accession no. AF013363). Moreover, molecular diagnostic assays designed for ITV-infected turkeys targeting the NS5 gene (Davidson et al., 1998, 2000) or amplifying NS5 and E genes simultaneously (Davidson et al., 2012) also detected Bagaza-infected birds. Conversely, the real-time PCR assay designed for the BAGV (Buitrago et al., 2012) detected ITV in virus-infected turkeys (unpublished observation). Despite all these observations, the current classification by the International Committee on Taxonomy of Viruses considers BAGV and ITV to be different viral species with different assigned virus codes (00.026.0.01.004.06.001. and 00.026.0.01.018.06.001., respectively) (King et al., 2012).

A comparison of full-length sequences of ITV and BAGV would undoubtedly shed light on their proximity and, particularly, on whether they are in fact different or the same virus species. However, until now the full-length sequences were only available from BAGV (NC_012534, HQ644144, HQ644143, AY632545, EU684972) (Aguiro et al., 2011; Bondre et al., 2009; Kuno & Chang, 2007) but not from ITV. To fill this gap, this work aimed to obtain complete genome sequences from different ITV isolates to facilitate a more comprehensive study and to clarify the phylogenetic relationships between BAGV and ITV, as well as their position with respect to other flaviviruses of the Ntaya serogroup.

Complete genome sequences were obtained from five ITV viral RNA samples purified from brain tissue of turkeys inoculated with: (1) ITV commercial attenuated vaccine virus (Biocvac Biological Laboratories) based on virus strain JQ4E4 (Ianconescu et al., 1975); (2) isolate 618 obtained in 1995 from a 10-week-old turkey; (3) isolate 107458 obtained in 2010 from a 18-week-old turkey; (4) isolate 106819 obtained in 2010 from a 14-week-old turkey; (5) isolate 105520 obtained in 2010 from a 10-week-old turkey (these five ITV isolates will be named, respectively, ITV-1 to ITV-5 hereafter). The sequencing strategy basically followed the original strategy employed for the BAGV full genome sequencing (Aguiro et al., 2011), although some additional primers had to be specifically designed to complete the sequencing (primer and sequencing information available upon request). SeqScape software (Applied Biosystems) was used to edit and assemble the obtained ITV sequences. CLUSTAL W2 was employed for multiple sequence alignments.

Using MEGA5 software, phylogenetic analysis was carried out on the five complete ITV sequences obtained, along with 42 additional complete genome sequences representative of the different flavivirus species, including BAGV and all available complete sequences belonging to the Ntaya serogroup. Maximum-likelihood trees were reconstructed using the optimal GTR+G+I substitution model (Fig. 1).

Five ITV full-length genome sequences were obtained which were between 10777 and 10794 nt long (GenBank accession nos KC734549–KC734553). Nucleotide homology within these new sequences was between 94 % and >99 %, differentiating two groups of isolates, one comprising ‘old’ strains, that is, the vaccine strain dating from 1975 (ITV-1) and one field isolate from 1995 (ITV-2), and a second group clustering around the three more recent field isolates from the year 2010 (ITV-3–ITV-5) (Davidson et al., 2012). These two groups will be named ‘old’ and ‘recent’ ITV isolates hereafter. Multiple alignments of the five ITV sequences from this study and of the complete available nucleotide sequences of BAGV had a homology of 92–96 %. The lowest value in this range (92 %) corresponded to the comparison between the ‘recent’ ITV isolates and the Indian BAGV strain (GenBank accession no. EU684972), whereas the highest homology (96 %) was assigned to the comparison between the ‘recent’ ITV isolates and the Spanish BAGV strain (GenBank accession no. HQ644143). At the amino-acid level, homology increased to 99.65 % between the Spanish BAGV and recent ITV strains (Table 1).

As expected, the phylogenetic analysis grouped ITV sequences together with BAGV in the same cluster (‘ITV + BAGV cluster’) within the Ntaya serogroup, but separate from a second cluster also belonging to this
serogroup consisting of sequences containing Tembusu and Tembusu-like viruses ('Tembusu cluster') (Fig. 1). The ‘ITV + BAGV cluster’, was further subdivided into two main clades, according to the nucleotide homologies described above, one comprising four ‘old’ isolates (i.e. isolated in 1996 or earlier) and another of ‘recent’ isolates (all originating from 2010). The ‘old isolates clade’ included two ITV isolates, namely, ITV-1 (vaccine strain, 1975) and ITV-2 (1995), and two BAGV isolates, namely, BAGV DakAr B209 (1966) and BAGV-India (1996), while the ‘recent isolates clade’ included three ITV isolates (ITV-3, ITV-4, ITV-5) and one BAGV (Spain/2010) (Fig. 1). The recent field ITVs were isolated in 2010 contemporary to BAGV emergence in Spain. In this work, a close phylogenetic relationship was found between isolates from these two 2010 epornitics, indicating that they derived from a close common ancestor and thus further emphasizing their relatedness. However, more data, particularly more viral sequences from diverse origins, are still needed to infer the origin of the viruses involved in each outbreak.

The phylogenetic analysis carried out in this work also reveals a high genetic homogeneity within the Tembusu virus cluster, which included very closely related flaviviruses recently described in China that affected ducks. These Tembusu-like viruses clustered on the same branch with nucleotide homologies of over 99% (Fig. 1), regardless of whether they are known as Tembusu, Baiyangdian or duck/layer (egg-drop syndrome) flavivirus, thereby suggesting that all these names correspond actually to the same Tembusu virus species.

Overall, these results indicate that both ITV and BAGV form a single phylogenetic group, with pairwise nucleotide identities ranging from 96 to >99% between their members, that is, above the level of homology required for a flaviviral species to be considered unique (Kuno et al., 1998). A few minor differences were observed within this group, with two main clades, comprising ‘old’ and ‘recent’ isolates, respectively. Both clades comprised ITV and BAGV sequences, thus emphasizing that these two denominations are arbitrary and correspond to the same virus species. Further supporting this concept, our unpublished data showed that antibodies raised in ITV-infected or vaccinated turkeys strongly cross-neutralized BAGV Spain/2010 isolate. Consequently, BAGV and ITV represent the same virus under different names. To resolve this question, the new name Avian meningoencephalomyelitis virus (abbreviation AMEV) is proposed. This virus has been detected in Africa, the Middle East, India and Spain, and affects phasianids such as turkeys, pheasants and partridges. It is presumably transmitted by mosquito bites and has been claimed to be potentially zoonotic (Bondre et al., 2009), although more evidence is needed to support this claim.

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### References


