VIRUSES OF FOREST TREES, VECTORS OF VIRAL TRANSMISSION
AND METHODS FOR STUDYING PLANT VIRUSES

Introduction. Forest decline has become a popular issue, especially in connection with air pollution emanating from naturalized viruses, but such diseases as forest diseases, especially of virus etiology [49] have been considered of minor importance. There are not so many studies focused on plant viruses as infection agents of forest trees in particular, but many focused more on mixed types of diseases, incorporating fungi, mycoplasma and other infection activators rather than viruses. There were numbers of scrutinies regarding forested areas and nurseries and they seem to confirm the expansion of viruses in many plants with virus associated symptoms. Viruses induce alterations in a tree’s metabolism and alter plants predisposition. Viruses reported in many forest ecosystems and recovered from forest tree species. Viruses are widespread pathogens in agricultural crops, weeds and forest trees in particular, as a result, they are considered to be important reservoirs of viruses [37]. Viruses can only be replicated in living cells, because they lack their own metabolic activities. Nowadays, plant pathologists and biologist commonly are not concerned with viruses of forest trees, instead focusing their attention on short-term agricultural and fruit crops, where virus impacts have been shown well in near-term outlook. In spite of this, viruses or virus-like particles (VLPs) of coniferous and deciduous forest tree species causing significant impact on plant communities [16]. They can be soil- and airborne, and are transmitted from one plant to the other by mechanical means through wounds, arthropods, nematodes or fungal vectors, and a considerable number by seed and pollen. The international transfer of contaminated plant material is another important factor of virus distribution. If the economic point of view is considered, we should be cognizant that virus diseased plants may increase production costs because of the possibly decreased growth of infected stock plants and that viruses may subsequently damage their market quality. Information based on the root tip [20]. Transmission vectors: fungi and nematodes. Fungus-borne viruses can be split in two categories: (i) viruses belonging to the Tom-assisted virus family, which have isometric particles and are transmitted by Olpidium bornovanus, and their chemical nature was identified as a glycoprotein. Some other viruses are transmitted as a result upon cell wall digestion and fungal penetration. In this case, the receptors of the vector were partially characterized. They have been shown to be distributed at the surface of the zoospore envelope, and inoculated into the host upon cell-wall infection. The conditions under which compounds are effective investigated for activity against plant-parasitic nematodes. Known vector nematodes belong to the families Longidoridae (longidorids) and Trichodoridae (trichodorids) and transmit nepoviruses and tobaviruses, respectively. These nematodes are ectoparasites that remain outside the roots while feeding on epidermal cells located just behind the root tip. Transmission occurs in noncirculative process in which virus particles are retained at specific sites on the surface of the esophagus. Nematodes can transmit virus even after serial feeds on noninfected tissues and retain virus for periods of months. However, it was shown that transfer of infection from nematodes to trees can be prevented in some way, thus forest pathologists are able to reduce spread of nematode-transmitted viruses by using plant metabolites. Many plant constituents have been investigated for activity against plant-parasitic nematodes. The conditions under which compounds are effective against nematodes vary with the compounds [60]. These active compounds, or precursors of active compounds, can often be applied to soil as organic amendments, or refined and developed as biopesticide compounds.
To date, only a few comprehensive studies have attempted to detect viruses in forest soils and somehow classify them. One of them was done in forest soils in New York State [20]. Objectives of the survey were to evaluate elution and bait plant methods to detect infectious to-bamoviruses. Soils were collected from two forest sites: Whiteface Mountain (WF) and Heibeck Forest (HF). The effectiveness of four buffers to elute tomato mosaic tobamovirus (ToMV) from organic and mineral fractions of WF soil amended with ToMV was tested, and virus content was assessed by enzyme-linked immunosorbent assay (ELISA). The effectiveness of Chenopodium quinoa (Willd.) bait plants to detect the virus also was tested. Both methods then were utilized to detect tobamoviruses in 11 WF and 2 HF soil samples. A phosphate buffer (100 mM, pH 7.0) eluted more ToMV from soil than the other buffers tested. Mineral soil bound more virus than organic soil. Virus recoveries from virus-amended organic and mineral soils were 3 and 10%, respectively, and the detection sensitivity was 10 to 20 ng/g of soil. Roots of plant species grown in all virus-amended soils tested positive by ELISA, and virus concentrations averaged 10 ng/g. Both ToMV and tobacco mosaic tobamovirus (TMV) were transmitted to C. quinoa by elution from one of two HF soil samples but not from the WF soil samples. A tobamovirus was detected by bait planting in 12 of 73 (16%) root extracts representing 5 of 13 soil samples (38%). Tobamovirus-like particles were seen by transmission electron microscopy in 6 of 12 infected root extracts. After the experiment was done, it was stated that Tobamoviruses occur in forest soils in New York State. A biotic soil transmission to trees may permit isolated spread and persistence of these viruses in forest ecosystems. Another experiment was conducted in the German forests (10). Precedence was next: samples were collected from the area near the base of trees and seeds of some of the herbaceous virus indicator hosts were then planted in the soil which contained the samples and these plants were indexed for viruses. In the end of the experiment the majority of viruses were identified as Potex-, Tobamo-, Potyviruses (potato virus Y group), and TNV isolates. More than half the recovered viruses were potexviruses. PVSi was recovered twice directly from the roots of European beech (Fagus sylvatica), and from soil/root samples from beech, pine, oak, and spruce forests (30%, 30%, 20%, and 18%, respectively). Other viruses were detected as well. Tobacco mosaic virus (ToMV) was detected in soil samples from pine, spruce, beech, and oak forests. It should be mentioned that there are always some factors which are significantly decrease quantity of viruses which could have been detected in soils. These are: (i) non-mechanically transmissible viruses were not surveyed, (ii) specific vectors (like nematodes or/and fungi) might have not been present in all soil samples, (iii) the indicator plants might have not been sensitive to all the viruses present in the samples, etc. In addition, there are many factors which determine the survival and spread of soilborne viruses. For example, soil qualities. Those may limit the spread of viruses with nematode vectors, so the survival of viruses in soil depends to a certain extent on their adsorption to clays. Adsorption may be affected by pH, with the consequence that low soil pH may prolong virus survival in soil in orested areas.

