Annex to:

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), More S, Bøtner A, Butterworth A, Calistri A, Depner K, Edwards S, Garin-Bastuji B, Good M, Gortázar Schmidt C, Michel V, Miranda MA, Nielsen SS, Raj M, Sihvonen L, Spoolder H, Thulke H-H, Velarde A, Willeberg P, Winckler P, Baldinelli F, Broglia A, Dhollander S, Beltrán-Beck B, Kohnle L, Morgado J and Bicout D, 2017. Scientific Opinion on the assessment of listing and categorisation of animal diseases within the framework of the Animal Health Law (Regulation (EU) No 2016/429): West Nile fever (WNF). EFSA Journal 2017;15(8):4955, doi:10.2903/j.efsa.2017.4955

Annex A – Mapped fact-sheet used in the individual judgement on West Nile Fever

Article 5

Question A(i)

Question A(i) scientific evidence indicate that the disease is transmissible **Answer** Y ☐ N ☐ na ☐		
Art. 7 criteria	**Art. 7 parameters**	**Assessment of the Art. 7 parameters from the fact-sheet**
(a)(vi) the routes and speed of transmission of the disease between animals and, when relevant, between animals and humans	(a)(vi) 1 types of routes of transmission from animal to animal (horizontal, vertical)	Results of experimental trials on WNV transmission routes in wild birds are summarized in table a.vi.1-2. in the Table section. Mosquito bites are the usual source of WNV for mammals, reptiles and amphibians however in some animals; there is also evidence for transmission by other routes. Carnivorous mammals and reptiles (e.g., cats and alligators) can be infected by eating contaminated tissues. Direct transmission during close contact has also been reported in

		alligators, possibly via fecal shedding of virus. Chipmunks, squirrels and raccoons can also shed WNV in feces, oral secretions and/or urine. WNV has been found in the urine of experimentally infected hamsters, and in very small amounts in the oral and/or cloacal fluids of experimentally infected North American bullfrogs (*Rana catesbeiana*) and green iguanas (*Iguana iguana*). Transplacental transmission was reported in experimentally infected sheep and mice, as well as in a horse that was fatally infected with a lineage 1 virus in Africa, and aborted in the final stage of the disease. The epidemiological significance (if any) of mammalian, reptilian and amphibian hosts in the maintenance or amplification of WNV remains to be established.
	(a)(vi) 2 types of routes of transmission from animal to humans (direct, indirect)	There is no evidence of natural direct transmission between vertebrates and humans. However, human infection from the exposure of conjunctival membranes (Fonseca et al., 2005) and/or percutaneous injury to the body fluids or tissues of WNV infected birds (CDC, 2002) has been described.

Question A(ii)

Question A(ii) animal species are either susceptible to the disease or vectors and reservoirs thereof exist in the Union
Interpretation: indicate if animal species susceptible to the disease or vector or reservoir are present in the Union
Answer Y ☐ N ☐ na ☐

Art. 7 criteria	Art. 7 parameters	Assessment of the Art. 7 parameters from the fact-sheet
(a)(i) animal species concerned by the disease	(a)(i) 1 naturally susceptible wildlife species	**Birds** **Order Anseriformes** Family Anatidae: Wood Duck-Aix sponsa; Eurasian Wigeon-Anas penelope (c); Bronze-winged Duck (Spectacled Duck)-Anas specularis (c); Canvasback-Aythya valisineria; Canada Goose-Branta Canadensis; Barnacle Goose-Branta leucopsis (c)(a); Emperor Goose-Chen canagica (c); Greater Magellan Goose (Andean Goose)-Chloephagapicta leucoptera (c)(a); Abyssinian Blue-winged Goose-Cyanochen cyanopterus (c)(a); Tundra Swan-Cygnus columbianus (c); Trumpeter Swan-Cygnus Cygnus buccinator (c)(a); Mute Swan-Cygnus olor; Rosybilled Duck-Netta peposaca (c)(a); Ruddy Duck-Oxyura jamaicensis **Order Apodiformes** Family Apodidae: Chimney Swift-Chaetura pelagica; Family Trochilidae; Ruby-throated Hummingbird-Archilochus colubris **Order Caprimulgiformes** Family Caprimulgidae: Common Nighthawk-Chordeiles minor **Order Casuariiformes** Family Dromaiidae: Emu-Dromaius novaehollandiae (c) **Order Charadriiformes** Family Charadriidae: Ruddy Turnstone-Arenaria interpres; Killdeer-Charadrius vociferous; Piping Plover-Charadrius melodus Family Laridae: Herring Gull-Larus argentatus; Laughing Gull-Larus atricilla; Ring-billed Gull-Larus delawarensis; Great Black-backed Gull-Larus marinus; Black Skimmer-Rhynchops niger; Grey Gull-Larus modestus (c)(a); Inca Tern-Larosterna inca (c)(a) **Order Ciconiformes** Family Ardeidae: Yellow-crowned Night-heron-Nyctanassa violacea (c); Black-crowned Night-heron-Nycticorax nycticorax (c); Great Blue Heron-Ardea Herodias; Green Heron-Butorides virescens; Least Bittern-Ixobrychus exilis Family Cathartidae: Turkey Vulture-Cathartes aura; Black Vulture-Coragyps atratus; King Vulture-Sarcoramphus papa (c)(a) Family Ciconiidae: Saddle-billed Stork-Ephippiorhynchos senegalensis (c)(a);Marabou Stork-Leptopilos crumeniferus (c)(a); Lesser Adjutant Stork-Leptoptilos javanicus (c)(a) Family Phoenicopteridae: Chilean Flamingo-Phoenicopterus chilensis (c); Greater Flamingo (American)-Phoenicopterus ruber ruber (c) Family Threskiornithidae: Scarlet Ibis-Eudocimus ruber (c); Waldrapp-Geronticus eremita (c)(a) **Order Columbiformes** Family Columbidae: White-crowned Pigeon-Columba leucocephala; Rock Dove (Feral Pigeon)-Columba livia; Mauritius Pink Pigeon-Columba mayeri (c)(a); Common Ground-Dove-Columbina passerina; Eurasian Collared-Dove-Streptopelia decaocto; White-winged Dove-Zenaida asiatica; Mourning Dove-Zenaida macroura; Luzon Pigeon (Bleeding Heart Pigeon)-Gallicolumba luzonica (c)(a); Inca Dove-Columbina inca **Order Coraciiformes** Family Alcedinidae: Belted Kingfisher-Ceryle alcyon **Order Cuculiformes**

		Family Cuculidae: Yellow-billed Cuckoo-Coccyzus americanus **Order Falconiformes** Family Accipitridae: Cooper's Hawk-Accipiter cooperii; Northern Goshawk-Accipiter gentilis; Sharp-shinned Hawk-Accipiter striatus; Golden Eagle-Aquila chrysaetos; Red-tailed Hawk-Buteo jamaicensis; Rough-legged Hawk-Buteo lagopus (c); Red-shouldered Hawk-Buteo lineatus; Broad-winged Hawk-Buteo platypterus; Swainson's Hawk-Buteo swainsoni; Northern Harrier-Circus cyaneus; Swallow-tailed Kite-Elanoides forficatus; Bald Eagle-Haliaeetus leucocephalus; Mississippi Kite-Ictinia mississippiensis; Osprey-Pandion haliaetus; Harris' Hawk-Parabuteo unicinctus (c) Family Falconidae: Merlin-Falco columbarius; Prairie Falcon-Falco mexicanus; Peregrine Falcon-Falco peregrinus; American Kestrel-Falco sparverius **Order Galliformes** Family Numididae: Crested Guineafowl-Guttera pucherani (c)(a) Family Odontophoridae: Northern Bobwhite-Colinus virginianus Family Phasianidae: Chukar-Alectoris chukar (c)(a); Ruffed Grouse-Bonasa umbellus; Green Junglefowl-Gallus varius (c)(a); Impeyan (Himalayan) Pheasant (Monal)-Lophophorus impeyanus (c); Bulwer's Wattled Pheasant-Lophura bulweri (c)(a); Ring-necked Pheasant-Phasianus colchicus; Mount Peacock-Pheasant-Polypectron inopinatum (c)(a); Crested Partridge-Rollulus roulroul (c)(a); Blyth's Tragopan-Tragopan blythii (c); Argus Pheasant (unspecified)-various (c)(a); Greater Sage Grouse-Centrocerus urophasianus **Order Gaviformes** Family Caprimulgidae: Common Loon-Gavia immer **Order Gruiformes** Family Gruidae: Demoiselle Crane-Anthropoides virgo (c)(a); West African Crowned Crane-Balearica pavonina pavonina (a); Wattled Crane-Bugeranus carunculatus (c)(a); Whooping Crane-Grus americana (c)(a); Mississippi Sandhill Crane-Grus canadensis pulla (c); Red-crowned Crane-Grus japonensis (c)(a); Siberian Crane-Grus leucogeranus (c)(a); Hooded Crane-Grus monacha (c)(a); White-naped Crane-Grus vipio (c)(a); Black-necked Crane-Grus nigricollis (c)(a) Family Rallidae: Virginia Rail-Rallus limicola **Order Musophagiformes** Family Musophagidae: Lady Ross' Turaco (Plantain-Eater)-Musophaga rossae (c)(a) **Order Passeriformes** Family Bombycillidae: Cedar Waxwing-Bombycilla cedrorum Family Cardinalidae: Northern Cardinal-Cardinalis cardinalis; Blue Grosbeak-Guiraca caerulea(a); Rose-breasted Grosbeak-Pheucticus ludovicianus; Dickcissel-Spiza americana Family Corvidae: Western Scrub-Jay-Aphelocoma californica; American Crow-Corvus brachyrhynchos; Common Raven-Corvus corax; Fish Crow-Corvus ossifragus; Blue Jay-Cyanocitta cristata; Steller's Jay-Cyanocitta stelleri; Black-billed Magpie-Pica hudsonia (c) Family Emberizidae: Song Sparrow-Melospiza melodia; Savannah Sparrow-Passerculus sandwichensis; Fox Sparrow-Passerella iliaca; Eastern Towhee-Pipilo erythrophthalmus; Field Sparrow-Spizella pusilla Family Estrildidae: Zebra Finch-Taeniophygia guttata (c) Family Fringillidae: American Goldfinch-Carduelis tristis; House Finch-Carpodacus mexicanus; Purple Finch-Carpodacus purpureus; Evening Grosbeak-Coccothraustes vespertinus; European Goldfinch-Carduelis carduelis (c) Family Hirundinidae: Barn Swallow-Hirundo rustica; Purple Martin-Progne subis; Tree Swallow-Tachycineta bicolor Family Icteridae: Red-Winged Blackbird-Agelaius phoeniceus; Rusty Blackbird-Euphagus carolinus; Brewer's Blackbird-Euphagus cyanocephalus; Baltimore Oriole-Icterus galbula; Brown-headed Cowbird-Molothrus ater; Boat-tailed Grackle-Quiscalus major; Great-tailed Grackle-Quiscalus mexicanus; Common Grackle-Quiscalus quiscula Family Laniidae: Loggerhead Shrike-Lanius ludovicianus Family Mimidae: Gray Catbird-Dumetella carolinensis; Northern Mockingbird-Mimus polyglottos; Brown Thrasher-Toxostoma rufum Family Paridae*:* Tufted Titmouse-Baeolophus bicolor; Varied Tit-Parus varius (c); Black-capped Chickadee-Poecile atricapilla; Carolina Chickadee-Poecile carolinensis Family Parulidae: Black-throated Blue Warbler-Dendroica caerulescens; Yellow-rumped Warbler-Dendroica coronate; Yellow Warbler-Dendroica petechial; Blackpoll Warbler-Dendroica striata; Common Yellowthroat-Geothlypis trichas; Kentucky Warbler-Oporornis formosus; Northern Parula-Parula Americana; Ovenbird-Seiurus aurocapillus; Northern Waterthrush-Seiurus noveboracensis; Nashville Warbler-Vermivora ruficapilla; Canada Warbler-Wilsonia Canadensis; Hooded Warbler-Wilsonia citrina Family Passeridae: House Sparrow-Passer domesticus Family Sylviidae: White-crested Laughingthrush-Garrulax leucolophus (c)(a) Family Sittadae: White-breasted Nuthatch-Sitta carolinensis Family Sturnidae: European Starling-Sturnus vulgaris

		Family Thraupidae: Palm Tanager-Thraupis palmarum (c) Family Troglodytidae: Carolina Wren-Thryothaurus ludovicianus; Winter Wren-Troglodytes troglodytes Family Turdidae: Veery-Catharus fuscescens; Hermit Thrush-Catharus guttatus; Gray-cheeked Thrush-Catharus minimus; Swainson's Thrush-Catharus ustulatus; Wood Thrush-Hylocichla mustelina; Eastern Bluebird-Sialia sialis; American Robin-Turdus migratorius Family Tyrannidae: Traill's Flycatcher-Empidonax traillii/alnorum; Eastern Phoebe-Sayornis phoebe; Scissor-tailed Flycatcher-Tyrannus forficatus; Eastern Kingbird-Tyrannus tyrannus Family Vireonidae: Black-whiskered Vireo-Vireo altiloquus; Warbling Vireo-Vireo gilvus; Red-eyed Vireo-Vireo olivaceus **Order Pelecaniformes** Family Pelecanidae: American White Pelican-Pelecanus erythrorhynchos; Brown Pelican-Pelicanus occidentalis (c)(a); Family Phalacrocoracidae; Double-crested Cormorant-Phalacrocorax auritus; Guanay Cormorant-Phalacrocorax bougainvillei (c) **Order Piciformes** Family Picidae: Red-headed Woodpecker-Melanerpes erythrocephalus; Downy Woodpecker-Picoides pubescens; Yellow-bellied Sapsucker-Sphyrapicus varius **Order Podicipediformes** Family Podicipedidae: Pied-billed Grebe-Podilymbus podiceps **Order Psittaciformes** Family Cacatuidae: Cockatoo (unspecified)-Cacatua spp. (c) ; Cockatiel-Nymphicus hollandicus (c) Family Psittacidae: Red-crowned Parrot-Amazona viridigenalis (c); Macaw (unspecified)-Ara spp. (c); Budgerigar-Melopsittacus undulatus (c); Lorikeet spp.-Tricheglossus spp. (c) **Order Sphenisciformes** Family Spheniscidae: Black-footed (Jackass) Penguin-Spheniscus demersus (c); Magellan Penguin-Spheniscus humboldti (c)(a) **Order Strigiformes** Family Strigidae: Northern Saw-whet Owl-Aegolius acadicus; Boreal Owl-Aegolius funereous (c); Short-eared Owl-Asio flammeus; Verreaux's Eagle Owl (Milky Eagle Owl)-Bubo lacteus(c)(a); Great Horned Owl-Bubo virginianus; Snowy Owl-Nyctea scandiaca (c); Eastern Screech Owl-Otus asio; Tawny Owl-Strix aluco(c); Great Grey Owl-Strix nebulosa (c); Spotted Owl-Strix occidentalis (c); Barred Owl-Strix varia; Northern Hawk Owl-Surnia ulula (c) Family Tytonidae: Barn Owl-Tyto alba **Order Struthioniformes** Family Struthionidae: Ostrich-Struthio camelis (c)(a) **Mammals** **Order Artiodactyla** Family Bovidae: Mountain Goat-Oreamnos americanus (c) Family Camelidae: Llama-Lama glama (c); Alpaca (Suri)-Lama pacos (c) Family Cervidae: White-tailed Deer-Odocoileus virgninianus; Reindeer-Rangifer tarnadus (c); Mule Deer-Odocoileus hemionus Family Suidae: Babirusa-Babyrousa babyrousa (c)(a) **Order Carnivora** Family Canidae: Timber Wolf-Canis lupus (c) Family Mustelidae: Striped Skunk-Mephitis mephitis Family Phocidae: Harbor Seal-Phoca vitulina (c) Family Procyonidae: Red Panda-Ailurus fulgens fulgens (c)(a) Family Ursidae: Black Bear-Ursus americanus(a) **Order Chiroptera** Family Vespertilionidae: Big Brown Bat-Eptesicus fuscus; Little Brown Bat-Myotis lucifugus **Order Perissodactyla** Family Rhinocerotidae: Great Indian Rhinoceros-Rhinoceros unicornis (c)(a) **Order Primata** Family Cercopithcidae: Barbary Macaque-Macaca sylvanus (c) Family Lemuridae: Ring-tailed Lemura-Lemur catta (c) **Order Proboscidea** Family Elephantidae: Indian (Asian) Elephant-Elephas maximus indicus (c)(a) **Order Rodentia** Family Sciuridae: Gray Squirrel-Sciurus carolinensis; Fox Squirrel-Sciurus niger; Eastern Chipmunk-Tamias striatus **Reptiles** **Order Crocodylia** Family Alligatoridae: American Alligator-Alligator mississippiensis (c) **Order Squamata** Family Varanidae: Crocodile Monitor-Varanus salvadorii (c)(a) ((c) denotes either a captive or farmed animal(s). Virus or viral RNA was detected in

