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Epidemiology and Pathology of Densovirinae

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Introduction

The parvovirus of invertebrates forms the Densovirinae subfamily within the Parvoviridae family [1]. Members of this group are commonly called Densonucleosis virus (DNV) first given to describe the characteristic histopathologic symptoms, i.e., hypertrophied and densely stained nuclei of sensitive cells in infected larvae. The name was subsequently shortened to Densovirus for all the group but this subfamily concists of three genera: Densovirus, Brevidensovirus and Iteravirus [2]. All the DNVs are characterized by their autonomous replication and the separate encapsidation of either of complementary single-stranded DNA strands. In this chapter we will review the pathology and the epidemiology of Densovirinae.

Pathology of Densoviruses

Distribution

The first DNV was isolated in France in 1964 from larvae of the greater wax moth, *Galleria mellonella*, used for fishing bait [3]. Subsequently, other members of Densovirinae or denso-like viruses were isolated all over the world, from insect orders, Lepidoptera, Dictyoptera, Diptera, Odonata and Orthoptera, as well as from Crustacea Decapoda (table 1) thus providing evidence of the ubiquity of DNVs in this phyllum of Arthropoda. In order

Table 1. Distribution of Densoviruses

Host	Name	Country of isolation	Year	Ref.
Insects				
Lepidoptera				
Agraulis vanillae	AvDNV	United Kingdom	1980	Kelly [4]
Bombyx mori	BmDNV-1	Japan	1975	Shimizu [5]
Bombyx mori	BmDNV-2	Japan	1983	Seki [6]
Casphalia extranea	CeDNV	Côte d'Ivoire	1981	Fédière [7]
Diatraea saccharalis	DsDNV	Guadeloupe	1977	Meynadier [8]
Euxoa auxilliaris	EaDNV	United States	1973	Sutter [9]
Galleria mellonella	GmDNV	France	1964	Meynadier [3]
Junonia coenia	JcDNV	United Kingdom	1972	Rivers [10]
Lymantria dispar (cell line)	LdiDNV	France	1982	Grignon [11]
Mythimna loreyi	MlDNV	Egypt	1995	Fédière [12]
Pieris rapae	PrDNV	China	1981	Sun [13]
Pseudoplusia includens	PiDNV	United States	1985	Chao [14]
Sibine fusca	SfDNV	Colombia	1977	Meynadier [15]
Dictyoptera				
Periplaneta fuliginosa	PfDNV	Japan	1979	Suto [16]
Diptera				
Aedes aegypti	AaeDNV	Soviet Union	1973	Lebedeva [17]
Aedes albopictus (cell line)	AalDNV	France	1993	Jousset [18]
Aedes pseudoscutellaris (cell line)	ApDNV	Venezuela	1980	Gorziglia [19]
Culex pipiens	CpDNV	France	1998	Baquerizo [20]
Haemagogus equinus (cell line)	HeDNV	United States	1995	O'Neill [21]
Simulium vittatum	SvDNV	United States	1976	Federici [22]
Toxorhynchites amboinensis (cell line)	TaDNV	United States	1995	O'Neill [21]
Odonata				
Leucorrhinia dubia	LduDNV	Sweden	1979	Charpentier [23]
Orthoptera				
Acheta domesticus	AdDNV	France	1977	Meynadier [24]
Crustacea				
Decapoda				
Carcinus mediterraneus	CmDNV	France	1988	Mari [25]
Macrobrachium rosenbergii	MrDNV	Malaysia	1990	Anderson [26]
Penaeus merguiensis	PmeDNV	Singapore	1985	Lightner [27]
Penaeus monodon	PmoDNV	Philippines	1985	Lightner [27]
Penaeus orientalis	PoDNV	China	1985	Lightner [27]
Penaeus semisulcatus	PseDNV	Kuwait	1985	Lightner [27]
Penaeus stylirostris	PstDNV	Hawaii	1989	Lu [28]

to obtain a uniform nomenclature of DNVs, they were identified by the two-letter abbreviation of the host name, such as GmDNV for the DNV from *Galleria mellonella*, or by three-letter abbreviation (one-letter abbreviation of the genus and two-letter abbreviation of the species) from insects with the same two-letter abbreviation, such as the DNVs from *Lymantria dispar* (LdiDNV) and *Leucorrhinia dubia* (LduDNV).

