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A HERPESVIRUS DISEASE OF FARMED GREEN TURTLE *(Chelonia mydas)*

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INTRODUCTION

The mariculture of fresh-water and salt-water animals has discovery of unique new infectious diseases which pose fascinating for the biologist but which are viewed with alarm by the commercial mariculturist. Bacteria, fungi parasites and viruses have all been found to exert harmful and sometimes devastating effects in marine populations, and the control or prevention of the diseases caused by these agents presents a major challenge to those of us working with diseases. Many factors contribute to the introduction and spread of infectious disease agents in a population of animals and, generally a complicated interrelationship exists between the disease agents and the conditions under which the animals are maintained. Thus, stress loads, transmission of agents by the water route, nutrition, animal movements turnover rates and other general factors, should all be considered in dealing with infectious diseases in aquacultured species along with the more specific questions of proper treatment or prevention for a given disease.

As with other marine species, the farming of the green turtle *Chelonia mydas*, has its own set of specific disease problems. One of these is a skin disease, caused by a herpes-type virus, which appears to be manifested only under the conditions present in aquaculture.

The commercial turtle farm, Mariculture Ltd., has been in about 5 years and currently has a stock of approximately 100,000 green turtles ranging from hatchling size to five years of age. These

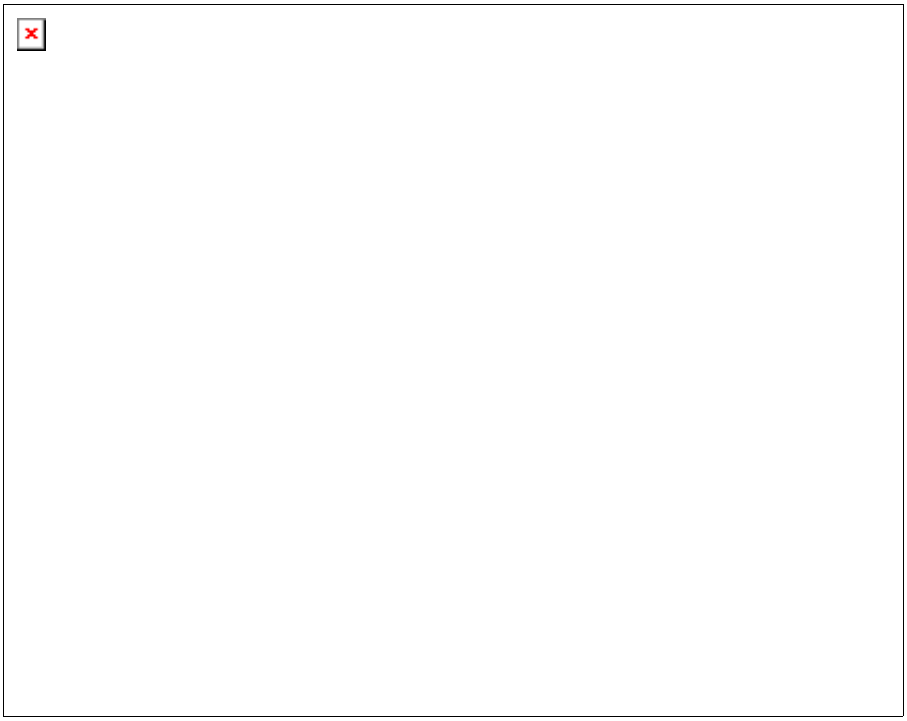
turtles are grown and maintained under stocking conditions which lead to an overall stress load on the animals. Changes in water flow and water temperature, the presence of organic material, and the routine introduction of new animals are all factors which add to the overall stress load on the farm at any given time. The skin of young turtles on the farm is usually marked by various types of lesions, one of which is extensive and spreading, and quite often leads to severe maceration of the skin and the death of the turtle. We call this particular skin condition grey-patch disease because of the grayish color of the lesions.