		animal tissue unless followed by an (a), which denotes detectable antibodies only have been reported (Source: USGS, National Wildlife Health Center (USGS, online)).
	(a)(i) 2 naturally susceptible domestic species	Family Phasianidae: Domestic Chicken (Red Junglefowl)-Gallus gallus; Turkey (domestic and wild)-Meleagris gallopavo Family Anatidae: Mallard-Anas platyrhynchos; Domestic Goose-Anser chinensis (c)(a) Family Bovidae: Domestic Cattle-Bos Taurus; Domestic (Suffolk) Sheep-Ovis aries Family Canidae: Domestic Dog-Canis familiaris Family Felidae: Domestic Cat (feral)-Felis catus Family Leporidae: Domestic Rabbit-Oryctolagus cuniculus Family Equidae: Domestic Horse-Equus equus przewalski caballus; Donkey-Equus asinus; Mule
	(a)(i) 3 experimentally susceptible wildlife species (a)(i) 4 experimentally susceptible domestic species	West Nile virus causes disease in humans, horses, and several species of birds. Most infected individuals show few signs of illness, but some develop severe neurological illness which can be fatal. West Nile Virus has an extremely broad host range. It replicates in birds, reptiles, amphibians, mammals, mosquitoes and ticks. Besides equids, details of susceptible domestic mammalian species are summarized in table a.i. (1) while table a.i. (2) summarizes outcomes of experimental infections of West Nile virus assessed in wild birds.
	(a)(i) 5 wild reservoir species	Birds, particularly passerine species (jays, finches, sparrows, and crows).
	(a)(i) 6 domestic reservoir species	West Nile virus causes disease in humans, horses, and several species of birds. Most infected individuals show few signs of illness, but some develop severe neurological illness which can be fatal. West Nile Virus has an extremely broad host range. It replicates in birds, reptiles, amphibians, mammals, mosquitoes and ticks. Besides equids, details of susceptible mammalian species are summarized in table a.i.(1) while table a.i. (2) summarizes outcomes of experimental infections of West Nile virus assessed in wild birds. Outside US, clinical symptoms to WNV infection has been reported in a scarce number of avian species in course of outbreaks: domestic geese (*Anser anser domesticus*) and white storks (*Ciconia ciconia*) during the WNV epidemic in Israel (Malkinson et al., 2002), goshawks (*Accipiter gentilis*) in Hungary (Bakonyi et al., 2006), eurasian jays (*Garrulus glandarius*), little owl (*Athene noctua*), mallard (*Anas plathyrynchos*), common buzzard (*Buteo buteo*) in Italy (Monaco et al., 2015). However, mass mortality of highly susceptible species (such as corvids or other species) is less frequently observed in the Old than in the New World although some species, as the jackdaws (*Corvus monedula*) could potentially function as sentinel (Lim et al., 2014). Surveillance activities carried out in Italy where WNV is endemic since 2008, pointed out the high susceptibility to the viral infection of three species of synantropic wild birds, namely carrion crow (*Corvus corone*), magpie (*Pica pica*) and eurasian jay (*Garrulus glandarius*) which justifies their use as sentinel in endemic areas (Italian Ministry of Health, 2016). WNV has been associated with sporadic disease in small numbers of other species, including squirrels, chipmunks, bats, dogs, cats, white-tailed deer, reindeer, sheep, alpacas, dromedary camels, alligators and harbour seals during intense periods of local viral activity. Some species of mammals including squirrels (*Sciurus* sp.), eastern chipmunks (*Tamias striatus*) and eastern cottontail rabbits (*Sylvilagus floridanus*) may be capable of transmitting WNV to mosquitoes, although their importance as reservoir hosts is still uncertain. Among reptiles, clinical signs were mainly reported during outbreaks in alligators, although there is also a report of neurological signs associated with WNV infection in a crocodile monitor (*Varanus salvadori*) lizard. Some infections in garter snakes (*Thamnophis sirtalis*) experimentally inoculated with WNV were also fatal. Green iguanas (*Iguana iguana*) can be infected. Amphibians including lake frogs (*Rana ridibunda*) and North American bullfrogs (*Rana catesbeiana*) can also be infected with WNV. Some alligators (e.g., American alligators, *Alligator mississippiensis*) and frogs (e.g., *Rana ridibunda* in Russia) may develop viremia sufficient to infect mosquitoes. As with mammals, their importance as reservoir hosts is still uncertain. Based on preliminary research carried out in Italy and Spain, only few bird species seem to play a major role as blood donor for the mosquitoes (Munoz et al., 2012; Hamer et al., 2009; Roiz et al., 2012, Spedicato et al., 2015). Unfortunately, the reservoir competence for many European bird species is still unknown even though the persistence of WNV in infected birds have been assessed in some species through experimental trials. House finches and House sparrows experimentally inoculated showed persistent infection in spleen and kidney 28 weeks p.i. The virus was still detected by real time RT-PCR in the spleen of two House sparrows at 36 weeks p.i. However, viral isolation attempts were unsuccessful (Wheeler et al., 2012). In a previous work (Nemeth et al., 2009a), a higher number of organs were analyzed in WNV-infected House

		sparrows, and viral RNA was detected in juvenile sparrows up to 65 days p.i in kidney and spleen, although infectious virus could be isolated at low titres only in one sparrow at 43 days p.i. Reisen and colleagues confirmed the persistent infection in five species of Passeriformes and in Common ground-dove (*Columbina passerina*) detecting the virus in spleen and kidney, but also in lung at >6 weeks p.i.

Question A(iii)

Question A(iii) disease causes negative effects on animal health OR poses a risk to public health due to its zoonotic character **Answer** Y ☐ N ☐ na ☐		
Art. 7 criteria	**Art. 7 parameters**	**Assessment of the Art. 7 parameters from the fact-sheet**
(a)(ii) morbidity and mortality rates of the disease in animal populations	(a)(ii) 1 Prevalence/ incidence	Refer to table a.ii in the Table section
	(a)(ii) 2 Case-morbidity rate (% clinically diseased animals out of infected ones)	
	(a)(ii) 3 Case-fatality rate	
(a)(iii) zoonotic character of the disease	(a)(iii) 1 report of zoonotic human cases	West Nile zoonotic transmission in known to be present in Europe since a long time: in the 1960s the virus emerged in southern France in the Camargue. Yet, the first large outbreak in humans was reported from Bucharest, Romania in 1996-1997. Since then, infection in humans and/or horses have been reported from the Czech Republic (1997), France (2000, 2003, 2004, 2006), Italy (1998, 2008, 2009), Hungary (2000-2009), Romania (1997-2001, 2003-2009), Spain (2004) and Portugal (2004). In 2010, the ecological parameters in Central European and Mediterranean countries were favorable for the transmission of WNV to humans. A human outbreak was reported from the Central Macedonia Region in northern Greece and human cases were reported from Romania, Hungary, Italy and Spain in August-September 2010. At the same time a large outbreak in humans was reported from Volgograd in Russia. Evidence of human cases have been in EU Countries have been listed in table b.ii.2 and a recent paper from the Italian Integrated WNV Surveillance Group (Rizzo et al., 2016) provide an example of geographical correlation between human and veterinary cases. For Figure **1** (Geographical distribution of West Nile neuroinvasive disease in horses (panel A) and humans (panel B), Italy 2008–2015 (Rizzo et al., 2016)) and Figure **2** (West Nile virus detections in the veterinary and human surveillance by month, Italy, 2008–2015 (Rizzo et al., 2016)) see Figures section.
(a)(iv) resistance to treatments, including antimicrobial resistance	(a)(iv) 1 resistant strain to any treatment even at laboratory level	Not applicable to WNV since there is no specific antiviral therapy
(b)(ii) Impact of the disease on human health	(b)(ii) 1 types of routes of transmission between animals and humans - *see (a)(vi)2*	Refer to table b.ii.2 in the Table section
	(b)(ii) 2 Incidence of zoonotic cases	
	(b)(ii) 3 Occasional or substantial?	WNV transmission through blood transfusion and organ transplantation is able to sustain community-level outbreak West Nile virus is most commonly transmitted to humans by mosquitoes even though additional routes of human to human transmission have also been documented as blood transfusions, organ transplants, exposure in a laboratory setting or the transmission from the mother to baby during pregnancy, delivery, or breastfeeding. It is important to note that these methods of transmission represent a very small proportion of cases thus sufficient to evoke only a sporadic occurrence of the disease.
	(b)(ii) 4 Epidemic or pandemic?	Sporadic potential
	(b)(ii) 5 DALY	As for most arthropod-borne diseases causing fever syndromes worldwide, the cumulative impact of WNV on global disease burden has not been fully assessed. Evaluations should include both the severe forms of the disease and the milder clinical manifestations which may result in neurological and ophthalmologic complications (Carson et al., 2006). WNV has been recognized able to induce a wide range of post-infection, long-term sequelae

		with the recovery of the affected patients within two years from the infection (Murray et al., 2008). However, a recent paper has emphasized that 40% of WNV affected patients continued to experience symptoms related to their WNV infection up to 8 years later demonstrating the health and economic impact of a result of prolonged recovery, continued morbidity, and related disability (Murray et al., 2014).
(b)(iii) Impact of the disease on animal welfare	(b)(iii) 1 severity of clinical signs at case level and related level and duration of impairment	The incubation period for equine WN encephalitis following mosquito transmission is estimated to be 3–15 days. A fleeting viraemia of low virus titre precedes clinical onset (Bunning et al., 2002). WN viral encephalitis occurs in only a small per cent of infected horses; the majority of infected horses do not display clinical signs (Ostlund et al., 2000). The disease in horses is frequently characterised by mild to severe ataxia. Additionally, horses may exhibit weakness, muscle fasciculation and cranial nerve deficits (Cantile et al., 2000; Ostlund et al., 2000; 2001; Snook et al., 2001). Fever is an inconsistently recognised feature. Treatment is supportive and signs may resolve or progress to terminal recumbency. The mortality rate is approximately one in three clinically affected unvaccinated horses. Many species of birds can become infected with WNV; the clinical outcome of infection is variable. Some species appear resistant while others suffer fatal neurologic disease. Neurologic disease and death have been documented in domestic geese in Israel and Canada, and in many native and exotic zoo birds in the USA during the emergence of WNV (Steele et al., 2000). WND associated cases have been described in European wild birds (Bakonyi et al. 2006, Höfle et al. 2008, Jiménez-Clavero et al. 2008). In 2011 during the Sardinian WND outbreak neurological disease has been reported in 2 wild birds as Eurasian jays (*Garrulus glandarius*) with clinical signs characterised by drowsy, incapability of flying or walking properly, ruffle feathers, pectoral atrophy, and absence of the flight instinct. Lethargy, head tremors, drooping wings and inability to fly due to the flaccid paralysis of the wing muscles were described in an adult common buzzard. The legs were kept flexed and the bird was not able to stand up. The podal reflex was lost whereas both, the pupillary and corneal reflexes were still present. The animals died within 24 hours from the admission to a veterinary clinic. A little owl (*Athene noctua*), was bought to a rehabilitation centre showing ataxia, incoordination, reluctance or inability to fly properly, head tilt and anisocoria. It was able to stand up by using the tail feather and the wings. In the second day, clinical signs became more severe. The corneal reflex was lost and the animal was not anymore capable of standing up although it still tried to fly when encouraged. It died at the end of the second day. Clinical signs have been described also in an adult male mallard (*Anas plathyrynchos*) with complete flaccid paralysis of the legs and even if still present, the instinct to escape was precluded by the leg paralysis. Neck and wing movements were still under control and the sensorium was still awake. In the following day, the bird progressively lost the wing muscle contractile capability and the instinct of escape. The third day after the onset of clinical symptoms the animal died. An ataxic adult common buzzard (*Buteo buteo*) showed irregular head tremors and had trouble in maintaining the upright position even if using the tail feather and the wings. The instinct of escape was lost and the podal reflex as well as the proprioception response on the left leg was slow. The droppings were of a fluid-like consistency and the feathers around the vent were matted with faeces. When recumbent in a sternal position, the bird was not able to stand up properly and, similarly, it was not able to open its wings even if it was able to flex them back at the elbow joint when forcedly opened. In the second day, the lethargy became more severe and the animal died (Monaco et al., 2015).
(c) potential to generate a crisis situation and its potential use in bioterrorism	(c) 1 listed in OIE/CFSPH classification of pathogens	Yes, listed among the diseases from potential bioterrorist agents
	(c) 2 listed in the Encyclopedia of Bioterrorism Defense of Australia Group	No
	(c) 3 included in any other list of potential bio-agro-terrorism agents	Not reported

Question A(iv)

Question A(iv) diagnostic tools are available for the disease Interpretation: diagnostic tools are available for the disease in the Union **Answer** Y ☐ N ☐ na ☐		
Art. 7 criteria	**Art. 7 parameters**	**Assessment of the Art. 7 parameters from the fact-sheet**
(a)(viii) existence of diagnostic and disease control tools	(a)(viii) 1 Existence of diagnostic tools	Viral nucleic acid and viral antigens can be demonstrated in tissues of infected animals by RT-PCR and immuno-histochemistry, respectively. Antibody can be identified in equine serum by IgM capture enzyme-linked immunosorbent assay (IgM capture ELISA), haemagglutination inhibition (HI), IgG ELISA, plaque reduction neutralisation (PRN) or virus neutralisation (VN). In some serological assays, antibody cross-reactions with related flaviviruses, such as St Louis encephalitis virus, Usutu virus, Japanese encephalitis virus, or tick-borne encephalitis (TBE) virus may be encountered. According to the OIE, the following tests are suitable methods for confirmation of clinical cases: Nested RT-PCR, Real time RT-PCR and IgM capture ELISA the first 2 tests are also suitable for identifying individual animal freedom from infection. The plaque reduction neutralisation and serum neutralisation tests are both suitable methods for detecting prevalence of infection, population freedom from infection and immune status in animals post vaccination (Table 1). Equine WNV-specific IgM antibodies are usually detectable from 7–10 days post-infection to 1–2 months post-infection. Most horses with WN encephalitis test positive in the IgM capture ELISA at the time that clinical signs are first observed. WNV neutralising antibodies are detectable in equine serum by 2 weeks post-infection and can persist for more than 1 year. Several PCR methods are available as commercial kits. In view of the continued evolution and possible emergence of new WNV strains, it is important that the designs of PCR tests are constantly monitored and updated when necessary. Within the EU, OIE reference laboratories exist in Italy.
	(a)(viii) 2 Existence of disease control tools	According to the OIE Terrestrial Animal Health Code (Chapter 8.18. West Nile fever) the following criteria define the occurrence of WN fever (WNF) in equids: a) WNV has been isolated from an animal that shows signs consistent with WNF; or b) viral antigen or viral ribonucleic acid (RNA) specific to WNV has been identified in samples from one or more animals that show clinical signs consistent with WNF, or that is epidemiologically linked to a confirmed or suspected outbreak of WNF; or c) antibodies to WNV have been identified in an unvaccinated animal that shows clinical signs consistent with WNF, or that is epidemiologically linked to a confirmed or suspected outbreak of WNF. In areas where the disease is endemic horses may be protected from the clinical signs by vaccination (refer to the table of the vaccines commercially available in EU -table d.ii.1). Control efforts are focused on mosquito abatement and repellents, although implementation of these strategies is difficult to achieve in many situations. Due to the low level viremia and lack of viral shedding, an infected horse appears to pose no direct risk to other animals, including humans, except for the manipulation of infected tissues during necropsy and laboratory handling. Therefore, apart from isolating the affected animal mainly for animal welfare reasons, no particular control measures apply. For the same reasons there are no trade restrictions for the importation of equines coming from WNF infected countries or zones although WNV infection in horses is a notifiable disease. In the infected areas strategies must be implemented to reduce the circulation of the virus through measures that modify the density of the vectors (reduction of stagnant water, performance of adulticidal and larvicidal treatments) and to reduce the possibilities of contact between the vectors and receptive hosts (application of repellent, mosquito netting etc.). Among biocidal products, the use of pyrethrin (6%) and piperonyl butoxide (60%) by aerial spray indicated that the odds of infection after spraying were around 6 times higher in the untreated area than in treated areas, and that the treatments successfully disrupted the WNV transmission cycle (Carney et al., 2008).

Question A(v)

Question A(v) the risk-mitigating measures and, where relevant, surveillance of the disease are effective and proportionate to the risks posed by the disease in the Union **Answer** Y ☐ N ☐ na ☐		
Art. 7 criteria	**Art. 7 parameters**	**Assessment of the Art. 7 parameters from the fact-sheet**
(a)(viii) existence of diagnostic and disease control tools	(a)(viii) 1 Existence of diagnostic tools	Viral nucleic acid and viral antigens can be demonstrated in tissues of infected animals by RT-PCR and immuno-histochemistry, respectively. Antibody can be identified in equine serum by IgM capture enzyme-linked immunosorbent assay (IgM capture ELISA), haemagglutination inhibition (HI), IgG ELISA, plaque reduction neutralisation (PRN) or virus neutralisation (VN). In some

		serological assays, antibody cross-reactions with related flaviviruses, such as St Louis encephalitis virus, Usutu virus, Japanese encephalitis virus, or tick-borne encephalitis (TBE) virus may be encountered. According to the OIE, the following tests are suitable methods for confirmation of clinical cases: Nested RT-PCR, Real time RT-PCR and IgM capture ELISA the first 2 tests are also suitable for identifying individual animal freedom from infection. The plaque reduction neutralisation and serum neutralisation tests are both suitable methods for detecting prevalence of infection, population freedom from infection and immune status in animals post vaccination (Table 1). Equine WNV-specific IgM antibodies are usually detectable from 7–10 days post-infection to 1–2 months post-infection. Most horses with WN encephalitis test positive in the IgM capture ELISA at the time that clinical signs are first observed. WNV neutralising antibodies are detectable in equine serum by 2 weeks post-infection and can persist for more than 1 year. Several PCR methods are available as commercial kits. In view of the continued evolution and possible emergence of new WNV strains, it is important that the designs of PCR tests are constantly monitored and updated when necessary. Within the EU, OIE reference laboratories exist in Italy.
	(a)(viii) 2 Existence of disease control tools	According to the OIE Terrestrial Animal Health Code (Chapter 8.18. West Nile fever) the following criteria define the occurrence of WN fever (WNF) in equids: a) WNV has been isolated from an animal that shows signs consistent with WNF; or b) viral antigen or viral ribonucleic acid (RNA) specific to WNV has been identified in samples from one or more animals that show clinical signs consistent with WNF, or that is epidemiologically linked to a confirmed or suspected outbreak of WNF; or c) antibodies to WNV have been identified in an unvaccinated animal that shows clinical signs consistent with WNF, or that is epidemiologically linked to a confirmed or suspected outbreak of WNF. In areas where the disease is endemic horses may be protected from the clinical signs by vaccination (refer to the table of the vaccines commercially available in EU -table d.ii.1). Control efforts are focused on mosquito abatement and repellents, although implementation of these strategies is difficult to achieve in many situations. Due to the low level viremia and lack of viral shedding, an infected horse appears to pose no direct risk to other animals, including humans, except for the manipulation of infected tissues during necropsy and laboratory handling. Therefore, apart from isolating the affected animal mainly for animal welfare reasons, no particular control measures apply. For the same reasons there are no trade restrictions for the importation of equines coming from WNF infected countries or zones although WNV infection in horses is a notifiable disease. In the infected areas strategies must be implemented to reduce the circulation of the virus through measures that modify the density of the vectors (reduction of stagnant water, performance of adulticidal and larvicidal treatments) and to reduce the possibilities of contact between the vectors and receptive hosts (application of repellent, mosquito netting etc.). Among biocidal products, the use of pyrethrin (6%) and piperonyl butoxide (60%) by aerial spray indicated that the odds of infection after spraying were around 6 times higher in the untreated area than in treated areas, and that the treatments successfully disrupted the WNV transmission cycle (Carney et al., 2008).
(b)(ii) Impact of the disease on human health	(b)(ii) 6 Availability of medical treatment and their effectiveness (therapeutic effect and any resistance)	There is no specific recommended treatment, other than supportive care, at present. Intensive care and mechanical ventilation may be required in some cases. Various therapies including interferon, antisense nucleotides and intravenous immunoglobulins (passive immunization) are being tested in clinical trials. While a few case reports suggest that some of these treatments may be promising, larger studies are still lacking. Screening for new drugs that may inhibit WNV is underway.
	(b)(ii) 7 Availability of vaccines and their effectiveness (reduced morbidity)	There are no vaccines available for human use in EU.

<table>
<tr><td rowspan="3">(d)(i) feasibility, availability and effectiveness of diagnostic tools and capacities</td><td>(d)(i) 1 officially/internationally recognised diagnostic tool, OIE certified</td><td>
<table>
<tr><th rowspan="2">Method</th><th colspan="5">Purpose</th></tr>
<tr><th>Population freedom from infection</th><th>Individual animal freedom from infection</th><th>Confirmation of clinical cases</th><th>Prevalence of infection – surveillance</th><th>Immune status in individual animals or populations post-vaccination</th></tr>
<tr><td colspan="6">Agent identification[1]</td></tr>
<tr><td>Nested RT-PCR</td><td>–</td><td>++</td><td>++</td><td>–</td><td>–</td></tr>
<tr><td>Real time RT-PCR</td><td>–</td><td>++</td><td>++</td><td>–</td><td>–</td></tr>
<tr><td>Isolation in tissue culture</td><td>–</td><td>++</td><td>++</td><td>–</td><td>–</td></tr>
<tr><td colspan="6">Detection of immune response</td></tr>
<tr><td>IgM capture ELISA</td><td>–</td><td>–</td><td>++</td><td>–</td><td>–</td></tr>
<tr><td>Plaque reduction neutralisation</td><td>++</td><td>–</td><td>+</td><td>++</td><td>++</td></tr>
<tr><td>Serum neutralisation</td><td>++</td><td>–</td><td>+</td><td>++</td><td>++</td></tr>
<tr><td>Immunohisto-chemistry</td><td>-</td><td>-</td><td>+</td><td>-</td><td></td></tr>
</table>
Key: +++ = recommended method; ++ = suitable method; + = may be used in some situations, but cost, reliability, or other factors severely limits its application; – = not appropriate for this purpose.
Although not all of the tests listed as category +++ or ++ have undergone formal validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.
RT-PCR = reverse-transcriptase polymerase chain reaction; IgM = immunoglobin;
ELISA = enzyme-linked immunosorbent assay.