Many plant viruses have been also recovered from rivers and lakes, primarily in Central Europe [32]. Potex-, tombus-, tobamovirus, and cucumoviruses (cucumber mosaic virus group) were detected along with ungrouped or as yet unidentified viruses [33]. The most important trees for forest ecosystems and forestry in particular include some of coniferous and deciduous trees so the main researches for identifying vi-
ruses specifically in these trees direct the need of observations. Some viruses of forest trees are listed below.

OAK. Yanwood & Hecht-Poinar [40] described a virus resembling TMV (Tobamovirus) in oak in California. Subsequently, the virus was detected in buds and young leaves of 11 symptomless species of Quercus and Lithocarpus [59]. The virus was transmitted from oak to herbaceous plants by conidia of the powdery mildew Sphaerotheca lanestris Harkn. [35]. In the mountains of the Rhine-
land, TMV was isolated from oaks displaying chlorotic flecking, mottling, and mosaic on deformed leaves. Viruses were observed in young oak seedlings after mechanical transmission [36]. TMV-like particles were detected in symptomatic oak in Germany, but mechanical transmission tests were unsuccessful. Similarly, Horvath and coworkers [29] detected TMV-like particles in malformed leaves of turkey oak (Q. cerris). A mosaic disease of blackjack oak (Q. marilandica) in the United States was graft-transmissible, but attempts at mechanical transmission were unsuccessful [6]. Other possible members of the group, such as potato mop-top virus, may be transmitted by root-infecting fungi (Plasmодиофорales: Spongospora subterranea Lagerh). Some virus-like symptoms such as distinct chlorotic lesions, ringspots and chlorotic mottle were observed on leaves of oak trees and seedlings (Quercus robur L) growing at several forest stands and nurseries in north-Germany. Investigations by serological means demonstrated that the agent of virus-like symptoms of oak were not tobacco mosaic virus, tobacco necrosis virus, brome mosaic virus and cherry leafroll virus but were related to the cryptic virus group [9].