Symptoms

The densoviruses are responsible for fatal diseases of their host. The symptoms of GmDNV infections has been studied extensively [3, 29]. Generally the first symptoms are anorexia and lethargy followed by flaccidity and the inhibition of moulting and metamorphosis. During the infection, larvae become whitish and progressively paralyzed, followed by a slow melanization [30]. This symptom is similar to that of AvDNV and MlDNV infections [4, 31]. The cockroach *P. fuliginosa* infected with the PfDNV displays very characteristic symptoms. Prior to the death, the hind legs are paralyzed and their movements uncoordinated. Interestingly, females are particulary affected by this DNV [16]. The abdomen is swollen with an hypertrophied fat body, colored milky white in contrast to the brownish-white tissues observed in an uninfected cockroach. More than half of the infected cockroach develop ulcers in the hindgut by a process of accumulation of hemocytes around injured hindgut epithelial cells [32]. Some other DNVs produce tumor lesions in the intestine of their hosts. Typical tumors were observed in heavily infected slug caterpillar, pests of oil paml, *S. fusca* and *C. extranea* [15, 33]. The midgut epithelial cells of diseased larvae undergo intensive proliferation and the progressive thickening and opacity of the gut wall screens off the intestinal content. In the case of *C. extranea*, the larval color changes from green to yellowish brown and the transparent gut becomes opaque [33]. The nymphs of the Swedish dragonfly *L. dubia* infected with LduDNV become sluggish and flaccid, but there is no other external sign of the disease [23]. When silkworm larvae are infected *per os* with BmDNV-1, they usually die after seven days showing body flaccidity as a major sign. The alimental canal of the diseased larvae is pale yellow with little internal content [5]. Mosquito larvae infected with DNV exhibit symptoms of paralysis. Interestingly, despite the lack of cytopathic effect in the mosquito cell culture, the DNVs isolated from cell lines proved to be pathogenic for mosquito larvae by *per os* infection [18, 21, 34, 35]. When first instar larvae of *A. aegypti* were infected with AalDNV, the symptoms of the disease appeared at stage IV. Affected larvae lost their mobility and hung near to the

water surface. Their bodies were distorted and curved. They lost their pigmentation and exhibited a whitish color. These symptoms appeared one day before death [34].

Histopathology Associated with Densoviruses

Most DNVs known so far are polytropic in tissue tropism. In AvDNV, DsDNV, GmDNV, MlDNV, PiDNV, AaeDNV and AalDNV infections, almost all larval tissues, i.e. fat body, hypodermis, central nervous system, silk gland, muscular membrane, tracheal cells, malpighian tubules, foregut, hindgut, hemocytes, ovaries and molting gland, are susceptible, with the exception of the midgut epithelium [4, 8, 14, 17, 18, 29, 30, 31, 36]. On the other hand, DNVs infecting *B. mori*, *C. extranea* and *S. fusca* multiply predominantly in the columnar cells of midgut epithelium [15, 33, 37].

The histopathological aspects of DNVs infections are characteristic. The main lesions occur in the nuclei of infected cells. The nuclei become greatly hypertrophied very rapidly and densely stained (eosinophilic) and Feulgen positive [29]. In the GmDNV infections, the first obvious pathological changes occur in cells of the fat body. A voluminous dense homogeneous structure appears in each of the infected nuclei. Later, all cells become progressively involved [29]. In the larval tissues of AalDNV-infected *A. aegypti*, no alteration was observed at 2, 3 and 4 days postinfection. Anomalies appeared at day 5 principally in cells of the fat body. Later, the dense nuclei appeared in almost all of the larval tissues [34]. Histopathological studies on the midgut epithelium of the silkworm infected with BmDNV-1 show that the infected nuclei were more than 2.5 times as large as normal nuclei. At the last stage of infection, the degenerated columnar cells were liberated into midgut lumen [6]. In the case of BmDNV-2 almost the same features were observed under light microscope [38].

Ultrastructure of Infected Cells

Ultrastructural studies have led to a comprehensive description of the pathogenesis of DNV infection in larval tissues of *G. mellonella* [30, 39, 40]. The first ultrastructural changes in GmDNV infections are observed both in the cytoplasm and the nucleus. In the cytoplasm, during the first six hours postinfection, polyribosomes disappear and the number of free ribosomes and the formation of microbody-like structures arising from the accumulation of small, spherical particles of 17–20 nm inside of vesicles, increased.