Table 1: Test methods available for the diagnosis of WNV and their purpose (Source: OIE, 2013).
</td></tr>
<tr><td>(d)(i) 2 Se and Sp of diagnostic test</td><td rowspan="2">Refer to table d.i. in the Table section</td></tr>
<tr><td>(d)(i) 3 type of sample matrix to be tested (blood, tissue, etc.)</td></tr>
<tr><td rowspan="5">(d)(ii) feasibility, availability and effectiveness of vaccination</td><td>(d)(ii) 1 types of vaccines available on the market</td><td rowspan="5">WNV vaccines approved by EMA are listed in table d.ii.1 in the Table section</td></tr>
<tr><td>(d)(ii) 2 availability / production capacity (per year)</td></tr>
<tr><td>(d)(ii) 3 Field protection as reduced morbidity (reduced susceptibility to infection and/or to disease)</td></tr>
<tr><td>(d)(ii) 4 Duration of protection</td></tr>
<tr><td>(d)(ii) 5 Way of administration</td></tr>
<tr><td rowspan="4">(d)(iii) feasibility, availability and effectiveness of medical treatments</td><td>(d)(iii) 1 types of drugs available on the market and/or allowed by the EU regulatory system</td><td rowspan="4">There is no specific recommended treatment, other than supportive care, at present.</td></tr>
<tr><td>(d)(iii) 2 availability / production capacity (per year)</td></tr>
<tr><td>(d)(iii) 3 therapeutic effect in the field (effectiveness)</td></tr>
<tr><td>(d)(iii) 4 Way of administration</td></tr>
<tr><td rowspan="3">(d)(iv) feasibility, availability and effectiveness of biosecurity measures</td><td>(d)(iv) 1 available biosecurity measures</td><td rowspan="3">The biosecurity measures preventing WNV entering or leaving any place where farm animals are present should be focused on fighting the vectors, major responsible for the viral transmission. Farm to farm movement of infected horses is not effective to spread the disease since they are neither able to transmit the virus to biting mosquitoes nor, directly, to vertebrates including humans.
To minimize the possibilities of contact between the vectors and receptive hosts it is advisable to use mosquito nets to avoid the vector entrance in the stables as well as the use of repellents on the animals. Data related to the efficacy of these substances</td></tr>
<tr><td>(d)(iv) 2 effectiveness of biosecurity measure</td></tr>
<tr><td>(d)(iv) 3 feasibility of biosecurity measure</td></tr>
</table>

		has been detailed in the paragraph "use and potential residual of biocides or medical drugs in environmental compartments ". Personnel involved in field collection of samples should considered the use of repellants (i.e. 20-30% DEET) and other precautions for mosquito avoidance as wearing long sleeved shirts, full length trousers, socks, light coloured clothing, high boots.
(d)(v) feasibility, availability and effectiveness of restrictions on the movement of animals and products, as control measure	(d)(v) 1 available restriction movement measures	No specific measures are mentioned in the EU legislation for WNV outbreak control.
	(d)(v) 2 effectiveness of restriction of animal movement in preventing the between farm spread	
	(d)(v) 3 feasibility of restriction of animal movement	
(d)(vi) feasibility, availability and effectiveness of killing of animals	(d)(vi) 1 available killing of animal measures	No specific measures are mentioned in the EU legislation for WNV outbreak control.
	(d)(vi) 2 effectiveness of killing animals (at farm level or within the farm) for reducing /stopping spread of the disease	
	(d)(vi) 3 feasibility of killing animals	
(d)(vii) feasibility, availability and effectiveness of disposal of carcasses and other relevant animal by—products	(d)(vii) 1 disposal options available	No specific measures are mentioned in the EU legislation for WNV outbreak control.
	(d)(vii) 2 effectiveness of disposal option	
	(d)(vii) 3 feasibility of disposal option	

Question B(i)

Question B(i) disease causes or could cause significant negative effects in the Union on animal health, OR poses or could pose a significant risk to public health due to its zoonotic character?
Answer Y ☐ N ☐ na ☐

Art. 7 criteria	Art. 7 parameters	Assessment of the Art. 7 parameters from the fact-sheet
(a)(ii) morbidity and mortality rates of the disease in animal populations	(a)(ii) 1 Prevalence/ Incidence	Refer to table a.ii in the Table section
	(a)(ii) 2 Case-morbidity rate (% clinically diseased animals out of infected ones)	
	(a)(ii) 3 Case-fatality rate	
(a)(iii) zoonotic character of the disease	(a)(iii) 1 report of zoonotic human cases	West Nile zoonotic transmission in known to be present in Europe since a long time: in the 1960s the virus emerged in southern France in the Camargue. Yet, the first large outbreak in humans was reported from Bucharest, Romania in 1996-1997. Since then, infection in humans and/or horses have been reported from the Czech Republic (1997), France (2000, 2003, 2004, 2006), Italy (1998, 2008, 2009), Hungary (2000-2009), Romania (1997-2001, 2003-2009), Spain (2004) and Portugal (2004). In 2010, the ecological parameters in Central European and Mediterranean countries were favorable for the transmission of WNV to humans. A human outbreak was reported from the Central Macedonia Region in northern Greece and human cases were reported from Romania, Hungary, Italy and Spain in August-September 2010. At the same time a large outbreak in humans was reported from Volgograd in Russia.

		Evidence of human cases have been in EU Countries have been listed in table b.ii.2 and a recent paper from the Italian Integrated WNV Surveillance Group (Rizzo et al., 2016) provide an example of geographical correlation between human and veterinary cases. For Figure **1** (Geographical distribution of West Nile neuroinvasive disease in horses (panel A) and humans (panel B), Italy 2008–2015 (Rizzo et al., 2016)) and Figure **2** (West Nile virus detections in the veterinary and human surveillance by month, Italy, 2008–2015 (Rizzo et al., 2016)) see Figures section.
(a)(iv) resistance to treatments, including antimicrobial resistance	(a)(iv) 1 resistant strain to any treatment even at laboratory level	Not applicable to WNV since there is no specific antiviral therapy
(b)(ii) Impact of the disease on human health	(b)(ii) 1 types of routes of transmission between animals and humans - *see (a)(vi)2*	Refer to table b.ii.2 in the Table section
	(b)(ii) 2 Incidence of zoonotic cases	
	(b)(ii) 3 Occasional or substantial?	WNV transmission through blood transfusion and organ transplantation is able to sustain community-level outbreak West Nile virus is most commonly transmitted to humans by mosquitoes even though additional routes of human to human transmission have also been documented as blood transfusions, organ transplants, exposure in a laboratory setting or the transmission from the mother to baby during pregnancy, delivery, or breastfeeding. It is important to note that these methods of transmission represent a very small proportion of cases thus sufficient to evoke only a sporadic occurrence of the disease.
	(b)(ii) 4 Epidemic or pandemic?	Sporadic potential
	(b)(ii) 5 DALY	As for most arthropod-borne diseases causing fever syndromes worldwide, the cumulative impact of WNV on global disease burden has not been fully assessed. Evaluations should include both the severe forms of the disease and the milder clinical manifestations which may result in neurological and ophthalmologic complications (Carson et al., 2006). WNV has been recognized able to induce a wide range of post-infection, long-term sequelae with the recovery of the affected patients within two years from the infection (Murray et al., 2008). However, a recent paper has emphasized that 40% of WNV affected patients continued to experience symptoms related to their WNV infection up to 8 years later demonstrating the health and economic impact of a result of prolonged recovery, continued morbidity, and related disability (Murray et al., 2014).

Question B(ii)

Question B(ii) disease agent has developed resistance to treatments WHICH poses a significant danger to public and/or animal health in the Union?
Interpretation: disease agent has developed resistance to treatments AND therefore poses a significant danger to public and/or animal health. If no treatment exists the answer should be na
Answer Y ☐ N ☐ na ☐

Art. 7 criteria	Art. 7 parameters	Assessment of the Art. 7 parameters from the fact-sheet
(a)(iv) resistance to treatments, including antimicrobial resistance	(a)(iv)1 list of any resistant strain to any treatment even at laboratory level	Not applicable to WNV since there is no specific antiviral therapy

Question B(iii)

Question B(iii) disease causes or could cause a significant negative economic impact affecting agriculture or aquaculture production in the Union?
Interpretation: disease and/or infection causes or could cause a significant negative economic impact affecting agriculture or aquaculture production in the Union if no intervention is in place
Answer Y ☐ N ☐ na ☐

Art. 7 criteria	Art. 7 parameters	Assessment of the Art. 7 parameters from the fact-sheet
(a)(ii) morbidity and mortality rates of the disease in animal populations	(a)(ii) 3 Case-fatality rate	Refer to table a.ii. in the Table section
(b)(i) the impact of the disease on agricultural and aquaculture	(b)(i) 1 Number of MSs where the disease is present	Since the beginning of the 2016 transmission season, the presence of WNV has been confirmed in MSs and neighbouring countries. As of 27th October 2016, 205 human cases of West Nile fever have been

production and other parts of the economy		reported in EU and 261 cases in neighbouring countries (Austria, Croatia, Cyprus, Egypt, Hungary, Italy, Israel, Portugal, Romania, Russian Federation, Serbia, Spain and Syrian Arab republic, Tunisia, Ukraine) (ECDC, 2016).
	(b)(i) 2 Proportion of production losses (%) by epidemic/endemic situation (milk, growth, semen, meat, etc.)	In European outbreaks WNV has not been associated to any mortality in domestic birds but has been limited to a few cases in wild birds (paragraph a.i). Outside EU, among poultry, young geese seem to be particularly susceptible to WNV, and have been affected in both Western and Eastern Hemispheres. In Israel, disease was reported in 3-8-week-old goslings, with morbidity and mortality rates of approximately 40%. During an outbreak in Canada, the mortality rate was 25% in 6-week-old goslings, but 15-month-old and 5-year-old geese seroconverted with no clinical signs. In experimental infections, up to 50–75% of geese may die. Ducks are not thought to be highly susceptible to WNV; however, an outbreak among captive lesser scaup (*Aythya affinis*) ducklings resulted in 70% mortality. During other outbreaks, the morbidity and mortality rates were 100% in Impeyan pheasants, and the mortality rate was 25% in chukar partridges. Similarly to geese, young partridges and pheasants seem to be more susceptible to disease. In contrast, both young and old chickens and turkeys are infected asymptomatically.

Question B(iv)

Question B(iv) disease has the potential to generate a crisis or the disease agent could be used for the purpose of bioterrorism **Answer** Y ☐ N ☐ na ☐		
Art. 7 criteria	**Art. 7 parameters**	**Assessment of the Art. 7 parameters from the fact-sheet**
(c) potential to generate a crisis situation and its potential use in bioterrorism	(c) 1 listed in OIE/CFSPH classification of pathogens	Yes, listed among the diseases from potential bioterrorist agents
	(c) 2 listed in the Encyclopaedia of Bioterrorism Defense of Australia Group	No
	(c) 3 included in any other list of potential bio-agro-terrorism agents	Not reported

Question B(v)

Question B(v) disease has or could have a significant negative impact on the environment, including biodiversity, of the Union **Answer** Y ☐ N ☐ na ☐		
Art. 7 criteria	**Art. 7 parameters**	**Assessment of the Art. 7 parameters from the fact-sheet**
(b)(iv) impact of the disease on biodiversity and the environment	(b)(iv) 1 endangered wild species affected: listed species as in CITES and/or IUCN list	Endangered wild species affected (CITES and/or IUCN) CITES (https://cites.org/sites/default/files/eng/app/2016/E-Appendices-2016-03-10.pdf) Phoenicopteridae spp. (App. II) *Falco rusticolus* (App.I) *Aquila adalberti* (App.I) Falconiformes spp. (App II)
	(b)(iv) 2 mortality in wild species	WNV outbreaks have been reported among domesticated geese in the Eastern Hemisphere, but generally there have been only sporadic reports of deaths in individual wild birds. It is uncertain whether this is related to the virulence of the viruses circulating in this region, host susceptibility, reduced transmission/ amplification or lack of surveillance. One recently introduced lineage 2 virus in Central Europe has affected significant numbers of wild and captive raptors. Species known to be susceptible to this isolate include sparrow hawks (*Accipiter nisus*), goshawks (*Accipiter gentilis*) and gyrfalcons (*Falco rusticolus*). The same virus was isolated from a dead collared dove (*Streptopelia decaocto*) in Italy, during an outbreak of mortality in collared doves and other species including blackbirds. Different lineages of the WNV have also been found occasionally in other dead birds including European robins (*Erithacus rubecula*), a raven (*Corvus corax*), common magpies (*Pica pica*), a Eurasian jay (*Garrulus glandarius*), house sparrows (*Passer domesticus*), a black redstart (*Phoenicurus ochruros*), a sedge warbler (*Acrocephalus schoenobaenus*) and a Savi's warbler (*Locustella luscinioides*).
	(b)(iv) 3 capacity of the pathogen to persist in the environment and	WNV is scarcely resistant in the environment thus its capability to survive during the vector-free period and, eventually, become endemic is still unknown. Different mechanisms have been claimed to explain WNV persistence. The duration of viremia in some bird species has been experimentally demonstrated (refer to the

	cause mortality in wildlife	paragraph "a.i - animal species concerned by the disease") as well as the chronic infection in birds with the persistence of WNV RNA within the organs (spleen, kidney, and lung) of several species of birds. To what extent the virus circulates in the bloodstream is difficult to say and may be influenced by stressful events as migration or mating. Also vertical transmission by *Culex* mosquitoes has been experimentally demonstrated in *Cx. tarsalis* (Reisen et al., 2006) as well as the overwintering of WNV demonstrated in in *Cx. pipiens* mosquitoes collected during the 2000 outbreak in New York city (Nasci et al., 2001).
(e)(iv) the impact of disease prevention and control measures, as regards the environment and biodiversity	(e)(iv) 2 Mortality in wild species	The main risk may be represented by the environmental residual of biocides which may interfere with ecology of wild species.

Article 9

Questions 1

Instruction to answer: The answer to the question 1CAq can be Y only for diseases affecting aquatic animal species, therefore do not assess this question for diseases affecting terrestrial animal species

Question 1A the disease is not present in the territory of the Union OR present only in exceptional cases (irregular introductions) OR present in only in a very limited part of the territory of the Union **Answer** Y ☐ N ☐ na ☐		
Question 1B the disease is present in the whole OR part of the Union territory with an endemic character AND (at the same time) several Member States or zones of the Union are free of the disease **Answer** Y ☐ N ☐ na ☐		
Question 1C the disease is present in the whole OR part of the Union territory with an endemic character **Answer** Y ☐ N ☐ na ☐		
Question 1CAq several Member States or zones of the Union are free of the disease **Answer** Y ☐ N ☐ na ☐		
Art. 7 criteria	**Art. 7 parameters**	**Assessment of the Art. 7 parameters from the fact-sheet**
(b)(i) the impact of the disease on agricultural and aquaculture production and other parts of the economy	(b)(i) 1 Number of MSs where the disease is present	Since the beginning of the 2016 transmission season, the presence of WNV has been confirmed in MSs and neighbouring countries. As of 27th October 2016, 205 human cases of West Nile fever have been reported in EU and 261 cases in neighbouring countries (Austria, Croatia, Cyprus, Egypt, Hungary, Italy, Israel, Portugal, Romania, Russian Federation, Serbia, Spain and Syrian Arab republic, Tunisia, Ukraine) (ECDC, 2016).
(a)(vii) the absence or presence and distribution of the disease in the Union, and, where the disease is not present in the Union, the risk of its introduction into the Union	(a)(vii) 1 Map of MSs where the disease is present (a)(vii) 2 Type of epidemiological occurrence	The geographic distribution of West Nile cases in Europe and in Mediterranean Basin from 2008 to 2016 shown in figure 3 (see "Figures" section). West Nile virus introduction and circulation have been demonstrated on multiple occasions in Southern Europe and Mediterranean basin since 1960s when seropositive animals or virus isolates were discovered in **France**, **Portugal**, and **Cyprus** (Filipe et al., 1969; Joubert et al., 1970), with WNV activity having dramatically increased over the last five years and spread to eastern territories without previous WNV records. If migratory birds have been associated to the introduction of viral strains from endemic areas (Calistri et al., 2010) the mechanism of virus persistence in animal hosts in Europe leading to endemization of the disease is still unknown. The circulation of WNV in Europe may occur silently for several months, or even years, before a spill over event occurs and viral circulation becoming evident. In Europe, WNV has mainly been reported in central and south-eastern Europe, regions in which WNV infections and virulence have recently increased, and the implicated viruses have spread to new areas, including **Bulgaria** and **Greece** in 2010, **Albania** and **Former Yugoslav Republic of Macedonia** in 2011, and **Croatia**, **Serbia**, and **Kosovo** in 2012. Accordingly, alarming outbreaks were reported in several European countries in 2010; 261 confirmed human cases, including 34 deaths, occurred in Greece, 57 cases and five deaths occurred in **Romania**, and 480 cases and six deaths occurred in **Russia** (Sirbu et al., 2011; Papa et al., 2010; Onishchenko et al., 2011). Sporadic occurrence of the disease has been reported in **France**

		since 1962, when it first appeared in Camargue. In the same region the WNV was detected in 2000, 2004 and, after a ten-year period, in 2015 (Bahuon et al., 2016). In **Italy** WNV epidemics have been registered since 2008 (Savini et al., 2008) caused by genetically divergent isolates and, to date, North Eastern regions as well as Sardinia and Sicily are considered endemic even though the endemic areas are modified every year according to the results of the surveillance activities (Italian Ministry of Health, 2016).
	(a)(vii) 3, 4, 5, 6, 7, 8, Risk of introduction (all related parameters)	Data not provided since the disease is already present in the Union.

