

# A New Look at Avian Flaviviruses

**Davidson, I.**

Division of Avian Diseases, Kimron Veterinary Institute, P.O. Box 12, 50250, Bet Dagan, Israel.

### ABSTRACT

The flaviviruses are important pathogens of wild birds, domestic poultry and humans, and several members are zoonotic. The review presents an update of the classification of this family of avian flaviviruses, describing their emergence, hosts and major disease features, dissemination patterns and control, as well as their molecular classification and genetic relatedness. A new perspective, based on the molecular identity of TMEV and BGAV throughout the entire genome, presents an innovative look at avian flaviviruses offering a global perspective on the presence of these avian flaviviruses and on the present view that TMEV exists only in Israel. Therefore, we suggest renaming TMEV and BGAV by a unified name, Avian Meningoencephalitis Virus – AMEV.

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**Keywords:** Avian Flaviviruses; Turkey Meningoencephalitis virus (TMEV); Bagaza virus (BGAV)

### INTRODUCTION

The flaviviruses (genus *Flavivirus*) are important pathogens of wild birds, domestic poultry and humans, and several members are zoonotically important. These viruses are distributed worldwide and cause widely diverse diseases varying from mild viral symptoms to severe and fatal hemorrhagic and neurological diseases. About 70 flavivirus species are known today, 40 of which cause infection in humans. The genus presents major concern due to its rapid rate of evolution, leading to the emergence of new viruses. The emergence of novel flaviviruses in wild birds and in poultry during the last years in several continents is a live reflection of that phenomenon, raising the necessity to reconsider our acquaintance with the flavivirus family.

The review presents a “who’s who” in avian flaviviruses, describing their emergence, hosts and major disease features, dissemination patterns and control, as well as their molecular classification and genetic relatedness. A new look at flaviviruses offers a global perspective on the presence of the avian flaviviruses.

### WHO’S WHO IN AVIAN FLAVIVIRUSES, DISEASES AND HOSTING AVIAN SPECIES?

During the year 2010 several avian flaviviruses brought renewed attention in several countries, attracting global interest.

#### Turkey Meningoencephalitis Virus (TMEV)

The virus was first described by Komarov and Kalmar in 1960 (1) and identified as a flavivirus by Porterfield in 1961 (2) and was considered to be confined only to Israel and to South Africa (3). TMEV re-emerged, or alternatively, the extent of affected commercial turkey flocks in Israel increased during the year 2010. The virus infection is controlled by vaccination with a live attenuated virus (4).

TMEV belongs to the mosquito-borne cluster, clade XI (5) and Ntaya Flavivirus Antigenic Complex VI (6). TMEV causes a neuroparalytic disease expressed as paresis, incoordination, dropping wings and mortality that can reach up to 80% of the flock in commercial turkeys (7). The first molecular characterization was initially described independently by Davidson (8,9) and Kuno (5), serving thereafter for

the diagnostic assay development (10, 11). Coincidentally, in the same year, several TMEV-similar flaviviruses, belonging to the Ntaya antigenic subgroup, have re-emerged in Spain, China, India and Malaysia, as detailed below.

### **Bagaza Virus (BAGV)**

A BGA outbreak in wild partridges and pheasants in Cadiz, Southern Spain was documented during late 2010 (12). The BAGV infection caused an increased rate of death in wild birds, following weakness, incoordination, ataxia, weight loss and diarrhoea. The causative role of BAGV was demonstrated experimentally, showing the virus' presence in most internal organs. However, the organs most affected were the central nervous system and the spleen.

### **Tembusu Virus (TMUV)**

TMUV (13,14) outbreaks in Chinese ducks emerged in China during the year 2010, although it was already described from China and Kuala Lumpur in 1955. Egg laying and 3-21 day-old ducklings were affected, showing retarded growth, high fever, loss of appetite, decreased egg production and extensive death.

### **Baiyangdian Virus (BYD)**

The BYD virus is a new Tembusu-related flavivirus discovered in China during the year 2010 (15). The disease is economically important due to its causing a severe drop in egg production in ducks. The experimental disease revealed the virus' affinity for the oviduct and geese ovaries.

### **Sitiawan Virus (SV)**

The SV virus affected Malaysian 4-6 weeks-old chicks (16), causing leg stretching and mobility impairment, as well as encephalitis and growth retardation. The clinical disease caused by both TMUV (in ducks) and SV (in chickens) was reproduced experimentally, showing viremia with a high affinity for the central nervous system at 6 days post infection.

### **Ntaya Virus (NTAV)**

The NTAV virus was first discovered in Uganda in the year 1951, but has lately attracted increased interest regarding its genomic sequencing (17). NTAV is neurotropic in wild birds, causes haemorrhages in the brain, lungs, liver, heart, ovaries and splenomegaly.