This step could represent the accumulation and transport of viral proteins to the nucleus. In the nucleus, the heterochromatin becomes very condensed and is localized at the nuclear membrane. The nucleolus undergoes hypertrophy which is accompanied by a segregation of its fibrillar and granular components. The development of the granular portion coincides with the synthesis of double-strand DNA of the replicative form. Simultaneously, a virogenic stroma appears in close vicinity to the nucleolus. As the infection progresses, the granular portion of the nucleolus regresses in favor of the fibrillar portion. After one or two days, the virions are assembled inside the virogenic stroma which invades the whole nucleus and leads to a nuclear hypertrophy. By day 4 or 5, mature virions replace progressively the virogenic stroma and paracrystalline viral concentration takes place, pushing the chromatine and the nucleolus to the nuclear periphery. At the end of infection, the nuclei are so hypertrophied that the nuclear envelope is disrupted, allowing the virions to accumulate in the cytoplasm and viral inclusions, often arranged in paracrystalline arrays, can then be observed. Similar ultrastructural changes of nuclei infected with other DNVs have been observed [8, 9, 16, 33, 34, 41]. In several DNV-infected insects, the formation of cytoplasmic paracrystalline virions arrays, occurs prior to or without destruction of the nuclear membrane [14, 23, 42]. Although both DNV-1 and DNV-2 multiply in the nucleus of columnar cells, difference in the ultrastructural studies of infected cells is obvious when the sections are observed in the electron microscope. On the contrary of GmDNV and BmDNV-1, the virogenic stroma of cell infected with BmDNV-2 is less electron-dense than the surrounding nuclear matrix and occupies most of the nucleus. Discrete sites where virions replicate in linear array appear early in infection. These increase in size with each round of multiplication until they eventually fuse [43].

Epidemiology of Densoviruses

Host Range

Investigations on the host range of DNVs indicate that it varies considerably. The GmDNV, CeDNV and AdDNV have a host range apparently restricted to their original hosts [44, 45, 46]. In contrast, other DNVs, also isolated from lepidoptera, have a broader host range. The JcDNV can replicate in *Aglais urticae*, *B. mori*, *Chrysodeixis chalcites*, *L. dispar*, *Mamestra brassicae*, *Mamestra oleracea*, *Scotia ipsilon*, *Spodoptera exigua*, *Spodoptera littoralis* but not in *G. mellonella* [10, 42]. Similary, the MlDNV is infectious for *Chilo agamemnon*, *G. mellonella*, *Ostrinia nubilalis*, *Pectinophora gossypiel-*

la, *Sesamia cretica*, *S. littoralis* [31]. According to their sequences and genome organization the MlDNV is very closely related to GmDNV (95% identity) [47]. The close relationship is interesting since their tropism differs greatly, GmDNV being monospecific on its host, whereas MlDNV is polyspecific and infects a large number of lepidoptera pests. The striking differences in tropism related to the short sequence differences offered an ideal system to study these sequence-function relationships and the allotropic determinants [48]. The host range of EaDNV extends to *Pseudaletia unipuncta* and *Heliothis zea* [9]. The host range of the PfDNV was shown to extend to at least four other species of the genus *Periplaneta*: *P. americana*, *P. australasiae*, *P. brunnea* and *P. japonica* [16]. The host range of DNVs infecting mosquitoes extends to different species. Larvae of *A. albopictus*, *Aedes cantans*, *Aedes caspius*, *Aedes geniculatus*, *Aedes vexans*, *Culex pipiens* and *Culiseta annulata* are all susceptible to *per os* infection with AaeDNV [17]. The AalDNV isolated from a chronically infected cell line of the C6/36 clone of *A. albopictus* proved to be very pathogenic for *A. aegypti* and *A. metallicus* larvae [34]. In the case of BmDNV-1, there is no information concerning its cross-infectivity to others insects except between *B. mori* and the pyralid, *Glyphodes pyloalis*, infecting the mulberry plantations of sericultural farms [49]. Of practical interest for sericulture was the demonstration that the susceptibility to DNV infections varied from one strain of silkworm to another and that resistant strains could be selected. Among the economically important silkworms strains, severals are suceptible to BmDNV-2. Almost all strains susceptible for BmDNV-1 are resistant to BmDNV-2 and reciprocally, strains resistant to BmDNV-1 are sensitive to BmDNV-2 [50]. The mode of inheritance of the resistance to BmDNV infections has been investigated and it was established that for each virus the nonsusceptibility is genetically controlled by a recessive gene that is not sex linked. A practical aspect of this result was to recommend the rearing of silkworm strains homozygous for the nonsusceptible gene, in order to avoid DNV epizootics in sericultural farms [50].

Finally, it is worth mentioning that despite their high virulence for their insect hosts, DNVs do not appear to be able to replicate in vertebrates or mammals, including humans. No pathogenic effect was detected following inoculation of GmDNV, JcDNV, CeDNV and MlDNV to mice or rabbits for production of antisera [31, 44]. Similary, no replication of AalDNV could be detected in monkey MA-104 and BGM cells and in human Hela cells [18].