Questions 2.1

Question 2.1A the disease is highly transmissible **Answer:** Y ☐ N ☐ na ☐		
Question 2.1BC the disease is moderately to highly transmissible **Answer** Y ☐ N ☐ na ☐		
Art. 7 criteria	**Art. 7 parameters**	**Assessment of the Art. 7 parameters from the fact-sheet**
(a)(vi) the routes and speed of transmission of the disease between animals and, when relevant, between animals and humans	(a)(vi) 3 Incidence between animals and, when relevant, between animals and humans	Not provided since literature search did not provide relevant results.
	(a)(vi) 4 Transmission rate (beta) (from R_0 and infectious period) between animals and, when relevant, between animals and humans	Transmission rate of West Nile Virus (WNV) infection between vector (mosquito) and avian population has been defined by using different mathematical models. Most of them included the disease basic reproduction number, R_0, which provides key insights into disease outbreak and control. It represents the average number of secondary infections deriving from the introduction of an infected individual into a susceptible population. Quantitatively, it has a threshold value of 1: when $R_0 > 1$ a disease outbreak can occur, and when $R_0 < 1$ it will not. Qualitatively, the expression for R_0 indicates which elements of the disease system can be manipulated to reduce the chance of an outbreak. The first WNV model was presented by Thomas and Urena (2001) to investigate the effectivity of pesticide spraying to reduce mosquito populations and in succession human WNV encephalitis in New York city after the outbreak in late summer 1999. Another WNV model was presented by Wonham et al. (2004), who suggested a theoretical framework including the derivation of R_0 and developing a single-season susceptible–infectious–removed (SIR) model of WN cross-infection between birds and mosquitoes, incorporating specific features unique to WN ecology. They demonstrated that mosquito control decreases, but bird control increases the chance of an outbreak. A similar WNV model was presented in a further theoretical study by Cruz-Pacheco et al. (2005). Their numerical results comprise the influence of mosquito vertical transmission on the WNV dynamics and estimated R0 values for 8 bird species. The work also finds the basic reproductive number R_0 in terms of measurable epidemiological and demographic parameters. Because the different WNV models result in different R_0 estimates, Wonham et al. (2006) compared the models cited above with respect to their disease transmission term. An age-structured WNV model was applied to the WNV dynamics in Southern Europe and Western Africa by Durand et al. (2010). A common feature of all existing WNV models is that they are formulated with constant parameters. Therefore, they are not able to describe the observed seasonal cycles of WNV cases and, consequently, have not been compared or verified with surveillance data. To overcome the above mention shortcomings two different models have been proposed: Laperriere and colleagues (2011) proposed an epidemic model for the simulation of the WNV dynamics of birds, horses and humans in the Minneapolis metropolitan area (Minnesota, US) to describe the observed seasonal cycles of WNV cases, incorporating epidemiological, entomological, climatic and environmental information. In the EU context Calistri et al. (2016) adapted the model developed by Rubel et al. (2008) to explain the Austrian epidemics of a close WNV related flavivirus, the Usutu virus by including the vertical (transovarial) transmission rate (VTR) in mosquitoes. Aiming to define the period at major risk for human infection, a simulation of the seasonal dynamic of WNV transmission was proposed and risk maps defined according to

		the mean values of R0 for the whole Italy (varied between 0.4 and 4.8, with values >1 from the end of May to the middle of September).

Question 2.2

Question 2.2AB there be possibilities of airborne or waterborne or vector-borne spread Interpretation: the disease or the infection can be transmitted via airborne or waterborne or vector-borne (mechanical or biological vector) spread **Answer** Y ☐ N ☐ na ☐		
Art. 7 criteria	**Art. 7 parameters**	**Assessment of the Art. 7 parameters from the fact-sheet**
(a)(vi) the routes and speed of transmission of the disease between animals and, when relevant, between animals and humans	(a)(vi) 1 types of routes of transmission from animal to animal (horizontal, vertical)	Results of experimental trials on WNV transmission routes in wild birds are summarized in table a.vi.1-2. in the Table section. Mosquito bites are the usual source of WNV for mammals, reptiles and amphibians however in some animals, there is also evidence for transmission by other routes. Carnivorous mammals and reptiles (e.g., cats and alligators) can be infected by eating contaminated tissues. Direct transmission during close contact has also been reported in alligators, possibly via fecal shedding of virus. Chipmunks, squirrels and raccoons can also shed WNV in feces, oral secretions and/or urine. WNV has been found in the urine of experimentally infected hamsters, and in very small amounts in the oral and/or cloacal fluids of experimentally infected North American bullfrogs (*Rana catesbeiana*) and green iguanas (*Iguana iguana*). Transplacental transmission was reported in experimentally infected sheep and mice, as well as in a horse that was fatally infected with a lineage 1 virus in Africa, and aborted in the final stage of the disease. The epidemiological significance (if any) of mammalian, reptilian and amphibian hosts in the maintenance or amplification of WNV remains to be established.

Question 2.3

Question: 2.3A the disease affects multiple species of kept and wild animals OR single species of kept animals of economic importance **Answer** Y ☐ N ☐ na ☐		
Art. 7 criteria	**Art. 7 parameters**	**Assessment of the Art. 7 parameters from the fact-sheet**
(a)(i) animal species concerned by the disease	(a)(i) 1 naturally susceptible wildlife species	**Birds** **Order Anseriformes** Family *Anatidae*: Wood Duck-Aix sponsa; Eurasian Wigeon-Anas penelope (c); Bronze-winged Duck (Spectacled Duck)-Anas specularis (c); Canvasback-Aythya valisineria; Canada Goose-Branta Canadensis; Barnacle Goose-Branta leucopsis (c)(a); Emperor Goose-Chen canagica (c); Greater Magellan Goose (Andean Goose)-Chloephagapicta leucoptera (c)(a); Abyssinian Blue-winged Goose-Cyanochen cyanopterus (c)(a); Tundra Swan-Cygnus columbianus (c); Trumpeter Swan-Cygnus Cygnus buccinator (c)(a); Mute Swan-Cygnus olor; Rosybilled Duck-Netta peposaca (c)(a); Ruddy Duck-Oxyura jamaicensis **Order Apodiformes** Family *Apodidae*: Chimney Swift-Chaetura pelagica; Family Trochilidae; Ruby-throated Hummingbird-Archilochus colubris **Order Caprimulgiformes** Family *Caprimulgidae*: Common Nighthawk-Chordeiles minor **Order Casuariiformes** Family *Dromaiidae*: Emu-Dromaius novaehollandiae (c) **Order Charadriiformes** Family *Charadriidae*: Ruddy Turnstone-Arenaria interpres; Killdeer-Charadrius vociferous; Piping Plover-Charadrius melodus Family *Laridae*: Herring Gull-Larus argentatus; Laughing Gull-Larus atricilla; Ring-billed Gull-Larus delawarensis; Great Black-backed Gull-Larus marinus; Black Skimmer-Rhynchops niger; Grey Gull-Larus modestus (c)(a); Inca Tern-Larosterna inca (c)(a) **Order Ciconiformes** Family *Ardeidae*: Yellow-crowned Night-heron-Nyctanassa violacea (c); Black-crowned Night-heron-Nycticorax nycticorax (c); Great Blue Heron-Ardea Herodias; Green Heron-Butorides virescens; Least Bittern-Ixobrychus exilis Family *Cathartidae*: Turkey Vulture-Cathartes aura; Black Vulture-Coragyps atratus; King Vulture-Sarcoramphus papa (c)(a) Family *Ciconiidae*: Saddle-billed Stork-Ephippiorhynchos senegalensis (c)(a);Marabou Stork-Leptopilos crumeniferus (c)(a); Lesser Adjutant Stork-Leptoptilos javanicus (c)(a) Family *Phoenicopteridae*: Chilean Flamingo-Phoenicopterus chilensis (c); Greater Flamingo (American)-Phoenicopterus ruber ruber (c)

		Family *Threskiornithidae*: Scarlet Ibis-Eudocimus ruber (c); Waldrapp-Geronticus eremita (c)(a) **Order Columbiformes** Family *Columbidae*: White-crowned Pigeon-Columba leucocephala; Rock Dove (Feral Pigeon)-Columba livia; Mauritius Pink Pigeon-Columba mayeri (c)(a); Common Ground-Dove-Columbina passerina; Eurasian Collared-Dove-Streptopelia decaocto; White-winged Dove-Zenaida asiatica; Mourning Dove-Zenaida macroura; Luzon Pigeon (Bleeding Heart Pigeon)-Gallicolumba luzonica (c)(a); Inca Dove-Columbina inca **Order Coraciiformes** Family *Alcedinidae*: Belted Kingfisher-Ceryle alcyon **Order Cuculiformes** Family *Cuculidae*: Yellow-billed Cuckoo-Coccyzus americanus **Order Falconiformes** Family *Accipitridae*: Cooper's Hawk-Accipiter cooperii; Northern Goshawk-Accipiter gentilis; Sharp-shinned Hawk-Accipiter striatus; Golden Eagle-Aquila chrysaetos; Red-tailed Hawk-Buteo jamaicensis; Rough-legged Hawk-Buteo lagopus (c); Red-shouldered Hawk-Buteo lineatus; Broad-winged Hawk-Buteo platypterus; Swainson's Hawk-Buteo swainsoni; Northern Harrier-Circus cyaneus; Swallow-tailed Kite-Elanoides forficatus; Bald Eagle-Haliaeetus leucocephalus; Mississippi Kite-Ictinia mississippiensis; Osprey-Pandion haliaetus; Harris' Hawk-Parabuteo unicinctus (c) Family *Falconidae*: Merlin-Falco columbarius; Prairie Falcon-Falco mexicanus; Peregrine Falcon-Falco peregrinus; American Kestrel-Falco sparverius **Order Galliformes** Family *Numididae*: Crested Guineafowl-Guttera pucherani (c)(a) Family *Odontophoridae*: Northern Bobwhite-Colinus virginianus Family *Phasianidae*: Chukar-Alectoris chukar (c)(a); Ruffed Grouse-Bonasa umbellus; Green Junglefowl-Gallus varius (c)(a); Impeyan (Himalayan) Pheasant (Monal)-Lophophorus impeyanus (c); Bulwer's Wattled Pheasant-Lophura bulweri (c)(a); Ring-necked Pheasant-Phasianus colchicus; Mount Peacock-Pheasant-Polypectron inopinatum (c)(a); Crested Partridge-Rollulus roulroul (c)(a); Blyth's Tragopan-Tragopan blythii (c); Argus Pheasant (unspecified)-various (c)(a); Greater Sage Grouse-Centrocerus urophasianus **Order Gaviformes** Family *Caprimulgidae*: Common Loon-Gavia immer **Order Gruiformes** Family *Gruidae*: Demoiselle Crane-Anthropoides virgo (c)(a); West African Crowned Crane-Balearica pavonina pavonina (a); Wattled Crane-Bugeranus carunculatus (c)(a); Whooping Crane-Grus americana (c)(a); Mississippi Sandhill Crane-Grus canadensis pulla (c); Red-crowned Crane-Grus japonensis (c)(a); Siberian Crane-Grus leucogeranus (c)(a); Hooded Crane-Grus monacha (c)(a); White-naped Crane-Grus vipio (c)(a); Black-necked Crane-Grus nigricollis (c)(a) Family *Rallidae*: Virginia Rail-Rallus limicola **Order Musophagiformes** Family *Musophagidae*: Lady Ross' Turaco (Plantain-Eater)-Musophaga rossae (c)(a) **Order Passeriformes** Family *Bombycillidae*: Cedar Waxwing-Bombycilla cedrorum Family *Cardinalidae*: Northern Cardinal-Cardinalis cardinalis; Blue Grosbeak-Guiraca caerulea(a); Rose-breasted Grosbeak-Pheucticus ludovicianus; Dickcissel-Spiza americana Family *Corvidae*: Western Scrub-Jay-Aphelocoma californica; American Crow-Corvus brachyrhynchos; Common Raven-Corvus corax; Fish Crow-Corvus ossifragus; Blue Jay-Cyanocitta cristata; Steller's Jay-Cyanocitta stelleri; Black-billed Magpie-Pica hudsonia (c) Family *Emberizidae*: Song Sparrow-Melospiza melodia; Savannah Sparrow-Passerculus sandwichensis; Fox Sparrow-Passerella iliaca; Eastern Towhee-Pipilo erythrophthalmus; Field Sparrow-Spizella pusilla Family Estrildidae: Zebra Finch-Taeniophygia guttata (c) Family *Fringillidae*: American Goldfinch-Carduelis tristis; House Finch-Carpodacus mexicanus; Purple Finch-Carpodacus purpureus; Evening Grosbeak-Coccothraustes vespertinus; European Goldfinch-Carduelis carduelis (c) Family *Hirundinidae*: Barn Swallow-Hirundo rustica; Purple Martin-Progne subis; Tree Swallow-Tachycineta bicolor Family *Icteridae*: Red-Winged Blackbird-Agelaius phoeniceus; Rusty Blackbird-Euphagus carolinus; Brewer's Blackbird-Euphagus cyanocephalus; Baltimore Oriole-Icterus galbula; Brown-headed Cowbird-Molothrus ater; Boat-tailed Grackle-Quiscalus major; Great-tailed Grackle-Quiscalus mexicanus; Common Grackle-Quiscalus quiscula Family *Laniidae*: Loggerhead Shrike-Lanius ludovicianus Family *Mimidae*: Gray Catbird-Dumetella carolinensis; Northern Mockingbird-Mimus polyglottos; Brown Thrasher-Toxostoma rufum Family *Paridae:* Tufted Titmouse-Baeolophus bicolor; Varied Tit-Parus varius (c);

		Black-capped Chickadee-Poecile atricapilla; Carolina Chickadee-Poecile carolinensis Family *Parulidae*: Black-throated Blue Warbler-Dendroica caerulescens; Yellow-rumped Warbler-Dendroica coronate; Yellow Warbler-Dendroica petechial; Blackpoll Warbler-Dendroica striata; Common Yellowthroat-Geothlypis trichas; Kentucky Warbler-Oporornis formosus; Northern Parula-Parula Americana; Ovenbird-Seiurus aurocapillus; Northern Waterthrush-Seiurus noveboracensis; Nashville Warbler-Vermivora ruficapilla; Canada Warbler-Wilsonia Canadensis; Hooded Warbler-Wilsonia citrina Family *Passeridae*: House Sparrow-Passer domesticus Family *Sylviidae*: White-crested Laughingthrush-Garrulax leucolophus (c)(a) Family *Sittadae*: White-breasted Nuthatch-Sitta carolinensis Family *Sturnidae*: European Starling-Sturnus vulgaris Family *Thraupidae*: Palm Tanager-Thraupis palmarum (c) Family *Troglodytidae*: Carolina Wren-Thryothaurus ludovicianus; Winter Wren-Troglodytes troglodytes Family *Turdidae*: Veery-Catharus fuscescens; Hermit Thrush-Catharus guttatus; Gray-cheeked Thrush-Catharus minimus; Swainson's Thrush-Catharus ustulatus; Wood Thrush-Hylocichla mustelina; Eastern Bluebird-Sialia sialis; American Robin-Turdus migratorius Family *Tyrannidae*: Traill's Flycatcher-Empidonax traillii/alnorum; Eastern Phoebe-Sayornis phoebe; Scissor-tailed Flycatcher-Tyrannus forficatus; Eastern Kingbird-Tyrannus tyrannus Family *Vireonidae*: Black-whiskered Vireo-Vireo altiloquus; Warbling Vireo-Vireo gilvus; Red-eyed Vireo-Vireo olivaceus **Order Pelecaniformes** Family *Pelecanidae*: American White Pelican-Pelecanus erythrorhynchos; Brown Pelican-Pelicanus occidentalis (c)(a); Family Phalacrocoracidae; Double-crested Cormorant-Phalacrocorax auritus; Guanay Cormorant-Phalacrocorax bougainvillei (c) **Order Piciformes** Family *Picidae*: Red-headed Woodpecker-Melanerpes erythrocephalus; Downy Woodpecker-Picoides pubescens; Yellow-bellied Sapsucker-Sphyrapicus varius **Order Podicipediformes** Family *Podicipedidae*: Pied-billed Grebe-Podilymbus podiceps **Order Psittaciformes** Family *Cacatuidae*: Cockatoo (unspecified)-Cacatua spp. (c) ; Cockatiel-Nymphicus hollandicus (c) Family *Psittacidae*: Red-crowned Parrot-Amazona viridigenalis (c); Macaw (unspecified)-Ara spp. (c); Budgerigar-Melopsittacus undulatus (c); Lorikeet spp.-Tricheglossus spp. (c) **Order Sphenisciformes** Family *Spheniscidae*: Black-footed (Jackass) Penguin-Spheniscus demersus (c); Magellan Penguin-Spheniscus humboldti (c)(a) **Order Strigiformes** Family *Strigidae*: Northern Saw-whet Owl-Aegolius acadicus; Boreal Owl-Aegolius funereous (c); Short-eared Owl-Asio flammeus; Verreaux's Eagle Owl (Milky Eagle Owl)-Bubo lacteus(c)(a); Great Horned Owl-Bubo virginianus; Snowy Owl-Nyctea scandiaca (c); Eastern Screech Owl-Otus asio; Tawny Owl-Strix aluco(c); Great Grey Owl-Strix nebulosa (c); Spotted Owl-Strix occidentalis (c); Barred Owl-Strix varia; Northern Hawk Owl-Surnia ulula (c) Family *Tytonidae*: Barn Owl-Tyto alba **Order Struthioniformes** Family *Struthionidae*: Ostrich-Struthio camelis (c)(a) **Mammals** **Order Artiodactyla** Family *Bovidae*: Mountain Goat-Oreamnos americanus (c) Family *Camelidae*: Llama-Lama glama (c); Alpaca (Suri)-Lama pacos (c) Family *Cervidae*: White-tailed Deer-Odocoileus virgninianus; Reindeer-Rangifer tarnadus (c); Mule Deer-Odocoileus hemionus Family *Suidae*: Babirusa-Babyrousa babyrousa (c)(a) **Order Carnivora** Family *Canidae*: Timber Wolf-Canis lupus (c) Family *Mustelidae*: Striped Skunk-Mephitis mephitis Family *Phocidae*: Harbor Seal-Phoca vitulina (c) Family *Procyonidae*: Red Panda-Ailurus fulgens fulgens (c)(a) Family *Ursidae*: Black Bear-Ursus americanus(a) **Order Chiroptera** Family *Vespertilionidae*: Big Brown Bat-Eptesicus fuscus; Little Brown Bat-Myotis lucifugus **Order Perissodactyla** Family *Rhinocerotidae*: Great Indian Rhinoceros-Rhinoceros unicornis (c)(a) **Order Primata** Family *Cercopithcidae*: Barbary Macaque-Macaca sylvanus (c)