### **Usutu Virus (USUV)**

USUV was first described during the years 2001 and 2012 in blackbirds (robins) in Germany (18, 19). The USUV-infected birds were asymptomatic with sudden deaths. During the year 2009 USUV affected several immunocompromised persons in Italy (20).

### **West Nile Virus (WNV)**

WNV is the most renowned flavivirus, representing one of the major zoonoses, affecting wild birds, duck, horses and humans (21). The virus was initially described during the year 1998 in Israel and in Romania and a year later in New York (22). WNV causes a broad spectrum of symptoms (from febrile rash to fatal encephalitis) and is basically a neuro-invasive disease. The WNV pathogenicity for birds differs, depending on the virus strain and the avian species affected.

## **CHARACTERISTICS OF FLAVIVIRUS**

Flaviviruses virions are spherical (about 50 nm) in diameter, consisting of a tightly adherent lipid envelope that may display glycoprotein spikes, surrounding a spherical nucleocapsid with icosahedral symmetry (23). The viral genome consists of a single molecule of linear positive-stranded RNA of about 10.5-11

Kkb, encodes for one long open reading frame that is further processed into 10 proteins. The mature viral proteins are created by co- and post-translational processing and cleavage into three structural proteins, the nucleocapsid (C), prM, a precursor glycoprotein that is cleaved during virus maturation to yield the trans-membranal protein (M) and the major spike glycoprotein envelope protein, which is also the major target for neutralizing antibodies (E). The seven non-structural proteins include the NS5, the RNA-dependent RNA polymerase, NS3, with several functions, including helicase, protease and contribution to the RNA polymerase complex activities. NS2B and NS3 are largely responsible for the polyprotein cleavage and the host-cell proteases which are accountable for the remainder of this processing. Additional NS proteins include NS1, NS2A, NS2B, NS4A and NS4B. Structural proteins are encoded in the 5' end of the genome, while the non-structural proteins are encoded in the 3'.

Flavivirus replication involves the synthesis of complementary negative-sense RNA, serving as a template for the genome-sense RNA synthesis, translating into a single

polyprotein that is cleaved and processed to form the various structural and non-structural proteins. For mosquito-transmitted flaviviruses, virion assembly occurs on membranes of the endoplasmic reticulum and plasma membrane in mosquito cells (23).

## DISSEMINATION OF FLAVIVIRUSES

Members of the genus *Flavivirus* are subdivided on the basis of their mode of transmission into 4 groups: (a) tick-borne viruses; (b) mosquito-borne viruses; (c) viruses with no known arthropod vector; (d) viruses with no known animal host (23). The first 2 groups are maintained in nature in alternate cycles between arthropods and vertebrates, including mammals and birds, as one or more hosts. The mosquito-borne flaviviruses revealed 2 distinct epidemiological groups: the neurotropic viruses, correlated with the *Culex* species vector and with bird reservoirs, and the non-neurotropic viruses, associated with haemorrhagic disease in humans, correlated with the *Aedes* species and primate hosts. Many viruses of the *Culex* clade cycle between mosquitoes and birds. Flaviviruses infect mosquitoes by a viremic blood meal. Viruses penetrate to the insect midgut, spread to other insect tissues and are then secreted by the saliva. Some innate immune responses and RNA interference mechanisms are potential mosquito antiviral defence mechanisms, conferring genetic variability in the flavivirus competence to establish mosquito infection. Co-adaptation of flaviviruses and vectors influences viral evolution. Phylogenetic analyses demonstrated the correlation between the viral molecular phylogeny and its ecological/epidemiological characteristics (24, 25).

An essential feature of emerging pathogens is their ability to spread to new and unusual geographic ranges. In the case of the flaviviruses, the introduction of WNV into the Americas in 1999 (22) and of the USUV into Europe in 2001 (19,20) exemplified the phenomenon. During a retrospective investigation of human encephalitis cases, that occurred during the year 1996 in the Kerala state in India, Bagaza virus was detected in mosquito pools and specific antibodies were identified in human sera (26), however, no evidences exists regarding BGAV's zoonotic potential.

Among the avian flaviviruses, viruses that have been identified in mosquitoes: NTAV was named by the locality in Africa in which the carrying mosquitoes were caught. USUV and TMEV were also demonstrated in mosquitoes

(27). TMUV was documented to be spread by wild house sparrows, as an intermediate host which is the most widely disseminated wild bird in China. The efficient TMUV distribution might be attributed to the wide dissemination of the secondary host bird that lives around poultry houses and coops. SV was revealed in the course of a wide survey of insects in search of animal and human flaviviruses (16).