ASH. Virus-like diseases of European ash consisting of mosaic and leaf deformation have been described in Europe [8,39]. Viruses are widespread in the ash population affected by decline. Tobacco ringspot virus (TRSV), tobacco mosaic virus (TMV), and tomato ringspot virus (TmRSV) occur in ash in New York State [12]. The first two viruses were associated with foliar viruslike symptoms on ash in the field, but virus infection was not correlated with dieback. Virus particles resembling TMV, however, were detected in Quercus spp. and Acer spp. [22] in conditions that suggest that natural spread might have occurred with the aid of the powdery mildew fungus (Sphaerotheca lanestris Harkn). As for TmRSV, it first was identified in stump sprouts of a white ash that had declined was associated with foliar symptoms [19]. CLRV is another example of infection agent in trees [5]. Nienhaus and Hamacher transmitted a CLRV isolate to white ash (F. americana) seedlings that subsequently developed chlorotic spots, ringspots, and line patterns. Similar symptoms on European ash in the U.K. were associated with ArMV [17]. The trees became infected when growing in soil infested with viruliferous nematodes. Similar symptoms on European ash in the U.K. were associated with ArMV. The trees became infected when growing in soil infested with viruliferous nematodes. ArMV was mechanically transmitted to seedlings of European ash and flowering ash (F. ornus L.), which developed chlorotic local lesions, systemic chlorotic chevrons, and a chlorotic mottle, respectively.

BIRCH. Birch decline is a serious disease in northeast-
ern USA and eastern Canada, affecting both white and yellow birch (Betula papyrifera and B.alganiensis). Most of the merchantable trees in the severely affected areas were killed in the 1935-55 period. Hansbrough [27] in 1953 transmitted a gold ringspot of white birch to seedlings but did not associate this virus with the decline. Later Berbee transmitted the line pattern of yellow birch to seedlings. The line pattern symptoms on both species consist of chlorotic lines forming oak-leaf designs, irregular rings or
linear flecks, sometimes accompanied by a mild mosaic. Until fully expanded, emerging leaves on infected trees generally are symptomless and some infected trees have a few, or no, foliar symptoms. These symptoms may be restricted to a few leaves on a few branches. The leaves remain on the trees until the end of the growing season. Chlorotic tissue turns nearly white during summer. The virus has been mechanically transmitted to Chenopodium, cowpea, cucumber, squash, and bean. Serological and host range studies demonstrate that this virus-causing line pattern of birch is a strain of apple mosaic. Finally, Berbee and Gottlieb concluded that apple mosaic virus (APMV) was responsible for such disease flow in symptomatic birch [23]. It has been also reported that Cherry leaf roll virus, CLRV is widely distributed in birch trees. The abnormalities, caused by CLRV were revied by Hamacher and others. In CLRV-infected birch, the area occupied by vascular bundles was reduced in comparison to healthy trees. Phloem cells were partly collapsed or disorganized and cell walls were thickened. Meristematic cells were deformed and reduced in size or their development completely inhibited. Sclerenchyma and collenchyma developed earlier than normal and chloroplasts were malformed. Tannin accumulated in the epidermis, palisade, and spongy mesophyll cells of the leaf laminae. Parenchyma cells of petioles, leaf laminae, and veins became necrotic. Young roots of diseased trees showed collapsed cells in the pericycle and endodermis and an accumulation of phenolic compounds [25]. It was also shown that CLRV is widely distributed in B. pendula and B. pubescens throughout the Finnish forest region [31]. Furthermore, dwarf birch, mountain birch, Kilopaa birch and curly birch were confirmed to be previously unknown hosts of CLRV [55]. The main route of CLRV dispersal in birch in natural habitats is assumed to be pollen and seed transmission, which has been studied in detail before [15].

ELM. Recent investigations in forest, nurseries and public gardens have shown that viruses are widely spread in deciduous trees including elm trees (Ulmus sp.). Biological, serological and electron microscopic assays showed that viruses of elm trees such as Cherry leaf roll virus (CLRV), Elm mottle virus (EMoV), Arabis mosaic virus (AmV) and Tobacco ringspot virus (TRSV) are present in some of the German parks and forests [3, 4]. Elm mottle virus of Ulmus minor was also reported to be found in Croatia [44]. In the United States, a graft-transmissible disease of American elm (U. americana L.) was reported in Ohio in 1927 [51].