<table>
<tr><td></td><td></td><td>Family Lemuridae: Ring-tailed Lemura-Lemur catta (c)
Order Proboscidea
Family Elephantidae: Indian (Asian) Elephant-Elephas maximus indicus (c)(a)
Order Rodentia
Family Sciuridae: Gray Squirrel-Sciurus carolinensis; Fox Squirrel-Sciurus niger; Eastern Chipmunk-Tamias striatus
Reptiles
Order Crocodylia
Family Alligatoridae: American Alligator-Alligator mississippiensis (c)
Order Squamata
Family Varanidae: Crocodile Monitor-Varanus salvadorii (c)(a)
(c) denotes either a captive or farmed animal(s). Virus or viral RNA was detected in animal tissue unless followed by an (a), which denotes detectable antibodies only have been reported (Source: USGS, National Wildlife Health Center).</td></tr>
<tr><td></td><td>(a)(i) 2 naturally susceptible domestic species</td><td>Family Phasianidae:
Domestic Chicken (Red Junglefowl)-Gallus gallus; Turkey (domestic and wild)-Meleagris gallopavo
Family Anatidae: Mallard-Anas platyrhynchos; Domestic Goose-Anser chinensis (c)(a)
Family Bovidae: Domestic Cattle-Bos Taurus; Domestic (Suffolk) Sheep-Ovis aries
Family Canidae: Domestic Dog-Canis familiaris
Family Felidae: Domestic Cat (feral)-Felis catus
Family Leporidae: Domestic Rabbit-Oryctolagus cuniculus
Family Equidae: Domestic Horse-Equus equus przewalski caballus; Donkey-Equus asinus; Mule</td></tr>
<tr><td></td><td>(a)(i) 3 experimentally susceptible wildlife species</td><td rowspan="2">West Nile virus causes disease in humans, horses, and several species of birds. Most infected individuals show few signs of illness, but some develop severe neurological illness which can be fatal. West Nile Virus has an extremely broad host range. It replicates in birds, reptiles, amphibians, mammals, mosquitoes and ticks. Besides equids, details of susceptible domestic mammalian species are summarized in table a.i. (1) while table a.i. (2) summarizes outcomes of experimental infections of West Nile virus assessed in wild birds.</td></tr>
<tr><td></td><td>(a)(i) 4 experimentally susceptible domestic species</td></tr>
<tr><td></td><td>(a)(i) 5 wild reservoir species</td><td>Birds, particularly passerine species (jays, finches, sparrows, and crows).</td></tr>
<tr><td></td><td>(a)(i) 6 domestic reservoir species</td><td>West Nile virus causes disease in humans, horses, and several species of birds. Most infected individuals show few signs of illness, but some develop severe neurological illness which can be fatal. West Nile Virus has an extremely broad host range. It replicates in birds, reptiles, amphibians, mammals, mosquitoes and ticks. Besides equids, details of susceptible mammalian species are summarized in table a.i.(1) while table a.i. (2) summarizes outcomes of experimental infections of West Nile virus assessed in wild birds.
Outside US, clinical symptoms to WNV infection has been reported in a scarce number of avian species in course of outbreaks: domestic geese (Anser anser domesticus) and white storks (Ciconia ciconia) during the WNV epidemic in Israel (Malkinson et al., 2002), goshawks (Accipiter gentilis) in Hungary (Bakonyi et al., 2006), eurasian jays (Garrulus glandarius), little owl (Athene noctua), mallard (Anas plathyrynchos), common buzzard (Buteo buteo) in Italy (Monaco et al., 2015). However, mass mortality of highly susceptible species (such as corvids or other species) is less frequently observed in the Old than in the New World although some species, as the jackdaws (Corvus monedula) could potentially function as sentinel (Lim et al., 2014). Surveillance activities carried out in Italy where WNV is endemic since 2008, pointed out the high susceptibility to the viral infection of three species of synantropic wild birds, namely carrion crow (Corvus corone), magpie (Pica pica) and eurasian jay (Garrulus glandarius) which justifies their use as sentinel in endemic areas (Italian Ministry of Health, 2016).
WNV has been associated with sporadic disease in small numbers of other species, including squirrels, chipmunks, bats, dogs, cats, white-tailed deer, reindeer, sheep, alpacas, dromedary camels, alligators and harbour seals during intense periods of local viral activity. Some species of mammals including squirrels (Sciurus sp.), eastern chipmunks (Tamias striatus) and eastern cottontail rabbits (Sylvilagus floridanus) may be capable of transmitting WNV to mosquitoes, although their importance as reservoir hosts is still uncertain.
Among reptiles, clinical signs were mainly reported during outbreaks in alligators, although there is also a report of neurological signs associated with WNV infection in a crocodile monitor (Varanus salvadori) lizard. Some infections in garter snakes (Thamnophis sirtalis) experimentally inoculated with WNV were also fatal. Green iguanas (Iguana iguana) can be infected.
Amphibians including lake frogs (Rana ridibunda) and North American bullfrogs (Rana catesbeiana) can also be infected with WNV. Some alligators (e.g., American alligators, Alligator mississippiensis) and frogs (e.g., Rana ridibunda in Russia) may develop viremia sufficient to infect mosquitoes. As with mammals, their importance</td></tr>
</table>

		as reservoir hosts is still uncertain. Based on preliminary research carried out in Italy and Spain, only few bird species seem to play a major role as blood donor for the mosquitoes (Munoz et al., 2012; Hamer et al., 2009; Roiz et al., 2012, Spedicato et al., 2015). Unfortunately, the reservoir competence for many European bird species is still unknown even though the persistence of WNV in infected birds have been assessed in some species through experimental trials. House finches and House sparrows experimentally inoculated showed persistent infection in spleen and kidney 28 weeks p.i. The virus was still detected by real time RT-PCR in the spleen of two House sparrows at 36 weeks p.i. However, viral isolation attempts were unsuccessful (Wheeler et al., 2012). In a previous work (Nemeth et al., 2009), a higher number of organs were analyzed in WNV-infected House sparrows, and viral RNA was detected in juvenile sparrows up to 65 days p.i in kidney and spleen, although infectious virus could be isolated at low titres only in one sparrow at 43 days p.i. Reisen and colleagues confirmed the persistent infection in five species of Passeriformes and in Common ground-dove (*Columbina passerina*) detecting the virus in spleen and kidney, but also in lung at >6 weeks p.i.

Questions 2.4

Instruction to answer: The answer to the question 2.4CAq can be Y only for diseases affecting aquatic animal species, therefore do not assess this question for diseases affecting terrestrial animal species

Question 2.4A the disease may result in high morbidity and significant mortality rates **Answer** Y ☐ N ☐ na ☐		
Question 2.4B the disease may result in high morbidity and in general low mortality **Answer** Y ☐ N ☐ na ☐		
Question 2.4C the disease usually does not result in high morbidity and has negligible or no mortality AND often the most observed effect of the disease is production loss **Answer** Y ☐ N ☐ na ☐		
Question 2.4CAq the disease may result in high morbidity and usually low mortality AND often the most observed effect of the disease is production loss **Answer** Y ☐ N ☐ na ☐		
Art. 7 criteria	**Art. 7 parameters**	**Assessment of the Art. 7 parameters from the fact-sheet**
(a)(ii) morbidity and mortality rates of the disease in animal populations	(a)(ii) 1 Prevalence/ Incidence (a)(ii) 2 Case-morbidity rate (a)(ii) 3 Case-fatality rate	Refer to table a.ii in the Table section
(b)(i) impact of the disease on agricultural and aquaculture production and other parts of the economy	(b)(i) 1 Number of MSs where the disease is present	Since the beginning of the 2016 transmission season, the presence of WNV has been confirmed in MSs and neighbouring countries. As of 27th October 2016, 205 human cases of West Nile fever have been reported in EU and 261 cases in neighbouring countries (Austria, Croatia, Cyprus, Egypt, Hungary, Italy, Israel, Portugal, Romania, Russian Federation, Serbia, Spain and Syrian Arab republic, Tunisia, Ukraine) (ECDC, 2016).
	(b)(i) 2 Proportion of production losses (%) by epidemic/endemic situation (milk, growth, semen, meat, etc.)	In European outbreaks WNV has not been associated to any mortality in domestic birds but has been limited to a few cases in wild birds (paragraph a.i). Outside EU, among poultry, young geese seem to be particularly susceptible to WNV, and have been affected in both Western and Eastern Hemispheres. In Israel, disease was reported in 3-8-week-old goslings, with morbidity and mortality rates of approximately 40%. During an outbreak in Canada, the mortality rate was 25% in 6-week-old goslings, but 15-month-old and 5-year-old geese seroconverted with no clinical signs. In experimental infections, up to 50–75% of geese may die. Ducks are not thought to be highly susceptible to WNV; however, an outbreak among captive lesser scaup (*Aythya affinis*) ducklings resulted in 70% mortality. During other outbreaks, the morbidity and mortality rates were 100% in Impeyan pheasants, and the mortality rate was 25% in chukar partridges. Similarly to geese, young partridges and pheasants seem to be more susceptible to disease. In contrast, both young and old chickens and turkeys are infected asymptomatically.

Questions 3

Question 3C the disease has a zoonotic potential with significant consequences for public health or possible significant threats to food safety **Answer** Y ☐ N ☐ na ☐
Question 3B the disease has a zoonotic potential with significant consequences on public health, including epidemic potential OR possible significant threats to food safety

Answer Y ☐ N ☐ na ☐		
Question 3A the disease has a zoonotic potential with significant consequences on public health, including epidemic or pandemic potential OR possible significant threats to food safety **Answer** Y ☐ N ☐ na ☐		
Art. 7 criteria	**Art. 7 parameters**	**Assessment of the Art. 7 parameters from the fact-sheet**
(a)(iii) zoonotic character of the disease	(a)(iii) 1 report of zoonotic human cases	West Nile zoonotic transmission in known to be present in Europe since a long time: in the 1960s the virus emerged in southern France in the Camargue. Yet, the first large outbreak in humans was reported from Bucharest, Romania in 1996-1997. Since then, infection in humans and/or horses have been reported from the Czech Republic (1997), France (2000, 2003, 2004, 2006), Italy (1998, 2008, 2009), Hungary (2000-2009), Romania (1997-2001, 2003-2009), Spain (2004) and Portugal (2004). In 2010, the ecological parameters in Central European and Mediterranean countries were favorable for the transmission of WNV to humans. A human outbreak was reported from the Central Macedonia Region in northern Greece and human cases were reported from Romania, Hungary, Italy and Spain in August-September 2010. At the same time a large outbreak in humans was reported from Volgograd in Russia. Evidence of human cases have been in EU Countries have been listed in table b.ii.2 and a recent paper from the Italian Integrated WNV Surveillance Group (Rizzo et al., 2016) provide an example of geographical correlation between human and veterinary cases. For Figure **1** (Geographical distribution of West Nile neuroinvasive disease in horses (panel A) and humans (panel B), Italy 2008–2015 (Rizzo et al., 2016)) and Figure **2** (West Nile virus detections in the veterinary and human surveillance by month, Italy, 2008–2015 (Rizzo et al., 2016)) see Figures section.
(a)(vi) the routes and speed of transmission of the disease between animals and, when relevant, between animals and humans	(a)(vi) 2 types of routes of transmission between animals and humans (direct and indirect including foodborne)	There is no evidence of natural direct transmission between vertebrates and humans. However, human infection from the exposure of conjunctival membranes (Fonseca et al., 2005) and/or percutaneous injury to the body fluids or tissues of WNV infected birds (CDC, 2002) has been described.
	(a)(vi) 3 Incidence between animals and, when relevant , between animals and humans	Not provided since literature search did not provide relevant results.
	(a)(vi) 4 Transmission rate (beta) (from R_0 and infectious period) between animals and, when relevant ,between animals and humans	Transmission rate of West Nile Virus (WNV) infection between vector (mosquito) and avian population has been defined by using different mathematical models. Most of them included the disease basic reproduction number, R_0, which provides key insights into disease outbreak and control. It represents the average number of secondary infections deriving from the introduction of an infected individual into a susceptible population. Quantitatively, it has a threshold value of 1: when $R_0 > 1$ a disease outbreak can occur, and when $R_0 < 1$ it will not. Qualitatively, the expression for R_0 indicates which elements of the disease system can be manipulated to reduce the chance of an outbreak. The first WNV model was presented by Thomas and Urena (2001) to investigate the effectivity of pesticide spraying to reduce mosquito populations and in succession human WNV encephalitis in New York city after the outbreak in late summer 1999. Another WNV model was presented by Wonham et al. (2004), who suggested a theoretical framework including the derivation of R_0 and developing a single-season susceptible–infectious–removed (SIR) model of WN cross-infection between birds and mosquitoes, incorporating specific features unique to WN ecology. They demonstrated that mosquito control decreases, but bird control increases the chance of an outbreak. A similar WNV model was presented in a further theoretical study by Cruz-Pacheco et al. (2005). Their numerical results comprise the influence of mosquito vertical transmission on the WNV dynamics and estimated R0 values for 8 bird species. The work also finds the basic reproductive number R_0 in terms of measurable epidemiological and demographic parameters. Because the different WNV models result in different R_0 estimates, Wonham et al. (2006) compared the models cited above with respect to their disease transmission term. An age-structured WNV model was applied to the WNV dynamics in Southern Europe and Western Africa by Durand et al. (2010). A common feature of all existing

		WNV models is that they are formulated with constant parameters. Therefore, they are not able to describe the observed seasonal cycles of WNV cases and, consequently, have not been compared or verified with surveillance data. To overcome the above mention shortcomings two different models have been proposed: Laperriere and colleagues (2011) proposed an epidemic model for the simulation of the WNV dynamics of birds, horses and humans in the Minneapolis metropolitan area (Minnesota, US) to describe the observed seasonal cycles of WNV cases, incorporating epidemiological, entomological, climatic and environmental information. In the EU context Calistri et al. (2014) adapted the model developed by Rubel et al. (2008) to explain the Austrian epidemics of a close WNV related flavivirus, the Usutu virus by including the vertical (transovarial) transmission rate (VTR) in mosquitoes. Aiming to define the period at major risk for human infection, a simulation of the seasonal dynamic of WNV transmission was proposed and risk maps defined according to the mean values of R0 for the whole Italy (varied between 0.4 and 4.8, with values >1 from the end of May to the middle of September).
(b)(ii) Impact of the disease on human health	(b)(ii) 5 Disability-adjusted life year (DALY)	As for most arthropod-borne diseases causing fever syndromes worldwide, the cumulative impact of WNV on global disease burden has not been fully assessed. Evaluations should include both the severe forms of the disease and the milder clinical manifestations which may result in neurological and ophthalmologic complications (Carson et al., 2006). WNV has been recognized able to induce a wide range of post-infection, long-term sequelae with the recovery of the affected patients within two years from the infection (Murray et al., 2008). However, a recent paper has emphasized that 40% of WNV affected patients continued to experience symptoms related to their WNV infection up to 8 years later demonstrating the health and economic impact of a result of prolonged recovery, continued morbidity, and related disability (Murray et al., 2014).
	(b)(ii) 6 Availability of medical treatment and their effectiveness (therapeutical effect and any resistance)	There is no specific recommended treatment, other than supportive care, at present. Intensive care and mechanical ventilation may be required in some cases. Various therapies including interferon, antisense nucleotides and intravenous immunoglobulins (passive immunization) are being tested in clinical trials. While a few case reports suggest that some of these treatments may be promising, larger studies are still lacking. Screening for new drugs that may inhibit WNV is underway.
	(b)(ii) 7 Availability of vaccines and their effectiveness (reduced morbidity)	There are no vaccines available for human use in EU.
(c) potential to generate a crisis situation and its potential use in bioterrorism	(c) 1 listed in OIE/CFSPH classification of pathogens	Yes, listed among the diseases from potential bioterrorist agents
	(c) 2 listed in the Encyclopaedia of Bioterrorism Defense of Australia Group	No
	(c) 3 included in any other list of potential bio- agro-terrorism agents	Not reported

Questions 4

Question 4AB the disease in question has a significant impact on the economy of the Union, causing substantial costs, mainly related to its direct impact on the health and productivity of animals Interpretation: due to the substantial costs related to the disease's direct impact on the health and productivity of animals, the disease has a significant impact on the economy **Answer** Y ☐ N ☐ na ☐		
Question 4C the disease has a significant impact on the economy of the Union, mainly related to its direct impact on certain types of animal production systems Interpretation: due to its direct impact on certain types of animal production systems, the disease has a significant impact on the economy **Answer** Y ☐ N ☐ na ☐		
Art. 7 criteria	**Art. 7 parameters**	**Assessment of the Art. 7 parameters from the fact-sheet**
(a)(ii) morbidity and mortality rates of the disease in animal	(a)(ii) 1 Prevalence/ Incidence	Refer to table a.ii. in the Table section
	(a)(ii) 2 Case-morbidity rate	

populations	(% clinically diseased animals out of infected ones)	
	(a)(ii) 3 Case-fatality rate	
(b)(i) impact on agricultural and aquaculture production and other parts of the economy	(b)(i) 1 Number of MSs where the disease is present	Since the beginning of the 2016 transmission season, the presence of WNV has been confirmed in MSs and neighbouring countries. As of 27th October 2016, 205 human cases of West Nile fever have been reported in EU and 261 cases in neighbouring countries (Austria, Croatia, Cyprus, Egypt, Hungary, Italy, Israel, Portugal, Romania, Russian Federation, Serbia, Spain and Syrian Arab republic, Tunisia, Ukraine) (ECDC, 2016).
	(b)(i) 2 Proportion of production losses (%) by epidemic/endemic situation (milk, growth, semen, meat, etc.)	In European outbreaks WNV has not been associated to any mortality in domestic birds but has been limited to a few cases in wild birds (paragraph a.i). Outside EU, among poultry, young geese seem to be particularly susceptible to WNV, and have been affected in both Western and Eastern Hemispheres. In Israel, disease was reported in 3-8-week-old goslings, with morbidity and mortality rates of approximately 40%. During an outbreak in Canada, the mortality rate was 25% in 6-week-old goslings, but 15-month-old and 5-year-old geese seroconverted with no clinical signs. In experimental infections, up to 50–75% of geese may die. Ducks are not thought to be highly susceptible to WNV; however, an outbreak among captive lesser scaup (*Aythya affinis*) ducklings resulted in 70% mortality. During other outbreaks, the morbidity and mortality rates were 100% in Impeyan pheasants, and the mortality rate was 25% in chukar partridges. Similarly to geese, young partridges and pheasants seem to be more susceptible to disease. In contrast, both young and old chickens and turkeys are infected asymptomatically.

Question 5a

Question 5a the disease has a significant impact on society, with in particular an impact on labour markets

Interpretation: the disease has a significant impact on society with (as the most important but not the only one) an impact on labour markets

Answer Y ☐ N ☐ na ☐

Art. 7 criteria	Art. 7 parameters	Assessment of the Art. 7 parameters from the fact-sheet
(b)(i) impact on agricultural and aquaculture production and other parts of the economy	(b)(i) 1 Number of MSs where the disease is present	Since the beginning of the 2016 transmission season, the presence of WNV has been confirmed in MSs and neighbouring countries. As of 27th October 2016, 205 human cases of West Nile fever have been reported in EU and 261 cases in neighbouring countries (Austria, Croatia, Cyprus, Egypt, Hungary, Italy, Israel, Portugal, Romania, Russian Federation, Serbia, Spain and Syrian Arab republic, Tunisia, Ukraine) (ECDC, 2016).
	(b)(i) 2 Proportion of production losses (%) by epidemic/endemic situation (milk, growth, semen, meat, etc.)	In European outbreaks WNV has not been associated to any mortality in domestic birds but has been limited to a few cases in wild birds (paragraph a.i). Outside EU, among poultry, young geese seem to be particularly susceptible to WNV, and have been affected in both Western and Eastern Hemispheres. In Israel, disease was reported in 3-8-week-old goslings, with morbidity and mortality rates of approximately 40%. During an outbreak in Canada, the mortality rate was 25% in 6-week-old goslings, but 15-month-old and 5-year-old geese seroconverted with no clinical signs. In experimental infections, up to 50–75% of geese may die. Ducks are not thought to be highly susceptible to WNV; however, an outbreak among captive lesser scaup (*Aythya affinis*) ducklings resulted in 70% mortality. During other outbreaks, the morbidity and mortality rates were 100% in Impeyan pheasants, and the mortality rate was 25% in chukar partridges. Similarly to geese, young partridges and pheasants seem to be more susceptible to disease. In contrast, both young and old chickens and turkeys are infected asymptomatically.