MORBIDITY AND MORTALITY

During the past 18 months we have observed five successive groups of young turtles at the farm during the immediate post-hatching period. The eggs from which the hatchlings were obtained were originally collected from the beaches of Surinam, Costa Rica and Ascension Island. Each group underwent an epidemic of gray-patch disease and from 70 to 95% in a given group contracted the disease approximately 8-12 weeks after hatching and the origin of the eggs did not seem to influence the incidence of gray-patch disease. It is difficult to obtain accurate figures on mortality due to gray-patch disease because of the large number of animals involved and because of the superimposition of other diseases in animals with gray-patch disease. However, we estimate that 2 to 25% of animals with gray-patch disease die, depending upon the conditions under which they are maintained. Figure 1 depicts the mortality seen in one group of green turtles approximately 8 weeks after onset of gray-patch disease. It should be pointed out that this mortality is chronologically associated with gray-patch disease and we have no direct proof, as yet, that gray-patch disease actually kills the turtles. However, in each of the 5 groups of animals that we have studied a definite increase in mortality occurred at approximately the same time after the appearance on the farm of gray-patch disease and in all likelihood death of the animals is a result of the disease.

The remainder of this report describes the skin lesions of gray-patch disease, the finding of a herpes-type virus in these skin lesions and the studies we have carried out to demonstrate that this virus is the causative agent of the disease.

Figure 1: Mortality associated with gray-patch disease;
peak incidence occurs approximately 9 weeks after onset of lesions.



GRAY-PATCH DISEASE

Figure 2 shows a young turtle with gray-patch lesions on the skin of the neck and flippers.(Figure 2 photo not available). These lesions consist of spreading plaques of superficial epidermal maceration with slightly raised borders. Lesions of often spread and in some turtles eventually cover all the surface of the skin. of the neck and flippers. Occasionally, less extensive lesions resolve spontaneously after about 6 weeks duration but most lesions continue to spread for months. Typical gray-patch lesions generally disappear before one year of age. These lesions can involve all superficial epidermal structures such as the skin and the shell segments of the turtles, the eyelid and conjunctival surfaces of the eye itself. Since these animals live in crowded contaminated environment secondary bacterial infections are common. There also seems to be another form of the same skin disease in which a small non-spreading papular-like lesion is seen. These small lesions generally do not develop into the extensive gray-patch type and, unlike gray-patch lesions, appear in turtles throughout life. The turtles of the groups that we have studied developed either the gray-patch or papular-like lesions before they were one year of age.

HISTOLOGY

Histological examination of the gray-patch and papular-type lesions shows inclusions in the nuclei of the keratinocytes in the upper layer of the epidermis. In Figure 3 the nuclei are enlarged and irregular in shape and are separated from the cytoplasm of the cells. Multinucleated gaint cells are also seen in these lesions. This histopathological picture is consistent with a herpes-type virus infection.
(Figure 3 photo not available)

ELECTRON MICROSCOPY

Herpes-type viruses were demonstrated in both gray pustular and gray- patch lesions by electron microscopy utilizing the negative staining and ultra-thin sectioning techniques. For negative

staining electron microscope grids were either touched directly onto the surface of the lesions or material taken from the lesions was resuspended in distilled water and placed directly onto the grids. For ultra-thin section tissue scrapings from the lesions were pelleted by low speed centrifugation, washed, fixed in osmium tetroxide, dehydrated, embedded in epon, sectioned and examined in the electron microscope. The microscope was a Phillips EM300 Figure 4 shows a section of scrapings taken from gray patch lesions. (Figure 4 photo not available). Numerous viral particles are seen in this lesion. Figure 5 is a higher magnification of these particles. (Figure 5 photo not available). These particles have a morphology which is typical of herpes viruses. This micrograph shows that the particles contain a dense inner-core surrounded by a capsid and two outer membrane. Their size is approximately 150 to 170 nm. in diameter, which is consistent with the general size of herpes viruses.
Figure 6 is an electron micrograph of negative stained enveloped viral particles. (Figure 6 photo not available). The negative staining technique provides a rapid identification method to determine if an individual lesion contains virus particles. Hundreds of lesions in turtles have been monitored by this technique and all lesions have been shown to contain the viruses by these negative stains. Particle sizes from negatively stained preparations correspond roughly to the diameter of the particles seen in previous thin sections.