MAPLE. Maple mosaic, maple line pattern or maple variegation virus reported from Europe, but apparently present in the northeastern United States also [2]. In 1980 it was reported that chlorotic spotting of the leaves was observed on several Acer saccharum seedlings, 2-3 years old, in Sainte-Anne-de-Bellevue, Canada. Symptoms induced on indicator plants inoculated with homogenate from affected leaves resembled those produced by tobacco mosaic virus. Concentrated preparations showed a UV spectrum like that of TMV and reacted with antisera to an ash strain of the virus. Rod shaped particles were detected in leaf preparations of Acer and tobacco and in purified preparations [34].

BEECH. The most economically important deciduous tree for some of the European forest industries is European beech (Fagus sylvatica L.). Thereby, necessity to inspect viral diseases of this plant family seems to be obvious for economical and biodiversity reasons. In the U.K. and East Germany beech displaying chlorotic leaf mottling and spotting were reported [48]. TBRV was identified in symptomatic trees. Nienhaus and coworkers [38] isolated potex- and potyviruses from trees with similar symptoms in West Germany. One isolate was serologically identical with PVY, another with PVSi, and a third with bean yellow mosaic virus (BYYMV) [58]. European beech trees infected with cherry leaf roll virus (CLRV) or brome mosaic virus (BMV) often exhibit irregular, meandering growth of branches and sometimes develop clavikele twigs with reduced internodes [26]. Single branches or twigs, particularly in the upper part of the canopy, may die, thus giving the tree a bristle appearance. The wood of virus-infected branches and twigs is brittle and dry. Leaf symptoms appear on single twigs or branches beginning during June. Young trees usually show more pronounced leaf symptoms than older trees. CLRV-infected leaves exhibit chlorotic line patterns, mosaic, or yellow stippling. Common leaf symptoms are small size, curling, and reduced growth of veins accompanied by chlorosis that becomes bright yellow.

CONIFERS. Viruses infect many conifer trees species in forest ecosystems [21]. Several coniferous species have been infected with viruses via the root system [28], where infection remained localized. There also have been reports of systemic virosis of conifers. Cech and coworkers [14] described an aphid-transmissible virosis of Norway spruce in Czech Republic. The disease was aphid- and graft-transmitted and rod-shaped particles were detected in needle and twig exudates in both symptomatic trees and inoculated seedlings. Biddle & Tinsley [7] observed VLPs in sap exudates of Silka spruce with needle chlorosis and defoliation in the U.K. Rod-shaped VLPs also were observed in western white pine (P. monticola) and Scots pine. In 2006 a new virus was found in Pinus sylvestris L. in different pine populations in Germany and Hungary [13]. On the basis of sequence comparison with different RNA viruses and phylogenetic analysis it was assumed that virus proteins from pine show highest similarity to the homologous proteins of Beet cryptic virus 3 and of a cryptic virus of Pyrus pyrifolia. It should be mentioned that Cryptovirus have not yet been reported to occur in Gymnosperms. There was also a report from USA, where scientists found tomato mosaic virus in red spruce trees on Whiteface Mountain, New York (54). By all above mentioned, we may conclude that viruses of conifers are widespread not only in Nothern and Central parts of Europe but also in transatlantic countries.

Preffered methods for studying viral diseases of forest trees.