Question 5b

Question 5b the disease has a significant impact on animal welfare, by causing suffering to large numbers of animals

Interpretation: due to the suffering of large numbers of animals caused by the disease, the disease has a significant impact on animal welfare

Answer Y ☐ N ☐ na ☐

Art. 7 criteria	Art. 7 parameters	Assessment of the Art. 7 parameters from the fact-sheet
(b)(iii) impact of the	(b)(iii) 1 severity of	The incubation period for equine WN encephalitis following mosquito

disease on animal welfare	clinical signs at case level and related level and duration of impairment	transmission is estimated to be 3–15 days. A fleeting viraemia of low virus titre precedes clinical onset (Bunning et al., 2002). WN viral encephalitis occurs in only a small per cent of infected horses; the majority of infected horses do not display clinical signs (Ostlund et al., 2000). The disease in horses is frequently characterised by mild to severe ataxia. Additionally, horses may exhibit weakness, muscle fasciculation and cranial nerve deficits (Cantile et al., 2000; Ostlund et al., 2000; 2001; Snook et al., 2001). Fever is an inconsistently recognised feature. Treatment is supportive and signs may resolve or progress to terminal recumbency. The mortality rate is approximately one in three clinically affected unvaccinated horses. Many species of birds can become infected with WNV; the clinical outcome of infection is variable. Some species appear resistant while others suffer fatal neurologic disease. Neurologic disease and death have been documented in domestic geese in Israel and Canada, and in many native and exotic zoo birds in the USA during the emergence of WNV (Steele et al., 2000). WND associated cases have been described in European wild birds (Bakonyi et al. 2006, Höfle et al. 2008, Jiménez-Clavero et al. 2008). In 2011 during the Sardinian WND outbreak neurological disease has been reported in 2 wild birds as Eurasian jays (*Garrulus glandarius*) with clinical signs characterised by drowsy, incapability of flying or walking properly, ruffle feathers, pectoral atrophy, and absence of the flight instinct. Lethargy, head tremors, drooping wings and inability to fly due to the flaccid paralysis of the wing muscles were described in an adult common buzzard. The legs were kept flexed and the bird was not able to stand up. The podal reflex was lost whereas both, the pupillary and corneal reflexes were still present. The animals died within 24 hours from the admission to a veterinary clinic. A little owl (*Athene noctua*), was bought to a rehabilitation centre showing ataxia, incoordination, reluctance or inability to fly properly, head tilt and anisocoria. It was able to stand up by using the tail feather and the wings. In the second day, clinical signs became more severe. The corneal reflex was lost and the animal was not anymore capable of standing up although it still tried to fly when encouraged. It died at the end of the second day. Clinical signs have been described also in an adult male mallard (*Anas plathyrynchos*) with complete flaccid paralysis of the legs and even if still present, the instinct to escape was precluded by the leg paralysis. Neck and wing movements were still under control and the sensorium was still awake. In the following day, the bird progressively lost the wing muscle contractile capability and the instinct of escape. The third day after the onset of clinical symptoms the animal died. An ataxic adult common buzzard (*Buteo buteo*) showed irregular head tremors and had trouble in maintaining the upright position even if using the tail feather and the wings. The instinct of escape was lost and the podal reflex as well as the proprioception response on the left leg was slow. The droppings were of a fluid-like consistency and the feathers around the vent were matted with faeces. When recumbent in a sternal position, the bird was not able to stand up properly and, similarly, it was not able to open its wings even if it was able to flex them back at the elbow joint when forcedly opened. In the second day, the lethargy became more severe and the animal died (Monaco et al., 2015).
(a)(ii) morbidity and mortality rates of the disease in animal populations	(a)(ii) 2 Case-morbidity rate (% clinically diseased animals out of infected ones)	Refer to table a.ii. in the Table section

Question 5c

Question 5c the disease has a significant impact on the environment, due to the direct impact of the disease OR due to the measures taken to control it

Interpretation: due to the direct impact of the disease OR to the impact of the measures taken to control it, the disease has a significant impact on the environment

Answer: Y ☐ N ☐ na ☐

Art. 7 criteria	**Art. 7 parameters**	**Assessment of the Art. 7 parameters from the fact-sheet**
(b)(iv) impact of the disease on biodiversity and the environment	(b)(iv) 1 endangered wild species affected: listed species as in CITES and/or IUCN list	Endangered wild species affected (CITES and/or IUCN) CITES (https://cites.org/sites/default/files/eng/app/2016/E-Appendices-2016-03-10.pdf) Phoenicopteridae spp. (App. II) *Falco rusticolus* (App.I) *Aquila adalberti* (App.I) Falconiformes spp. (App II)
	(b)(iv) 2 Mortality in wild species	WNV outbreaks have been reported among domesticated geese in the Eastern Hemisphere, but generally there have been only sporadic reports of deaths in

		individual wild birds. It is uncertain whether this is related to the virulence of the viruses circulating in this region, host susceptibility, reduced transmission/ amplification or lack of surveillance. One recently introduced lineage 2 virus in Central Europe has affected significant numbers of wild and captive raptors. Species known to be susceptible to this isolate include sparrow hawks (*Accipiter nisus*), goshawks (*Accipiter gentilis*) and gyrfalcons (*Falco rusticolus*). The same virus was isolated from a dead collared dove (*Streptopelia decaocto*) in Italy, during an outbreak of mortality in collared doves and other species including blackbirds. Different lineages of the WNV have also been found occasionally in other dead birds including European robins (*Erithacus rubecula*), a raven (*Corvus corax*), common magpies (*Pica pica*), a Eurasian jay (*Garrulus glandarius*), house sparrows (*Passer domesticus*), a black redstart (*Phoenicurus ochruros*), a sedge warbler (*Acrocephalus schoenobaenus*) and a Savi's warbler (*Locustella luscinioides*).
(e)(iv) the impact of disease prevention and control measures	(e)(iv) 2 Mortality in wild species	The main risk may be represented by the environmental residual of biocides which may interfere with ecology of wild species.

Question 5d

Question 5d The disease has a significant impact on the long term on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds Interpretation: the consequences of the impact of the disease can even lead to the possible disappearance or long-term damage of endangered species or breeds **Answer** Y ☐ N ☐ na ☐		
Art. 7 criteria	**Art. 7 parameters**	**Assessment of the Art. 7 parameters from the fact-sheet**
(b)(iv) impact of the disease on biodiversity and the environment	(b)(iv) 1 endangered wild species affected: listed species as in CITES and/or IUCN list	Endangered wild species affected (CITES and/or IUCN) CITES (https://cites.org/sites/default/files/eng/app/2016/E-Appendices-2016-03-10.pdf) Phoenicopteridae spp. (App. II) *Falco rusticolus* (App.I) *Aquila adalberti* (App.I) Falconiformes spp. (App II)
	(b)(iv) 2 Mortality in wild species	WNV outbreaks have been reported among domesticated geese in the Eastern Hemisphere, but generally there have been only sporadic reports of deaths in individual wild birds. It is uncertain whether this is related to the virulence of the viruses circulating in this region, host susceptibility, reduced transmission/ amplification or lack of surveillance. One recently introduced lineage 2 virus in Central Europe has affected significant numbers of wild and captive raptors. Species known to be susceptible to this isolate include sparrow hawks (*Accipiter nisus*), goshawks (*Accipiter gentilis*) and gyrfalcons (*Falco rusticolus*). The same virus was isolated from a dead collared dove (*Streptopelia decaocto*) in Italy, during an outbreak of mortality in collared doves and other species including blackbirds. Different lineages of the WNV have also been found occasionally in other dead birds including European robins (*Erithacus rubecula*), a raven (*Corvus corax*), common magpies (*Pica pica*), a Eurasian jay (*Garrulus glandarius*), house sparrows (*Passer domesticus*), a black redstart (*Phoenicurus ochruros*), a sedge warbler (*Acrocephalus schoenobaenus*) and a Savi's warbler (*Locustella luscinioides*).
	(b)(iv) 3 Capacity of the pathogen to persist in the environment and cause mortality in wildlife	WNV is scarcely resistant in the environment thus its capability to survive during the vector-free period and, eventually, become endemic is still unknown. Different mechanisms have been claimed to explain WNV persistence. The duration of viremia in some bird species has been experimentally demonstrated (refer to the paragraph "a.i - animal species concerned by the disease") as well as the chronic infection in birds with the persistence of WNV RNA within the organs (spleen, kidney, and lung) of several species of birds. To what extent the virus circulates in the bloodstream is difficult to say and may be influenced by stressful events as migration or mating. Also vertical transmission by *Culex* mosquitoes has been experimentally demonstrated in *Cx. tarsalis* (Reisen et al., 2006) as well as the overwintering of WNV demonstrated in in *Cx. pipiens* mosquitoes collected during the 2000 outbreak in New York city (Nasci et al., 2001).

Question D

Question D The risk posed by the disease in question can be effectively and proportionately mitigated by measures concerning movements of animals and products in order to prevent or limit its occurrence and spread **Answer** Y ☐ N ☐ na ☐		
Art. 7 criteria	**Art. 7 parameters**	**Assessment of the Art. 7**

		parameters from the fact-sheet
(d)(v) feasibility, availability and effectiveness of restrictions on the movement of animals and products, as control measure	(d)(v) 1 available restriction movement measures	No specific measures are mentioned in the EU legislation for WNV outbreak control.
	(d)(v) 2 effectiveness of restriction of animal movement in preventing the between farm spread	
	(d)(v) 3 feasibility of restriction of animal movement	

Tables

Table a. i (1).: Summary outcomes of systematic review of experimental infections with WNV (papers published up to January 2016).

Species	Refs	Number of animal groups[A]	Agent detection [B]		Observation of clinical signs[C]		Clinical sings (and number of groups in which were reported)
			Min day	Max day	*Min day*	*Max day*	
Cats	(Austgen et al., 2004)	3 (19 animals)	Virus isolation from blood: 1 (0.5-3)	Virus isolation from blood: 7 (4.5-8)	1	6	No clinical signs observed (2), fever (1), depression/apathy (1)
							0 dead animals
Dogs	(Austgen et al., 2004; Karaca et al., 2005)	2 (19 animals)	Virus isolation from blood: 1.3 (0.5-2)	Virus isolation from blood: 5.3 (4.5-6)	1	1	No clinical signs observed (1), fever (1)
							0 dead animals
Horses	(Bunning et al., 2002; Castillo-Olivares et al., 2011; Shirafuji et al., 2009)	4 (17 animals)	Virus isolation from blood (3 groups): 3(1-4)	Virus isolation from blood (3 groups): 6 (6-7)	6.5 (3-8)	10 (9-11)	No clinical signs observed (1), twitching/tremors (1), neurological signs (2), fever (1)
			PCR from blood (1 group): 3	PCR from blood (1 group): 7			1 dead animal in 1 group
Pigs	(Teehee et al., 2005)	2 (12 animals)	Virus isolation from blood: 1.5 (1.5-4.5)	Virus isolation from blood: 5 (4.5-5)	Not reported		No clinical signs observed (1), not reported (1)
							0 dead animals
Rabbits	(Suen et al., 2015)	2 (27 animals)	Not reported		1	Not reported	No clinical signs observed (1), fever (1)
							0 dead animals
Sheep	(Barnard and Voges, 1986)	1 (2 animals)	Virus isolation from blood: 3	Virus isolation from blood: 11	3	3	Fever

A—All data were analysed at animal group level, reflecting the animal groups followed and reported in the individual references. Some references reported more than one animal group.

B—Min= first day (in dpi) that pathogen/RNA was detected in a sample for each reported animal group; Max= last day (in dpi) that virus/RNA was detected in a sample for each reported animal group. Min and Max were recorded individually for each animal group, and median (min-max) for each of those values were calculated from all group data (each group representing one observation, with no weighting based on the size of the animal groups). Contact transmission groups were not included in the summary.

C—Min= first day (in dpi) in which clinical signs were observed in each whole animal group reported; Max= last day (in dpi) in which clinical signs were observed in each whole animal group reported. Min and Max were recorded individually for each animal group, and median (min-max) for each of those values were calculated from all group data (each group representing one observation, with no weighting based on the size of the animal groups). Contact transmission groups were not included in the summary.

Table a.i. (2): Summary outcomes of experimental infections of West Nile virus performed in wild birds (adapted from Perez-Ramirez et al., 2014).

Order	Family	Species	Strain	Mortality	Viremia	Distribution	References
Passeriformes	Turdidae	American robin (*Turdus migratorius*)	NY	<20%	H	AM	(Komar et al., 2003; VanDalen et al., 2013)
		Swainson's thrush (*Catharus ustulatus*)	NY	<20%	M	AM	(Owen et al., 2006)
		Clay-colored thrush (*Turdus grayi*)	TEC/TAB	20-50%/<20%	M	AM	(Guerrero-Sánchez et al., 2011)
	Corvidae	Carrion crow (*Corvus corone*)	FR/ISR	20-50%/>50%	L	EUR/ASIA	(Dridi et al., 2013)
		American crow (*Corvus brachyrhynchos*)	NY/TEX/MEX	>50%	H	AM	(McLean et al., 2001; Komar et al., 2003; Brault et al., 2004; Weingartl et al., 2004; Kinney et al., 2006; Kipp et al., 2006; Brault et al., 2007; Brault et al., 2011; Nemeth et al., 2011)
			KEN/KUN	20-50%/<20%	M		
		Fish crow (*Corvus ossifragus*)	NY	>50%	H	AM	(Komar et al., 2003; Kipp et al., 2006; Nemeth et al., 2011)
		Little raven (*Corvus mellori*)	NY	<20%	M	OCE	(Bingham et al., 2010)
			KUN	<20%	L		
		Hooded crow (*Corvus cornix*)	EGY	>50%	H	EUR/ASIA/AFR	(Work et al., 1955)
		Western scrub-jay (*Aphelocoma californica*)	NY	>50%	H	AM	(Reisen et al., 2005)
		Blue jay (*Cyanocitta cristata*)	NY	>50%	H	AM	(Komar et al., 2003; Weingartl et al., 2004)
		Black-billed magpie (*Pica hudsonia*)	NY	>50%	H	AM	(Komar et al., 2003
		Jungle crow (*Corvus macrorhynchos*)	NY	>50%	H	ASIA	(Shirafuji et al., 2008)
	Passeridae	House sparrow (*Passer domesticus*)	NY/CA/KEN/EGY/TAB/TEC/SP/IT09	>50%	H	WORLD WIDE	(Work et al., 1955; Komar et al., 2003; Komar et al., 2005; Langevin et al., 2005; Reisen et al., 2005; Reisen et al., 2006; Nemeth et al., 2008; LaPointe et al., 2009; Nemeth et al., 2009a; Brault et al., 2011; Guerrero-Sánchez et al., 2011; Wheeler et al., 2012; Del Amo et al., 2014)
			TEX/KUN/IT08	<20%	M		
			MEX	<20%	L		
		Cape sparrow (*Passer melanurus*)	SA*	Und	L	AFR	(McIntosh et al., 1969)
	Icteridae	Red-winged blackbird (*Agelaius phoeniceus*)	NY	<20%	M/L	AM	(Komar et al., 2003; Reisen and Hahn, 2007; Nemeth et al., 2009b)
		Brown-headed cowbird (*Molothrus ater*)	NY	<20%	L	AM	(Reisen et al., 2006; Reisen and Hahn, 2007)
		Brewer's blackbird (*Euphagus cyanocephalus*)	NY	<20%	H	AM	(Reisen et al., 2006; Reisen and Hahn, 2007)

		Tricolored blackbird (*Agelaius tricolor*)	NY	<20%	H	AM	(Reisen and Hahn, 2007)
		Common grackle (*Quiscalus quiscula*)	NY	20-50%	H	AM	(Komar et al., 2003)
		Great-tailed grackle (*Quiscalus mexicanus*)	TAB/TEC	>50%/ 20-50%	H	AM	(Guerrero-Sánchez et al., 2011)
		Bay-winged cowbird (*Agelaioides badius*)	ARG	<20%	L	AM	(Diaz et al., 2011)
		Shiny cowbird (*Molothrus bonariensis*)	ARG	<20%	L	AM	(Diaz et al., 2011)
	Emberizidae	Song sparrow (*Melospiza melodia*)	NY	<20%	M	AM	(Reisen and Fang, 2007)
		White-crowned sparrow(*Zonotrichia leucophrys*)	NY	Und	na	AM	(Reisen et al., 2006)
	Fringillidae	Hawai'i 'amakihi (*Hemignathus virens*)	NY	20-50%	H	AM	(LaPointe et al., 2009)
		House finch (*Haemorhous mexicanus*)	NY	>50%	H	AM	(Komar et al., 2003; Reisen et al., 2005; Fang and Reisen, 2006; Reisen et al., 2006)
	Ploceidae	African masked weaver (*Ploceus velatus*)	SA*	Und	M	AFR	(McIntosh et al., 1969)
		Red-billed quelea (*Quelea quelea*)	SA*	Und	L	AFR	(McIntosh et al., 1969)
		Red bishop (*Euplectes orix*)	SA*	Und	M	AFR	(McIntosh et al., 1969)
	Hirundinidae	Cliff swallow (*Petrochelidon pyrrhonota*)	NY	<20%	M	AM	(Oesterle et al., 2009; Oesterle et al., 2010)
	Mimidae	Gray catbird (*Dumetella carolinensis*)	NY	<20%	M	AM	(Owen et al., 2006)
		Northern mockingbird (*Mimus polyglottos*)	NY	<20%	H	AM	(Komar et al., 2005)
	Sturnidae	European starling (*Sturnus vulgaris*)	NY	<20%	M	WORLD WIDE	(Komar et al., 2003; Reisen et al., 2006)
	Cardinalidae	Northern cardinal (*Cardinalis cardinalis*)	NY	<20%	H	AM	(Komar et al., 2005; Owen et al., 2012)
	Paridae	Tufted titmouse (*Baeolophus bicolor*)	NY	>50%	H	AM	(Kilpatrick et al., 2013)
	Troglodytidae	Carolina wren (*Thryothorus ludovicianus*)	NY	20-50%	H	AM	(Kilpatrick et al., 2013)
Falconiformes	Falconidae	Gyrfalcon (*Falco rusticolus*)	AUS*	20-50%	H	AM/EUR/AS	(Ziegler et al., 2013)
			NY	20-50%	M		
		Hybrid falcon (*Falco rusticolus x Falco cherrug*)	NY	<20%	L	WORLD WIDE	(Busquets et al., 2012)
		American kestrel (*Falco sparverius*)	NY	<20%	H	AM	(Komar et al., 2003; Nemeth et al., 2006a)
		Common kestrel (*Falco tinnunculus*)	EGY	<20%	L	EUR/AS/AFR	(Work et al., 1955)
Accipitriformes	Accipitridae	Red-tailed hawk (*Buteo jamaicensis*)	NY	<20%	H	AM	(Nemeth et al., 2006a)
Strigiformes	Tytonidae	Barn owl (*Tyto alba*)	NY	<20%	L	WORLD WIDE	(Nemeth et al., 2006a)
	Strigidae	Great horned owl (*Bubo virginianus*)	NY	<20%	H	AM	(Komar et al., 2003; Nemeth et al., 2006a)
		Eastern screech-owl (*Megascops asio*)	NY	>50%	H	AM	(Nemeth et al., 2006a)
Galliformes	Odontophoridae	California quail (*Callipepla californica*)	NY	<20%	L	AM	(Reisen et al., 2005; Reisen et al., 2006)