## GENETIC RELATEDNESS OF AVIAN FLAVIVIRUSES

The genus *Flavivirus*, family *Flaviviridae* comprises over 70 viruses, most of them are serologically related and classified into 8 antigenic complexes (6) (Table 1). However, many viruses have been added since the antigenic classification and the extensive geographic, host and vector specificity may still

**Table 1:** *Flavivirus* genus antigenic and phylogenetic classification based on a 1 kb NS5 gene fragment, according to Kuno G. *et al.* (1998) and Calisher C.H. *et al.* (1989).

Group	Virus	Phylogenetic Clade	Antigenic Cluster
Non-Vector Cluster			
	Apot	I	Modoc
	Sun Perlita, Jutiapa	II	Modoc
	Montana bat, Dakar bat, Rio Bravo, Carey Island, Phanom Penh bat, Batu Cave	III	Rio Bravo
Tick Borne Cluster			
	Gadget Gully, Royal Farm, Pow, Karshi, KFD, Langat, Omsk HF, TBE-far eastern, RSSE, TBE-CE, Negishi	IV	TBE
	Kadam, Tyulenly, Saumarez Reef, Meaban	V	Tyulenly
Mosquito-borne cluster			
	Edge Hill, Bouboni, Uganda, S. Banzi, Jugra, Saboya, Potiskim	VI	Uganda S.
	Sepik YF	VII	
	Sokuluk, Entebbe bat, Yokose	VIII	
	Den 1-4	IX	DEN
	Kedougou		
	Zika, Spondweni	X	
	TMEV, Bagaza, Tembusu, Ntaya, THCA, , SLE, Rocio, Ihleus,	XI	Ntaya
	Naranjal, Bussuquara, Aroa, Iguape	XII	
	Kokobera, Stratford	XIII	JE
	Cacipore, Kontago, Kunjin, WNV, Alfuy, Murrey Valley Encephalitis, Usutu	XIV	JE

introduce new features. Kuno *et al.* (5) provided a comprehensive phylogenetic classification, based on a 1 kb fragment of the flavivirus 3' terminus of the NS5 gene (Table 1).

Accordingly, the various flaviviruses diverged from a putative ancestor into two branches, non-vector and vector-borne virus clusters, while the vector-borne cluster subdivides into tick-borne and mosquito-borne clusters. Within each cluster, the phylogenetic clades correlate significantly with the existing antigenic complexes. Separate species were defined as virus classes with higher than 84% nucleotide sequence identity among them. Kuno's classification was based on the NS5 gene, as this gene, encoding for a function linked to virus replication, is considered to be more conserved than the envelope gene.

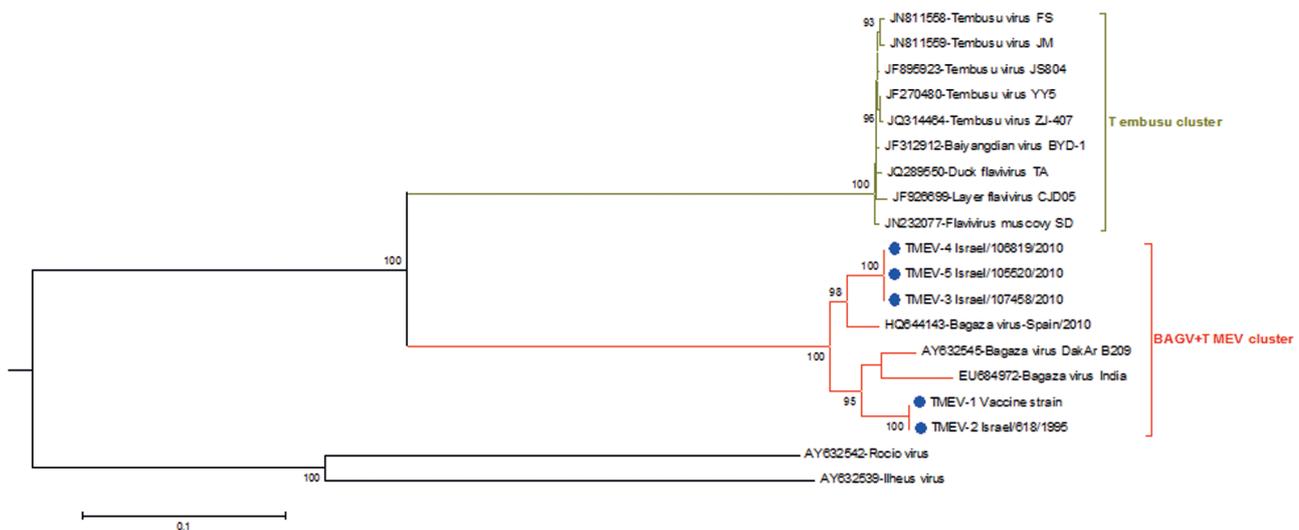
### THE "NEW LOOK" AT FLAVIVIRUSES

The increased attention to avian flaviviruses of veterinary importance, which occurred independently in Israel and Spain, commenced with a dramatic increase in turkey, partridges and pheasant rate of diseases and mortality. In addition, the 3 available genomic sequences of TMEV (2 different sequences of the NS5 gene - AF098456 and AF013377 (9) and the E gene sequence - AF372415), had a 93-95% homology to BGAV genome. According to the Kuno statement (5) mentioning that flaviviruses with sequence homologies higher than 84% are considered to be the same species, the relatedness of TMEV and BGAV was suspected.