Viruses of woody forest plants and of their seeds are known to be difficult to detect due to phenolic compounds in plant extracts and an often irregular distribution or the low concentration of the pathogens within the plants and seeds when using serological methods as a tool. But still these methods have to be regarded as important diagnostic tools when considering the restricted capacity of the test. The objectivity of electron microscopy has increased by the use of immunological reagents, but more up-to-date methods of detection are preferable for searching viruses in woody plants. The ELISA is one of the most sensitive immunological system. It was reported [18] that poplar mosaic virus was successfully detected in infected trees using ELISA method. Methods which detect nucleic acid are often essential when the pathogen being sought lacks protein. A new perspective for the diagnosis gives the combination of grafting plants to transmit the pathogen to host plants and a modified technique of the polymerase chain reaction (PCR) as well as the hybridization technique to detect the assumed pathogen. The PCR and hybridization technique are sensitive methods to detect viruses of smallest amount. Werner et al. [57] evaluated a method for detecting cherry leaf roll virus (CLRV) in seeds of birch and concluded that PCR followed by immunocapture-reverse transcriptase is the most sensitive way to detect the viral RNAs without a radioactive detection, which makes the system cheaper
and more reliable for routine use. In comparison to ELISA techniques RT-PCR approaches represent a remarkable improvement in sensitivity [41]. The advantages of PCR as a diagnostic tool include exceptional sensitivity, speed, and versatility. PCR is generally 102 to 105 times more sensitive than enzyme-linked immunosorbent assay, the widely used serological diagnostic benchmark. Sensitivity is of particular importance when viruses occur at low concentrations (dormant plant tissues, woody tissue) or are unevenly distributed. Applications of PCR-based plant virus diagnosis usually include the following:

1. Germplasm screening;
2. Field surveys to determine virus incidence and geographic distribution;
3. Provision of virus-free planting material;
4. Domestic and international plant quarantine;
5. Detection of mixed virus infections;
6. Analysis of virus distribution in different plant tissues;
7. Identification of alternative host plants;
8. Evaluation of virus-resistant or -tolerant cultivars;
9. Analysis of virus transmission by insect, nematode, or fungal vectors.

In the early 1990s, the newest method of DNA amplification, the polymerase chain reaction (PCR), was introduced for plant pathogen detection. It provides a method of exponentially amplifying specific DNA sequences by in vitro DNA synthesis. Depending on the specificity of the primers, the amplification products can provide both narrow and broad detection capabilities for various isolates of a pathogen. For the application of viral RNA sequences to PCR, cDNA is synthesized by reverse transcription (RT) and amplified by PCR (RT-PCR). With the availability of nucleotide sequences for many plant viruses and their strains, the development of RT-PCR assays for the detection and diagnosis of viruses in plant tissues and vectors has become feasible [50]. PCR methodology has been extensively applied to detect viroids, viruses, bacteria, mycoplasma-like organisms, fungi, and nematodes infecting various plant species [24]. This has opened new avenues for epidemiological studies such as for analysis of molecular virus-vector interactions and virus localization in the vector and it may enable the development of novel approaches for the control of virus spread by vectors. Successful virus detection of tobra- and nepoviruses has been reported in viruliferous soil-inhabiting nematodes [53]. RT-PCR for the detection of viruses is severalfold more sensitive than ELISA. Whereas ELISA detects virus concentrations in the lower nanograms or picograms, RT-PCR is capable of detecting viral nucleic acids in femtograms (fg). Thus, the prospect of detecting plant viruses by RT-PCR has increased, especially those which occur in very low concentrations in their vectors, e.g., nonpersistently transmissible viruses.

Inhibitors of RT or PCR can be effectively eliminated by capture of virus particles (Virus-Capture PCR) from crude plant tissue or vector extracts by the surface of polypropylene PCR tubes or microtiter plates or by polystyrene ELISA plates. Components of crude plant extracts that would otherwise inhibit RT-PCR are washed away. Immuncapture PCR appears to be the method of choice when specific antisera and available and highest sensitivity is required [30, 46], e.g., for certification of virus-free planting material. Immuncapture of serologically diverse virus isolates or multiple strains/species requires broad-spectrum antisera or a mixture of different antibodies. Immuncapture PCR or RT-PCR works reliably for the entire spectrum of plant viruses, including enveloped tospoviruses [56].

These biological and molecular techniques have given a large tool kit for the detection and diagnosis of plant viruses, thus researches may be done in various ways. But if one wishes to determine whether a plant is virus infected, say for quarantine purposes, one does not necessarily need a sophisticated technique that identifies a virus strain. On the other hand, if one is studying the durability of a potential resistance gene (or transgene) it is very useful to have an understanding of the range of variation of the virus. Thus, one has to select the best technique for what is wanted.