		Gambel's quail (Callipepla gambelii)	NY	<20%	L	AM	(Reisen et al., 2006)
		Northern bobwhite (*Colinus virginianus*)	NY	<20%	L	AM	(Komar et al., 2003)
	Phasianidae	Red-legged partridge (*Alectoris rufa*)	SP/MO	20-50%/>50%	H	EUR	(Sotelo et al., 2011b)
			NY	>50%	L		(Escribano-Romero et al., 2013)
		Japanese quail (*Coturnix japonica*)	NY	<20%	L	WORLD WIDE	(Komar et al., 2003)
		Ring-necked pheasant (*Phasianus colchicus*)	NY	<20%	L	WORLD WIDE	(Komar et al., 2003)
		Greater sage-grouse (*Centrocercus urophasianus*)	NY	>50%	M	AM	(Clark et al., 2006)
Pelecaniformes	Ardeidae	Rufous night-heron (*Nycticorax caledonicus*)	KUN	<20%	L	OCE	(Boyle et al., 1983b; Boyle et al., 1983a)
		Little egret (*Egretta garzetta*)	KUN	<20%	L	EUR/AS/AFR/OCE	(Boyle et al., 1983a; Boyle et al., 1983b)
		Intermediate heron (*Mesophoyx intermedia*)	KUN	<20%	L	AFR/AS	(Boyle et al., 1983a; Boyle et al., 1983b)
		Cattle egret (*Bubulcus ibis*)	SA*/EGY	Und/<20%	L	WORLD WIDE	(Work et al., 1955; McIntosh et al., 1969)
	Threskiornithidae	African sacred ibis (*Threskiornis aethiopicus*)	SA*	Und	L	AFR/AS	(McIntosh et al., 1969)
Columbiformes	Columbidae	Rock pigeon (*Columba livia*)	SA*/NY/TEC/TAB	Und/<20%	L	WORLD WIDE	(McIntosh et al., 1969; Guerrero-Sánchez et al., 2011)
		Ring-necked dove (*Streptopelia capicola*)	SA*	Und	L	AFR	(McIntosh et al., 1969)
		Eurasian collared-dove (*Streptopelia decaocto*)	NY/CO	<20%/<20%	M	AM/EUR/AS/AFR	(Panella et al., 2013)
		Laughing dove (*Spilopelia senegalensis*)	SA*/EGY	Und/<20%	L	AFR/AS	(Work et al., 1955; McIntosh et al., 1969)
		Common ground-dove (*Columbina passerina*)	NY	Und	na	AM	(Reisen et al., 2006; Reisen et al., 2008)
		Mourning dove (*Zenaida macroura*)	NY	<20%	M	AM	(Komar et al., 2003; Reisen et al., 2005; Reisen et al., 2006)
		Picui ground-dove (*Columbina picui*)	ARG	<20%	M	AM	(Diaz et al., 2011)
Gruiformes	Rallidae	American coot (*Fulica americana*)	NY	<20%	L	AM	(Komar et al., 2003)
		Crested coot (*Fulica cristata*)	SA*	Und	L	AFR/EUR	(McIntosh et al, 1969)
	Gruidae	Sandhill crane (*Grus canadensis*)	NY	<20%	L	AM	(Olsen et al., 2009)
Anseriformes	Anatidae	Common goose (*Anser anser*)	SA*	>50%	M	WORLD WIDE	(Banet-Noach et al., 2003)
		Canada goose (*Branta canadensis*)	NY	<20%	M	AM/EUR	(Komar et al., 2003)
		Mallard (*Anas platyrhynchos*)	NY	<20%	H	WORLD WIDE	(Komar et al., 2003)
		Yellow-billed duck(*Anas undulata*)	SA*	Und	L	AFR	(McIntosh et al, 1969)
		Red-billed teal (*Anas erythrorhyncha*)	SA*	Und	L	AFR	(McIntosh et al, 1969)
		Southern pochard (*Netta erythrophthalma*)	SA*	Und	L	AFR	(McIntosh et al, 1969)
Charadriiformes	Charadriidae	Killdeer (*Charadrius vociferus*)	NY	<20%	H	AM	(Komar et al., 2003)
	Laridae	Ring-billed gull (*Larus delawarensis*)	NY	>50%	H	AM	(Komar et al., 2003)
Psittacif	Psittacid	Monk parakeet	NY	<20%	L	AM	(Komar et al., 2003)

ormes	ae	(*Myiopsitta monachus*)					
		Budgerigar (*Melopsittacus undulatus*)	NY	<20%	L	OCE	(Komar et al., 2003)
Piciformes	Picidae	Northern flicker (*Colaptes auratus*)	NY	<20%	M	AM	(Komar et al., 2003)

CA: California 04; NY: New York 99; CO: Colorado 08; SA: South Africa; ARG: Argentina 06; EGY: Egypt; KUN: Kunjin; SP: Spain 07; MO: Morocco 03; AUS: Austria 09; MEX: Mexico 03; TEX: Texas 03; KEN: Kenya 3829; FR: France 00; ISR: Israel 98; TEC: Tecato (Mexico); TAB: Tabasco (Mexico); IT08: Italy 08; IT09: Italy 09. * Lineage 2.
Mortality:
L: Low viremia (mean peak viremia ≤ 104 PFU/mL); **M**: Medium viremia (mean peak viremia 104‑106 PFU/mL); **H**: High viremia (mean peak viremia > 106 PFU/mL); **na**: Data not available.
AFR: Africa; AM: America; AS: Asia; EUR: Europe; OCE: Oceania.
Und: Undetermined

Table a.i 5-6: List of wild and domestic WNV reservoir/sentinel animal species

Family	**Reservoir**	**Sentinel**	**Notes**
Turdidae	ND	Y	Intense viremia and clinical signs developed by infected birds
Corvidae	Potential	Y	Intense viremia and clinical signs developed by the infected birds with high mortality
Passeridae	Y	Y	Intense and long viremia and clinical signs developed by infected birds
Anatidae	-	Y	Intense viremia and clinical signs developed by infected birds
Columbidae	Y	-	Common ground-dove (Columbina passerina): WNV detection in spleen and kidney and lung at >6 weeks p.i
Frigillidae	Y	-	Persistant infection in house finches (Haemorhous mexicanus) House finches
Falconidae	-	Y	Intense viremia and clinical signs developed by infected birds
Phasianidae	-	Y	Viremia short and scarce, asymptomatic infection, detectable serological response
Laridae	-	Y	Intense viremia and clinical signs developed by infected birds
Strigidae		Y	Intense viremia and clinical signs developed by infected birds
Equidae	-	Y	Viremia short and scarce, development of clinical symptoms, detectable serological response
Canidae	-	Potential	Viremia short and scarce, rare development of clinical symptoms, detectable serological response . Potential use as sentinel in hurban areas
Felidae	-	Potential	Viremia short and scarce, rare development of clinical symptoms, detectable serological response . Potential use as sentinel in hurban areas
Cricetidae	Potential	-	Persistant infection and viral shedding

Table a.ii: WNV morbidity and mortality rate in horses (2010-2016 EU outbreaks)

				Equids in outbreaks						
Country	**Year**	**N. outbreaks**	**N. outbreaks with clinical symptoms**	**N. horses present**	**N. total cases**	**N. horses with symptoms**	**Died/Culled**	**Prevalence of total cases**	**Prevalence of clinical cases**	**Lethality**
Italy	2008	273	18	1941	563	32	5	29.01%	1.65%	0.89%
	2009	137	32	1398	223	37	9	15.95%	2.65%	24.32%
	2010	67	11	415	128	11	5	30.84%	2.65%	45.45%
	2011	91	41	881	197	58	14	22.36%	6.58%	24.14%
	2012	30	13	313	63	15	3	20.13%	23.81%	20.00%

	2013	35	11	308	50	12	1	16.23%	24.00%	8.33%
	2014	17	6	257	27	6	2	10.51%	22.22%	33.33%
	2015	26	6	302	30	6	5	9.93%	20.00%	16.67%
	2016*	33	13	310	37	13	4	7.25%	35.14%	10.81%
Portugal	2016	1	1	2	1	1	0	50%	50%	0%
	2015	3	3	82	4	4	0	4.88%	4.88%	0%
	2010	2	2	71	2	2	1	2.82%	2.82%	1%
Spain	2011	5	Unknown	44	11	Unknown	1	25.00%	Unknown	9%
	2010	31	2	845	39	2	2	4.62%	0.24%	5%
France	2015	35	26	262	49	34	5	18.70%	12.98%	0-5,26%
	2006	4	1	63	4	1	1	6.35%	1.59%	25%
Croatia	2014	1	0	2	1	0	0	50.00%	0%	0%
	2012	11	0	87	12	0	0	13.79%	0%	0%
Greece	2014	4	0	51	4	0	0	7.84%	0%	0%
	2013	10	2	559	15	2	1	2.68%	0%	7%
	2012	14	3	100	15	3	0	15.00%	3.00%	0%
	2011	17	0	374	23	0	1**	6.15%	0%	0%
	2010	27	3	559	30	3	3	5.37%	1%	10%
Romania	2010	3	Unknown	9	6	Unknown	0	66.67%	Unknown	Unknown
Former Yugoslav Republic of Macedonia	2011	4	0	51	10	0	0	19.61%	0%	0%
Bulgaria	2010	2	0	118	8	0	0	6.78%	0%	0%

Source: Italian National information system; (OIE, online)
*2016 Italian data: updated to 14th October 2016
** death may have been the result of conditions other than West Nile virus infection (possible snake bite reported)

Table a.v.4.: Detailed outcomes of systematic review on survival time of WNV in different matrixes at different temperatures.

Matrix	**Target**	**Species**	**Test**	**Temperature**	**Maximum detection**
Mosquito	Nucleic acid	na	RT-PCR	4°, 20°, 70°C	14 days
Mosquito	virus	na	Culture	4°, 20°, 70°C	2 days

Table ii 2: Number of cases (confirmed and probable) of West Nile Disease in Europe and in Mediterranean Basin (updated to 2nd December 2016)

COUNTRY	**YEAR**	**SPECIES**	**No. TOTAL CASES[1]**	**No. CONFIRMED CASES[2]**	**SOURCE**
Albania	2011	Human	2		(ECDC, online)
Algeria	2012	Human	1	1	(ECDC, online)
Austria	2016	Human	2	2	(ECDC, online)
	2015	Human	3	3	

	2014	Human	1	1	
Bosnia and Herzegovina	2014	Human	13	0	(ECDC, online)
	2013	Human	3	3	
Bulgaria	2016	Human	1	1	(ECDC, online)
	2015	Human	2	0	
Croatia	2016	Human	1	0	(ECDC, online)
	2013	Human	16	1	(ECDC, online)
	2012	Human	5	3	(ECDC, online)
	2013	Horses	-	12	(OIE, online)
Cyprus	2016	Human	1	1	(ECDC, online)
Egypt	2016	Human	1	1	(ECDC, online)
France	2015	Human	1	1	(ECDC, online)
Former Yugoslav Republic of Macedonia	2013	Human	1		(ECDC, online)
	2012	Human	6	1	
Greece	2014	Human	15	13	(HCDCP, online)
	2014	Horses	4	4	(OIE, online)
	2013	Human	86	58	(HCDCP, online)
	2013	Horses	-	15	(OIE, online)
	2012	Human	161	47	(HCDCP, online)
	2012	Horses	-	15	(OIE, online)
	2011	Human	101	-	(HCDCP, online)
	2011	Horses	23	-	(OIE, online)
	2010	Human	261	-	(HCDCP, online)
	2010	Horses	30	-	(OIE, online)
Hungary	2016	Human	39	16	(ECDC, online)
	2015	Human	18	13	(ECDC, online)
	2014	Human	11	3	(ECDC, online)
	2013	Human	31	6	(ECDC, online)
	2012	Human	12	7	(ECDC, online)
	2011	Human	3	-	(ECDC, online)
	2010	Human	3	-	(ECDC, online)
Israel	2016	Human	80	47	(ECDC, online)
	2015	Human	123	89	
	2014	Human	17	7	
	2013	Human	63	28	
	2012	Human	59	31	
	2011	Human	39	-	
Italy	2016	Human	71	71	(ISS, online)
	2016	Horses	51	51	(IZSAM, online)
	2015	Human	61	61	(ISS, online)
	2015	Horses	30	30	(IZSAM, online)
	2014	Human	24	24	(ISS, online)
	2014	Horses	27	27	(IZSAM, online)

	2013	Human	70	70	(ISS, online)
	2013	Horses	-	50	(IZSAM, online)
	2012	Human	50	39	(ISS, online)
	2012	Horses	-	63	(IZSAM, online)
	2011	Human	-	15	(ISS, online)
	2011	Horses	197	-	(IZSAM, online)
Kosovo	2012	Human	4	0	(ECDC, online)
Former Yugoslav Republic of Macedonia	2011	Human	4	-	(ECDC, online)
	2011	Horses	10	-	(OIE, online)
Montenegro	2013	Human	4	-	(ECDC, online)
	2012	Human	1	1	
Morocco	2010	Horses	25	-	(OIE, online)
Palestine	2014	Human	1	1	(ECDC, online)
	2012	Human	2	1	
Portugal	2016	Horses	1	1	(OIE, online)
	2015	Human	1	1	(ECDC, online)
	2015	Horses	4	4	(OIE, online)
Romania	2016	Human	93	80	(ECDC, online)
	2015	Human	18	18	(ECDC, online)
	2014	Human	23	22	(ECDC, online)
	2013	Human	24	22	(ECDC, online)
	2012	Human	14	13	(ECDC, online)
	2011	Human	11	-	(ECDC, online)
	2010	Human	52	-	(Sirbu et al., 2011)
	2010	Horses	6	-	(OIE, online)
Russian Federation	2016	Human	135	135	(ECDC, online)
	2015	Human	39	39	(ECDC, online)
	2014	Human	29	-	(ECDC, online)
	2013	Human	177	-	(ECDC, online)
	2012	Human	447	-	(ECDC, online)
	2011	Human	153	-	(ECDC, online)
	2010	Human	480	-	(Promed, online)
Serbia	2016	Human	41	41	(ECDC, online)
	2015	Human	28	28	
	2014	Human	76	56	
	2013	Human	302	200	
	2012	Human	70	41	
Spain	2016	Human	3	3	(Andalucia Ministry of Agriculture, online)
	2016	Horses	70	70	
	2015	Horses	18	18	
	2013	Horses	40	-	
	2011	Horses	12	-	
Syrian Arab Republic	2016	Human	2	1	(ECDC, online)

Tunisia	2016	Human	1	1	(ECDC, online)
	2015	Horses	1	1	(OIE, online)
	2013	Human	6	6	(ECDC, online)
	2012	Human	63	33	(ECDC, online)
	2011	Human	3	-	(ECDC, online)
Turkey	2014	Horses	1	1	(OIE, online)
	2011	Human	3	-	(ECDC, online)
	2010	Human	7	-	(ECDC, online)
Ukraine	2016	Human	1	0	(ECDC, online)
	2013	Human	1	-	
	2012	Human	12	-	
	2011	Human	8	-	

1. For EU countries, probable and confirmed cases, as per EU case definition
2. For EU countries, confirmed cases as per EU case definition http://ec.europa.eu/health/ph_threats/com/docs/1589_2008_ en.pdf

Table d.ii.1: Vaccines authorized for commercialization in the EU by the European Medicines Agency (updated in October 2016) and their efficacy as emerged from a systematic review (updated to January 2016)

Commercial name of vaccine	**Type of vaccine**	**Way admin**	**Doses**	**Species for which authorized**	**Countries in which authorized**	**Manufacturer**	**Efficacy**	Field protection	yearly availability/production capacity	Ref.
Proteq West Nile	West Nile recombinant canarypox virus, vCP2017 virus	Intramuscular		Horses	All EU	Merial	NA	NA	NA	
Equilis West Nile	inactivated chimaeric flavivirus strain YF-WN	Intramuscular		Horses	All EU	Intervet International BV	NA	NA	NA	
Equip WNV (previously Duvaxyn WNV)	inactivated West Nile virus, strain VM-2	Intramuscular	2 doses (21 days apart)	Horses	All EU	Zoetis Belgium SA	Viruses could be isolated from 8 out of 10 non-vaccinated animals up to 14 days after challenge, but only 1 vaccinated animals. Sixty percent of the controls had to be euthanized after challenge compared to none of the vaccinates. From 10 non-vaccinated animals, all presented, up to 21 days after challenge, pyrexia, head tremors or muscle fasciculations, and anxiety, and 9 showed mild paresis. In controls these	Experimental trial	NA	(Bowen et al., 2014)

							numbers were 2,2,6 and 2, respectively			

NA: data not available

Table a.vi.1-2: Experimental data on WNV transmission in wild birds

Direct *	**Indirect ****	**Horizontal**	**Vertical**	**Species**	**Notes**	**Reference**
C	Y	Y	NT	American crow (*Corvus brachyrhynchos)*		(Komar et al., 2003)
C	Y	Y	NT	Blue jay (*Cyanocitta cristata*)		(Komar et al., 2003)
C	Y	Y	NT	Black-billed magpie (*Pica hudsonia*)		(Komar et al., 2003)
C	Y	Y	NT	Ring- billed gull (*Larus delawarensis*)		(Komar et al., 2003)
C	Y	Y	N	Chicken (*Gallus gallus domesticus*)	only 1 animal in 16 in contact hens	(Langevin et al., 2001)
C	NT	Y	N	Domestic Geese (*Anser anser domesticus*)		(Swayne et al., 2001)
C	NT		NT	Common goose (*Anser anser domesticus*)		(Banet-Noach et al., 2003)
C	NT	Y	NT	Red-legged partridge (*Alectoris rufa*)		(Sotelo et al., 2011b)
NT	Y	NT	NT	Canada Goose (*Branta canadensis*)		(Komar et al., 2003)
N	Y	N	NT	Mallard (*Anas platyrhynchos*)		(Komar et al., 2003)
O	Y	Y	NT	American Kestrel (*Falco sparverius*)		(Komar et al., 2003) (C); (Nemeth et al., 2006a) (O)
N	Y	N	NT	Northern Bobwhite (*Colinus virginianus*)		(Komar et al., 2003)
N	Y	N	NT	Japanese Quail (*Coturnix japonicus*)		(Komar et al., 2003)
NT	Y	NT	NT	Ring-necked Pheasant (*Phasianus colchicus*)		(Komar et al., 2003)
N	Y	N	NT	American Coot (*Fulica americana*)		(Komar et al., 2003)
NT	Y	NT	NT	Killdeer (*Charadrius vociferus*)		(Komar et al., 2003)
N	Y	N	NT	Mourning Dove (*Zenaida macroura*)		(Komar et al., 2003)
N	Y	N	NT	Rock Dove (*Columba livia*)		(Komar et al., 2003)
N	Y	N	NT	Monk Parakeet (*Myiopsitta monachus*)		(Komar et al., 2003)
N	Y	N	NT	Budgerigar (*Melopsittacus undulatus*)		(Komar et al., 2003)
O	Y	Y	NT	Great Horned Owl (*Bubo virginianus*)		(Komar et al., 2003) (C); (Nemeth et al., 2006a) (O)
NT	Y	NT	NT	Northern Flicker (*Colaptes auratus*)		(Komar et al., 2003)

N	Y	N	NT	Fish Crow (*Corvus ossifragus*)		(Komar et al., 2003)
N	Y	N	NT	American Robin (*Turdus migratorius*)		(Komar et al., 2003)
N	Y	N	NT	European Starling (*Sturnus vulgaris*)		(Komar et al., 2003)
NT	Y	NT	NT	Red-winged Blackbird (*Agelaius phoeniceus*)		(Komar et al., 2003)
N	Y	N	NT	Common Grackle (*Quiscalus quiscula*)		(Komar et al., 2003)
N	Y	N	NT	House Finch (*Carpodacus mexicanus*)		(Komar et al., 2003)
N	Y	N	NT	House Sparrow (*Passer domesticus*)		(Komar et al., 2003)
N	NT	N	NT	Red-tailed hawk (*Buteo jamaicensis*)		(Nemeth et al., 2006a)
N	NT	N	NT	Song sparrow (*Melopiza melodia*)		(Reisen and Fang, 2007)
O	NT	Y	NT	Eastern Screech Owls (*Megascops asio*)		(Nemeth et al., 2006b)

C: Contact transmission
O: oral transmission
N: no evidence of direct transmission
NT: not tested
** Mosquitoes-exposed

Table d.i.1: Test methods available for the diagnosis of WNV and their purpose.