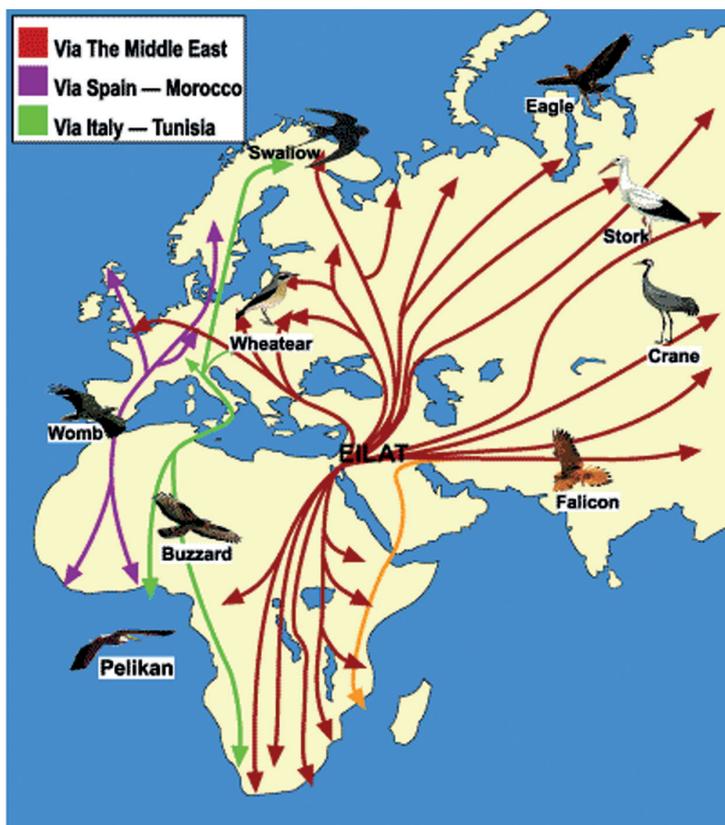
To verify the linkage between the two viruses, 3 BGAV

isolates and 4 TMEV isolates (one dated from the year 1995 and other 3 from the year 2010, as well as the vaccine strain) were studied molecularly. By conventional and by real-time PCR amplification of TMEV and of BGAV RNAs using assays for both viruses, TMEV and BGAV were amplified similarly. No other flavivirus, like USUV, JEV, WNV, KV and no unrelated virus, like AIV, NDV, BFDV and MDV were reactive (11, 28). Further, full genomes and polyprotein amino acid sequences of all viruses were elucidated and compared (29). A close relationship between all viruses was revealed; a genomic homology of 92-96% existed between BGAV and TMEV isolates, whereas the amino acid homology was much higher. These results confirmed that BGAV and TMEV are synonymous viruses, and may represent the same virus under different names, as shown in the full phylogenetic tree (29) and emphasized by zooming on the Ntaya clade (Fig. 1). Whether BGAV is able to cause disease in poultry, and whether TMEV is able to cause a similar disease in wild birds is still unknown.

In spite of the presently existing perception that TMEV exists only in Israel and in South Africa (3) it seems unlikely that other Mediterranean countries, with similar climatic conditions and insects do not have TMEV/BGAV-like flaviviruses. Probably TMEV is not restricted to Israel, and most probably TMEV/BGAV-like viruses are distributed Northbound by bird migration, as their two major routes from Africa passes through the Middle East and Spain (Fig. 2). In an attempt to trace TMEV in the wild, a survey of



**Figure 1:** Phylogenetic tree of the full genomes of several avian flaviviruses (adapted from ref. 29)



**Figure 2:** Migratory routes of wild birds between Africa and Europe

81 wild birds caught during all seasons of the last 2 years in Israel, including different types of wild birds (raptors, song and water birds), but no TMEV positive brain tissue RNA was detected (Davidson et al., unpublished). However, although the low statistical chance of detecting TMEV, the possible spread of TMEV via migratory birds cannot be excluded.

Finally, the disease emergence in these two global locations and the molecular similarities directs us towards “A New look at Flaviviruses”, giving “food of thought” and clues for a novel perception that additional similar viruses might be revealed in the future.

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