**Conclusion.** Recent researches found that viruses are predisposing factors leading to early senescence of trees. Senescence reduces the regeneration capacity of the host plants, and the juvenile metabolic vigor is lost. Viruses predispose trees to other damaging factors and lead to premature senescence. Under abiotic stress conditions the infected trees have less potential for recovery from inciting factors than non-infected trees. All these events may and should be controlled by measures, which prevent or at least decrease extent of impact of diseases caused by or/and associated with viruses. When decisions are made over which control measures (biological, chemical, phytosanitary, etc.) to deploy and whether to use a measure alone or together with others, the implementation of chosen methods should be done with full responsibility. Some virus control measures are generic, while others are so specific that they only apply to particular pathosystems in certain agro-ecological situations. In fact, to be adopted control measures also need to be ecologically sustainable, robust, affordable and compatible with standard agricultural practices. For example, a particular type of control measure may be unsuitable for environmental or socio-economic reasons as chemical control may cause build up of toxic residues that are harmful to mankind, domestic animals and wildlife or there is unforeseen accumulation of damaging pests or other pathogens and its use is prohibited entirely in true 'organic' production systems [42]. If nurseries are considered, elimination of virus-infected nursery stock can prevent the introduction of viruses into new areas. Vector control should be considered in nurseries. For sure, if we chose plants from nurseries without testing them on presence or propagation of any kind of viruses or virus potentially-related vectors it might later expand into infection over a large area (for example, public gardens, national reserves, etc.). The spread of viruses in seed can be prevented by appropriate indexing programs and the production of seed and propagating material from virus-free trees.

**Virus infections in forest plants pose a worldwide challenge to achieving satisfactory yields and quality of produce.** But nowadays, the role of viruses which affect and persist in forest trees and presented in forest soils is neglected. As were mentioned in this article, many viruses of forests haven't been identified yet or their influence on the flow of some associated with viruses diseases haven't been studied properly. Thus, we can not estimate correctly what are the main causes of diseases – whether they caused by microbiological agents or by virological agents. Consequently, the role of viruses may be far underestimated for forest biodiversity, forest protection services and forestry-based industries. Knowledge of viral processes is necessary for the nature of plant ecosystems and this basic concept illustrates our vision very well – the more we know about viruses the more we can affect them and regulate their pathogenesis. Hopefully, an increasingly sophisticated and diverse range of molecular control methods are becoming available to meet the challenge for virological detection and identification. For example, the development of molecular techniques reveals the characterization of DNA or RNA virus genomes and their properties. From the taxonomic viewpoint, this has led to a large increase in the number of plant virus species and genera that have been distinguished and also to the establishment of quantitative
criteria to delimit different species [1]. Concerning plant pathology, these methods have given a huge toolbox for scientists for fast and accurate work with yet unknown infection agents. In conclusion, maintaining the vigor of our forests by using modern techniques and practices for identification, control and prevention of virus-related processes in trees and soils should be main priority for modern day plant pathologists across the globe.

VIRAL INFECTIONS OF REPTILES:
A REAL THREAT FOR HEALTH OF HUMANS AND WARM-BLOODED ANIMALS

Introduction. Viral diseases of poikilotherm animals as well as viral agents transmitted or/and persisted in poikilotherm reservoir animals are now an almost unstudied and actual problem of contemporary virology. Investigations of reptilian pathology being today carried out bearing on relatively new scientific trends which have become to develop rapidly during these last years [2, 13]. Research of disease agents able to infect both cold- and warm-blooded hosts is mostly focused on reptiles participation in epidemiology of diseases caused by viruses belonging to families mentioned above. It should also emphasize the importance of reptile viral disease research for veterinary specialists dealing with animal patients kept at home, the studies in this field being also essential from the scientific point of view concerning taxonomic investigations and virus evolution. In Ukraine there are almost no investigations of viral diseases whose victims and/ or vectors are reptiles.

Object of this work is to draw attention to the importance of reptile viral infection problem as a possible source of virus spreading among animals and humans. In addition, the aim of our experimental research is to elaborate some protocols permitting to obtain virus susceptible cell systems adequate for isolation of reptile viruses.