Test	Target	Se	Sp	Matrix	Reference	Notes
NS1-antigen protein microarray	Antibodie s	95%	100%	Serum	(Cleton et al., in press)	differential diagnosis of flavivirus infections in horses
Real-time RT-PCR	Antigen	from 1,5 to 15 copies per reaction	100%	viral strains, human samples (cerebrospinal fluid, biopsies, serum and plasma) and mosquito pools	(Vázquez et al., 2016)	specificity evaluated using viral RNA from a panel of different flaviviruses and other encephalitic viruses belonging to several viral families
Real-time RT-PCR	Antigen	80 genome copies	100%	Viral strains Lineages 1 and 2	(Faggioni G, 2014)	specificity evaluated using TBE, Usutu, Dengue 1, Dengue 4, YF, JEV
SYBR Green I-based real-time RT-PCR	Antigen	20 copies	100%	Human serum/plasma	(Kumar et al., 2014)	specificity evaluated using DEN-1-4, JEV, YFV, SLEV
Antigen capture ELISA	Antigen	90%	98%	Human serum	(Saxena et al., 2013)	detection of NS1 antigen
Real-time RT-PCR	Antigen	10 copies	100%	Viral strains	(Barros et al., 2013)	detection and differentiation between WNV and JEV; specificity evaluated using DEN-1-4, JEV, YFV, ZIKAV, Ntaya, TBEV, USUV, Toscana, CHIKV
Real-time RT-PCR	Antigen	1.26 TCID50/ml for WNV-L1, 6.3 TCID50/ml for WNV-L2	100%	Tissue, feathers, oropharyngeal and cloacal swabs and blood from wild birds, samples from mice	(Del Amo et al., 2013)	detection and differentiation between WNV and USUV; specificity evaluated using SLEV, MVEV, JEV,

				infected experimentally		BAGV, DEN-1, TBEV, VEEV, VSV, AIV, EIV, NDV, AHS4
Competitive ELISA	Antibodies	100%	wild birds: 79.5% compared to VNT	Sera from mammals and wild birds	(Sotelo et al., 2011a)	
			horses: 96.5% compared to VNT			
			South african mammals: 79.5% compared to HAI			
			giraffas: 67% compared to HAI			
IgM capture ELISA	Antibodies	91.7%	99.2%	horse sera	(Long et al., 2006)	
Real-time RT-PCR	Antigen	2–4 genome copies of WNV	100%	Viral strains	(Eiden et al., 2010)	In OIE manual. For simultaneous detection and differentiation of WNV Lineage 1 and Lineage 2. Specificity evaluated using TBEV, YFV, JEV
Nested RT-PCR	Antigen	10-8.0/100 µL	ND	Equine brain, blood, and cerebrospinal fluid; avian brain tissues	(Johnson et al., 2001)	In OIE manual
Real-time RT-PCR	Antigen	0.1 PFU	100%	human serum, CSF, brain tissue, mosquito pools, and avian tissues	(Lanciotti et al., 2000)	In OIE manual. Specificity evaluated using DEN-2, JEV, YFV, SLEV, Lacrosse virus, Powassan virus, MVE, WEEV, EEEV

Table e.iv.1.: Biocidal products targeting mosquito control (genus Culex), for which reports were found in a systematic review of available treatments against the vectors of vector borne infections (papers published up to January 2016).

Active substance	Ref	Intended use (route investigated in the study)	Study findings
Studies not targeting any particular host			
deltamethrin	(Marcombe et al., 2011)	**Fogging**	Efficacy was assessed by monitoring mortality rates of naturally resistant and laboratory susceptible mosquitoes placed in sentinel cages. Results showed high mortality rates of susceptible sentinel mosquitoes (64%) while resistant mosquitoes exhibited very low mortality (10%)
		vehicle-mounted thermal foggers (1g/Ha)	
Studies focused on vector control in housing/ environment			
deltamethrin	(Akogbeto et al., 2010)	**Indoor spraying**	Deterrence rate[1]: Anopheles gambiae (31.25%, 24.75%, 30 and 60 dpt; Culex sp. and Mansonia sp. 30 dpt 46.15%).
		Huts were treated with insecticides. The absorption of the walls was 112 ml of insecticide per m^2 and that of the ceiling (polyethylene), the entry slits, and the door (painted metal) was in total 53.13 ml/m^2.	Exophily rate[2]: Anopheles gambiae (45.4%, 26.3%, 30 and 60 dpt; Culex sp. and Mansonia sp. 30 dpt 33.3%).

			Blood-feeding rate[3]: Anopheles gambiae (18.2%, 23.7%, 30 and 60 dpt; Culex sp. and Mansonia sp. 30 dpt, 14.3%).
			Immediate mortality[4]: Anopheles gambiae (32.7%, 15.8%,30 and 60 dpt; Culex sp. and Mansonia sp. 30 dpt, 8.5%).
			Overall mortality[5]: Anopheles gambiae (72.7%, 31.6%, 30 and 60 dpt; Culex sp. and Mansonia sp. 30 dpt, 21%).
deltamethrin	(Badolo et al., 2014)	**Treated mosquito nets** Concentration of 55mg/m^2	Mortality of mosquitoes was 90.5 (86-94)% in unwashed nets (3 min exposure, 24h-mortality), and remained above 90% after 5 washes. Average mortality after 10, 15 and 20 washes were 81 (75-86)%, 68.7 (63-75)% and 66.3 (60-72)%, respectively.
deltamethrin	(Dabire et al., 2006)	**Treated mosquito nets** Concentrations of 25 mg/m^2 and 2% w/w	Mosquito entrance rate was 10-fold higher in control houses than in houses with Long Lasting Impregnated Nets (LLINs) and there was no difference between the 2 tested net types. Among mosquitoes found in the houses, 36% were dead in LLIN houses compared to 0% in control houses. Blood feeding rate was 80% in control houses compared to 43% in LLIN houses. The type of net did not significantly impact any of these parameters.
deltamethrin	(Darriet et al., 2000)	**Treated mosquito nets** Concentration of 25 mg/m^2	The 24h mortality was 56% for *An gambiae ss* females, and 45% for *Culex spp* females (compared to 4 and 6% in controls)
deltamethrin	(Moosa-Kazemi et al., 2007)	**Treated mosquito nets** Concentrations of 25 mg/m^2	Recorded 24h-mortality was 100% even after 9 months.
deltamethrin	(Muller et al., 2002)	**Treated mosquito nets** Concentrations from 55 mg/m^2 (unwashed) to 1.6 mg/m^2 (18 months old and washed 3 times)	Mortality of mosquitoes was 97% in washed nets, and reduced to 84%, 54% and 7% after 6, 12 and 18 months (with respective average of times washed of 1.1, 1.9 and 3)
deltamethrin	(Van Roey et al., 2014)	**Treated mosquito nets** Concentrations of 55 and 68 mg/m^2	A positive control (commercial product PermaNet® 2.0, 55 mg a.i./m^2) was able to kill over 90% of mosquitoes (3 min exposure, 24h-mortality) for up to 30 months, while the observed mortality with the experimental product (Netprotect®, 68 mg a.i./m^2) was 85.7% after 12 months, and remained below 90%.
diflubenzuron	(Cetin et al., 2006)	**Septic tank water treatment** 0.01, 0.02, and 0.03 mg (AI)/liter, using a 25% wettable powder or a 4% granular formulation in wastewater tank	Recorded adult inhibition for *Culex pipiens* was always 100% in the first 2 weeks, for all concentrations tested, and remained at 100% for up to 4 weeks with 30g/L, and 2 weeks with 10g/L.
lambda-cyhalothrin	(Okumu et al., 2012)	**Indoor spraying** 0.03 g/m^2 sprayed on mud walls	Mortality (24h mortality of *Anopheles arabiensis*) was 90% after 30 days but reduced to 35% after 60 days.
lambda-cyhalothrin	(Trout et al., 2007)	**Outdoors Spraying** Mist (concentration of 62.52ml/L) directly applied to vegetation in the backyard of houses, and other resting sites.	The reduction in *Aedes albopictus* in sites was of 89.5% compared to controls, and in laboratory bioassays exposing mosquitoes to treated leaves, mortality varies from 80% after 2 weeks, to 35% after 8 weeks. In contrast, *Culex spp.* was not reduced.

permethrin	(Rozendaal et al., 1989)	**Treated mosquito nets** Concentrations of 125-1000 mg/m^2	Cotton cloth impregnated with permethrin at a rate of 0.5 g/m^2 killed all *An. darlingi* females exposed for 2 min, but after the material had been washed twice in soapy water the bioassay mortality fell to only 21.4%. Bioassays with *Culex quinquefasciatus Say* females showed that sprayed nets were less effective than nets impregnated by soaking (at equivalent dosages of 0.16-1.34 g/m^2)
permethrin	(Soleimani-Ahmadi et al., 2012)	**Treated mosquito nets** The nets were blended with 1000 mg a.i/m2 (2%, w/w), and final concentrations varied from 814 to 937 mg/m^2	Mortality of mosquitoes was 100% in the first 90 days, 92.4% (88-97) after 5 months, and reduced to 81.6% (75-88) after 9 months, and 72.3% (65-79) after 12 months.
Studies focused on humans as the host species (personal protection)			
DEET	(Soonwera and Phasornkusolsill, 2015)	**External use - topic/spray** DEET 20% (w/w), 0.1mL applied on a 3 cm × 10 cm area on the ventral portion of the forearm	DEET was used as control when evaluating other (non-ECHA approved) substances. The formulation gave protection for up to 182 min, and 98.5% protection from bites of **Aedes aegypti** and ***Culex quinquefasciatus***
DEET	(Gupta et al., 1987)	**Treated clothes and topic applications of repellent**, in different concentrations and combinations	The field trials were arranged in a four-way factorial design which compared fabric types, permethrin treatment and repellent treatments over a 14-hour test period. The repellent formulations and the permethrin-treated clothing used as one system provided better protection (81% mortality) than the repellent formulations or permethrin-treated clothing used separately.
DEET+permethrin	(Mani et al., 1991)	**External use - soap** containing 20% deet and 5% permethrin	Percentage repellency (reduction in biting rates) was 96% for ***Culex vishnui***, 89.6% for **Culex tritaeniorhynchus** and 94.8% for ***Culex pseudouishnui***
metofluthrin	(Dame et al., 2014)	"Clip-on" **spatial repellent device** 31.20%	Efficacy in reduction of *Anopheles quadrimaculatus*, in 2 study years, compared to control, were 16% and 8%); 19% and 8% for *Psorophora columbiae* and 69% for *Culex erraticus*. Total mosquito reduction was 13%.
Metofluthrin	(Revay et al., 2013)	**External use** Clip-On Metofluthrin (31.2%)	Biting on the arms of volunteers was reduced by 96.28% for ***Ae. albopictus***, and by 94.94% for ***Cx. pipiens***.

[1] percentage of reduction in the number of mosquitoes caught in treated hut relative to the number caught in the control hut
[2] percentage of mosquitoes that have escaped the hut and have taken refuge in the veranda trap divided by the total number of mosquitoes collected in the hut
[3] percentage of blood fed mosquitoes collected divided by the total of mosquitoes collected in verandah and hut
[4] percentage of dead mosquitoes collected in the morning compared to total mosquitoes collected in the hut
[5] immediate mortality plus delayed mortality recorded after 24 h.

Figures

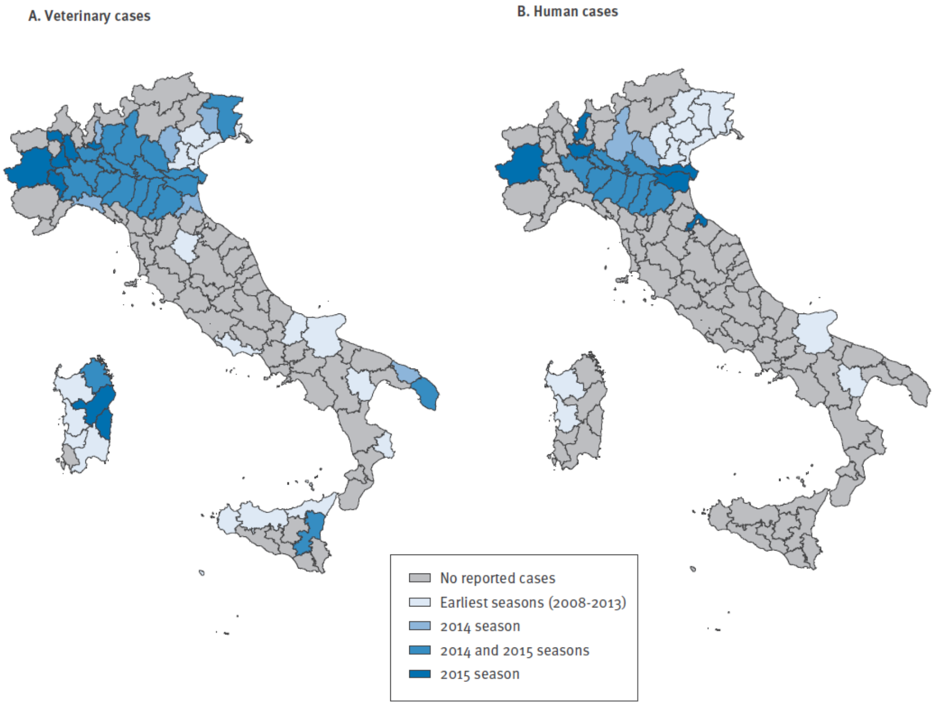

Figure 1: Geographical distribution of West Nile neuroinvasive disease in horses (panel A) and humans (panel B), Italy 2008–2015 (Rizzo et al., 2016) (http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=22580)

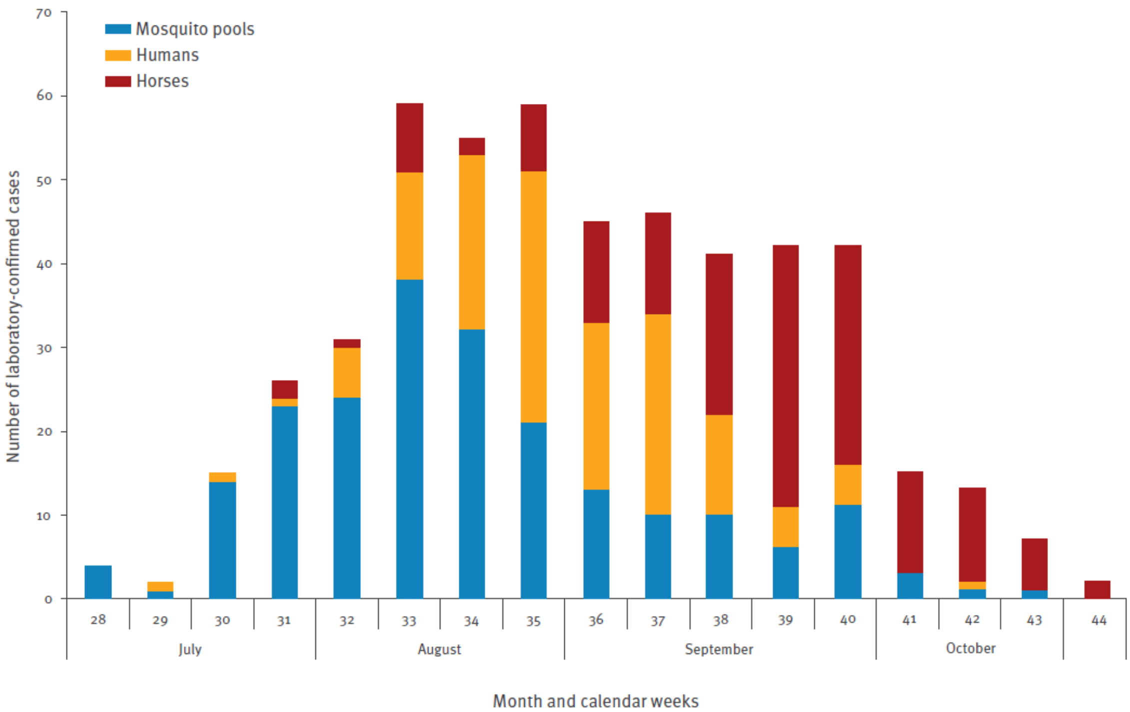

Figure 2: West Nile virus detections in the veterinary and human surveillance by month, Italy, 2008–2015 (Rizzo et al., 2016) (http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=22580)

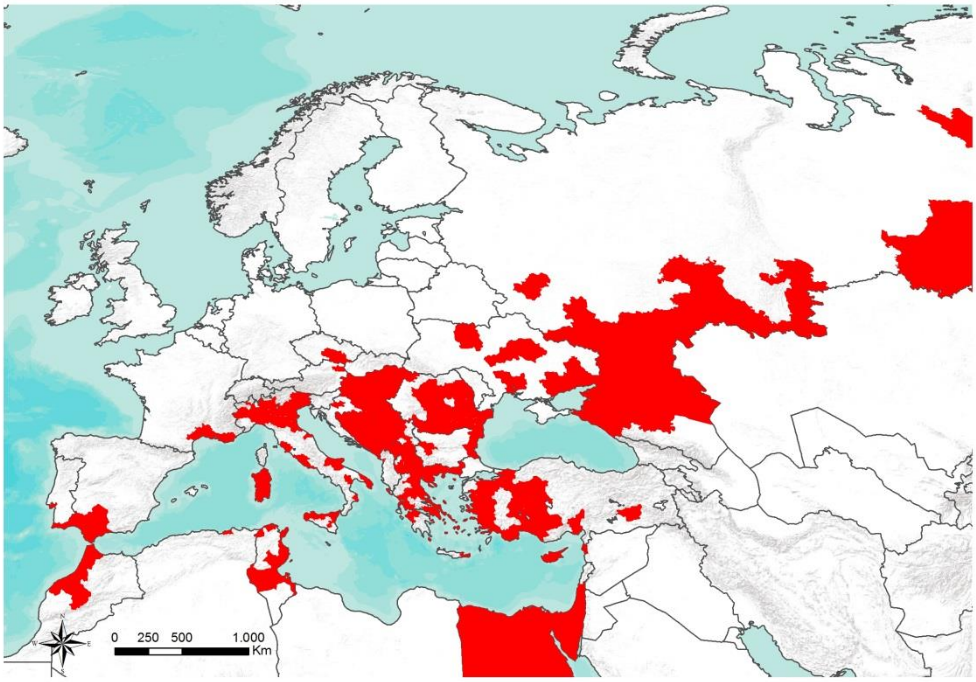

Figure 3: Geographic distribution of cases (confirmed and probable) of West Nile Disease in humans and animals (horses, wild birds, sentinel chickens, vectors) in Europe and in Mediterranean Basin (2008-2016) (source Arbozoonet: https://arbozoonet.izs.it/arbozoonet).