TRANSMISSION OF GRAY-PATCH DISEASE

One proof of infectious disease etiology comes from the ability to transmit the disease agent to an uninfected animal and reproduce the disease in that animal. We have transmitted gray-patch disease in turtles by scratch-inoculating lesion-free turtles with gray-patch material from infected animals. Figure 7 gives the results of transmission experiments in several groups of animals. In these experiments gray-patch material was scraped from infected animals suspended in saline and either inoculated directly into the epidermis of uninfected animals or was treated with antibiotics, (chloramphenicol and gentamyacin) to remove contaminating bacteria and then inoculated. As controls, animals were either mock-scratched with a sterile needle or inoculated with sterile saline. Groups of uninoculated animals were also placed into experimental tanks with inoculated animals. As shown in figure 7 all turtles that were inoculated with untreated or antibiotic treated gray-patch material developed new gray-patch lesions. These new lesions were fully developed within 3 weeks. All control animals remained lesion-free. The turtles used in these experiments were selected and inoculated before they had acquired "natural" gray-patch disease
An interesting observation is that the control animals contracted "natural" gray-patch disease several weeks after the conclusion of the experiments.
Figure 8 shows an animal that has been scratch-inoculated on a flipper with gray-patch disease material. (Figure 8 photo not available). A large typical gray-patch is seen along the scratch line and when examined by electron microscopy this type of lesion always contained herpes-type viruses. In general only animals below 1 year of age were susceptible to gray-patch disease by inoculation. This is in agreement with the observation that natural gray-patch disease is seldom seen in animals over one year of age. The reason for resistance to gray-patch disease in older animal is not known but it is likely that the older animals have developed circulating anti-bodies as a direct result of having survived the disease in the first year of life and this acquired immunity protects them from recurrence of the disease.

Figure 7: Transmission of gray-patch disease to uninfected animals

Turtle Groups			
Gray Patch Material	**Inoculated**	**Mock Inoculated**	**Uninoculated**

Untreated # 1	10 / 10	0 / 10	0 / 10
Untreated # 2	10 / 10	0 / 10	0 / 10
Antibiotic treated	10 / 10	0 / 10	0 / 10
Saline Control	0 / 10	0 / 10	0 / 10

DISCUSSION

We have demonstrated a herpes-type virus in gray-patch disease of young green turtles and, by transmission experiments, have shown that this virus is the causative agent of gray-patch disease. Although this is the first finding of a herpes virus in the green turtle, herpes viruses occurs in many animals in nature, including man, and have various effects in their different animal hosts. One common feature of the herpes group of viruses is their ability to become latent or dormant in their hosts and exist in individual animals for long periods. Most often these viruses persist in their hosts with no obvious harm but under certain conditions such as stress the virus can re-establish itself and produce overt clinical disease. An example of this is herpes simplex virus-induced fever blister in man which occur after various types of stress such as exposure to sunlight, hormonal changes in females, psychological disorder and various other unknown factors. It is of considerable interest that one of the tissues that herpes virus commonly affect in this manner is the skin, a fact that makes the herpes-type virus taken from the skin lesion of turtles even more interesting.

Recently, two previously-undescribed herpes viruses have been shown to be present in the marine environment. One of these was demonstrated by electron microscopy in oysters (1) and the other in a marine fungus (2). A new herpes virus has also been isolated from kidney cell cultures taken from Iguanas (3). The herpes-type virus that we see in turtles apparently represents yet another new herpes virus from the marine environment and the finding of this type of viruses in turtle skin posses several interesting questions.

Is this virus a natural virus of turtles or is it a virus which is introduced into the turtle population and one which may persist?

Are the gray-patch lesions due to the presence of this virus a natural occurrence in the life history of turtles, or do they represent an induced disease state which is only serious under the conditions under which the turtles are farmed?

The answer to these questions, we believe, will better enable us to control and treat gray-patch disease of farmed green turtles.

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