Today representatives of all Reptilia classes are known to be possible intermediate hosts or reservoirs of different pathogens [2, 4, 5, 13]. Numerous viruses isolated from these animals have been described including those belonging to the arbovirus group (togaviruses, flaviviruses, rhabdoviruses, and bunyaviruses) transmitted by arthropods and causing infections of humans as well as of other mammals and birds [4,6,10]. Numerious studies prove arboviruses to possess enormous pathogenic possibilities. During last two-three decades, a lot of jointed demographic, socio-economic, and ecological factors led to a unique combination of previously absent conditions being especially favorable for increasing of epidemic potencies for numerous arboviruses (yellow fever, West Nile, dengue fever, and Chikungunya viruses) as well as for their spreading on territories where they had been previously absent, such spreading being accompanied by devastating epidemics [3,4]. The scientific data have already proved different members of the arbovirus group to be able to infect any reptilian species [26]. Under certain temperature conditions, viremia can appear in these animals. The recent studies demonstrate some arboviruses are able to persist in reptilian organisms during the winter period. Such a factor plays an outstanding role in infective epidemiology of diseases caused by these viruses. The West Nile virus (WNV) causes an arboviral disease endemic for Africa, Asia Europe, Europe, and Oceania, the infection being transmitted by mosquitoes of the Culicidae family. This virus infects susceptible mammalian species including humans as well as avian species causing the development of meningoencephalitis. The first epizootic outbreak due to this virus activity among birds was registered in the USA (New-York) in 1999 [12]. Before 2002 the virus spread on territories of all states; 120 humans fell ill in the same years, 11 of them died [2]. In 2001 first cases of alligator disease were reported from the state of Florida alligator farms; next year such reports were from farms of Georgia as well as from a Nile crocodile farm in Israel and from Mexico where the disease had been detected among wild crocodiles [2,10]. Until 2005, the disease was registered in states Texas, Louisiana, and Idaho. In Louisiana, 5000 young alligators perished, four cases of human infection among the farm workers have been found [30]. Seropositive individuals were detected in epizootic nidi among wild alligators [2]. The West Nile virus is a typical emergent infection for Crocodylidae; it is probably due to introduction of a viral strain being pathogenic for humans, birds, and horses. There are some data proving a high viremia degree in diseased alligators associated with marked virus secretion. Taking into consideration the peculiarities of epizootic outbreaks it is foreseen the infective agent to be spread among sensible Crocodylidae species by other ways, not only by transmission. It has been recently reported about parenteral and oral virus inoculation to alligators [13]. These data suggest a possible danger of infection for humans being in direct contact with animal feces and tissues [2,14].

The Japanese encephalitis virus (JEV) has been isolated from Chinese rat snakes (Elaphe rufo dor sata) in Korea [15]. And the antibodies to JEV has been found in cobras (Naja naja) in Hong Kong. Transmission studies with Japanese encephalitis virus have shown that lizards can be infected with this virus both by parenteral inoculation and, in some species, by feeding on infected mosquitoes. Infected animals develop viremia, and the development of viremia is temperature dependent [205]. No clinical symptoms were reported in the lizards in that study. Direct virus detection in naturally infected reptiles by isolation in cell culture or by RT-PCR has been described less frequently [13].

Most alphaviruses can infect a wide range of vertebrates, mostly birds and mammals, but several have also been reported in reptiles. Studies on alphaviruses in reptiles have mostly focused on the possible role of these animals for the transmission of alphaviruses to humans and livestock. This has led to a focus on persistence of alphaviruses in reptiles, particularly viral persistence over winter in temperate regions in the absence of mosquito activity. Evidence of infections with eastern equine encephalitis (EEE) virus and western equine encephalitis (WEE) virus, either by isolation or serology, have been reported in a variety of chelonians, lizards (including members of the families Lacertidae, Teiidae, Iguanidae, Agamidae, and Gekkonidae) and snakes (including members of the families Colubridae, Elapidae, and Crotalidae) [10]. Transmission studies have been performed in ginger snakes to determine whether the virus can overwinter in snakes, can result in antibody production, and be infectious to mosquitoes. Environmental (body) temperature affects viremia, with no viremia detected in experimentally infected snakes during torpor, and a lag time of several days required to detect the virus after an animal emerges and is