Natural Epizootiology of Densovirus in Insect Populations

In the case of GmDNV, the virus is so virulent and contagious that epizootic cause a gradual decline in the mass rearing of *G. mellonella* larvae for fishing bait and became problematic [3]. In natural conditions, the horizontal viral transmission of GmDNV in beehives infested with a population of *G. mellonella*, is due to cannibalism of dying larvae and to the excretion of virus-contaminated cells. The role of parasites of insects has also been demonstrated [30].

The spatio-temporal dynamics of larval population of *C. extranea* and the incidence of the CeDNV in this population were analysed in an oil palm plantation at Eloka (Côte d'Ivoire). The sampling of larval population and diagnosis of infection over a period exceeding ten years revealed that the virus maintained as endemic infection and contributed significantly to the regulation of its host's population. During outbreaks of *C. extranea*, the artificial spreading of the virus proved very efficient to control this pest. In natural conditions, the propagation of the epizootics was correlated with the larval density, the higher the density of the host, the more efficient was the spreading of the virus. From an initial focus of penetration, the dissemination of the pest in the plantation follows the direction of the dominant wind and the dissemination of the viruses follows the same gradient [51, 52].

In Japan, the densonucleosis disease of the silkworm *B. mori* was prevalent in some sericultural farms and caused great economic damage. On the occurrence of BmDNV-1 in the Nagano prefecture, epizootiological investigations were made immunologically [53]. An enzootic was only noted at a few farms, mainly due to the rearing of silkworm strains nonsusceptible or highly resistant to BmDNV-1 infection. However, the DNV antigen was detected generally in the dusts from mulberry leaves of every farm. It has also been found that BmDNV-1 detected in the dust originates from mulberry leaves contaminated due to a chronic infection of the mulberry pyralid *G. pyloalis*, infecting the mulberry plantations. The results suggest that the epizootic of densonucleosis in sericultural farms is caused by the rearing of silkworm strains suceptible to BmDNV-1 and by the infestation of mulberry pyralid infected with DNV. The result also suggests that the virus isolated as BmDNV-1 is originally derived from a DNV of the mulberry pyralid [53].

Our current studies in Egypt on the natural and experimental epizootiology of the MlDNV in different populations of the cotton leafworm *S. littoralis* should provide insights for the important mechanism of viral persistence which occur in nature, and contribute to a long-term control of insect pests.

Potential Use of Densovirus for Biological Control of Insect Pests

It is known that occluded insect viruses belonging to the Baculoviridae family are efficiently used as biological control agents of insect pests. Despite their high virulence and infectivity for their natural hosts (most of them being economically or medically important insect pests), the use of DNVs as viral pesticide has not yet been investigated in detail because of safety considerations for vertebrates. However, it is very important to mention the successful control of insect pests.

The first report concerns the introduction of GmDNV-infected cadavers of G. mellonella to control beehives heavily infested with this pest [54].

In Colombia, where *S. fusca* causes damage to oil-palm trees, suspensions of SfDNV-infected larvae, collected from the field, were sprayed by plane on these plantations at three different concentrations equivalent to 1–5 infected larvae per hectare. Following the applications, a mortality rate at 15 days postinfection of 95% was obtained at the highest dose and 73% at the lowest dose. After one month, 100% mortality was recorded at all three doses. Furthermore, parasites of the pest were not affected and contributed to further disseminate the virus [55].

In Côte d'Ivoire, field experiments were conducted with CeDNV against larvae of *C. extranea*, an important leaf-eater of oil-palm and coconut trees. Two aerial treatments of plantations by helicopter were made with suspensions of infected larvae at the concentration of 50–100 dead caterpillars per hectare. The viral disease controlled efficiently the outbreak of the moth, caused 92% mortality of the insects two weeks after the treatment [56].

The AaeDNV has been used to control natural populations of mosquito larvae in different areas of the former Soviet Union and a commercial formulation (Viroden) has been developed [35, 57].

These spectacular field results could be encouraging for utilization of other DNVs in biological control. The exposure of animals and man to DNVs is a common phenomenon since cultivated crops are frequently infested with insects. In spite of that, up to now, no human disease related to strict DNVs has been reported. It is important to point out that morphology and biochemical properties do not necessarily have any relation with the biology of viruses. Recent results on the molecular biology of DNVs indicate that they use a different expression strategy and may be less harmful than suspected [1]. The sequence homologies between DNV and vertebrate parvovirus genomes raised a concern about safety for the use of DNVs as biopesticides [2]. As previously stated, inoculation of mice and rabbits with DNVs did not induce any pathological condition. The AaeDNV and

AalDNV did not produce pathogenic effects when inoculated intracerebrally into suckling mice [18, 57]. However, safety tests should be thoroughly performed to confirm harmlessness to mammals and useful, nontarget insects before approval of a DNV as a pesticide is granted.

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