

A Herpesvirus Disease of Green Sea Turtles in Aquaculture

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ABSTRACT—Hatchling green sea turtles, *Chelonia mydas*, in aquaculture are affected, early in life, by a herpesvirus disease called gray-patch disease. Gray-patch disease is primarily a cutaneous disorder which may, in some animals, become severe enough to cause death. The cutaneous lesions typically occur in epizootic fashion and upwards of 60-95 percent of separate groups of hatchlings may be affected. There is no cure and the lesions generally resolve spontaneously in animals that do not die. The morbidity and mortality level is dependent upon the degree of stress which the animals undergo. Elevated water temperature appears to be the most important of several possible stress factors. An inactivated viral vaccine has been developed which holds promise for prevention.

For the past 9 years, there has been a serious attempt to commercially farm the green sea turtle, *Chelonia mydas*, on a large farm Cayman turtle farms (e.g., Mariculture, LTD.¹), on the Island of Grand Cayman in the British West Indies. This farm is raising green sea turtles from hatchlings to marketable size in a total period of approximately 3 years. For the most part, the turtles are raised under stressful conditions of intensive crowding, high organic pollution, and fluctuating water temperatures, conditions which favor the outbreak of infectious diseases.

Over the past 4 years, we have studied some of these diseases to identify the etiologic agents and establish programs of prevention or control.

Table 1 lists the major diseases seen on the turtle farm and their presumed etiologic agents, if known. By far the most intensively studied of these diseases has been an herpesvirus disease which affects the turtles during their first year of life. Before describing the herpesvirus disease, it is important to point out the nature of the farm facilities

and describe some of the farm operations which may contribute to individual outbreaks.

TURTLE FARM FACILITIES AND OPERATIONS

The commercial turtle farm is located on approximately 4 hectares (10 acres) of land. The holding facilities are composed of 170 concrete and plastic tanks and pens of varying capacities ranging from 454 liters to 340,650 liters (120 gallons to 90,000 gallons). At the same site, there are 7.57 million liter (2 million gallon), man-made breeding ponds which hold large breeding ani-

mals. A beach for egg-laying breeders, a slaughterhouse, a hatchery, a newborn tank area, and the stock farm are also located on the 4-hectare site, along with administrative facilities and a tourist shop. There is a pumping system which circulates approximately 9.46 million liters (2.5 million gallons) per hour of fresh seawater, drawn directly from the Caribbean, through the pens and tanks. The water is circulated through the farm, and allowed to empty into the ocean at an effluent canal approximately 400 m (0.25 mile) from the inflow pipes. At any given time, there are upwards of 50,000-100,000 green sea turtles of all ages on the farm. The stocking density is high: large numbers of turtles are housed in each tank, some of the larger tanks holding up to 2,000 turtles.

During daily operations, turtles are frequently moved from tank to tank, and various groups of animals may be mixed. Feeding is done on a daily basis, and uneaten food often settles to the bottom of pens or tanks where residue is removed by mechanical means.

The general operations of the farm and handling of the turtles appear to play an important role in disease production. In the past, wild turtle eggs were brought in from various beaches (Costa Rica, Surinam, and Ascension Island), and young turtles were hatched on the farm from these eggs. It is possible that some of the organisms currently found on the farm were incidentally transported with the eggs from various parts of the world. In addition, turtles derived from the different natural beaches were often mixed after hatching, a situation which may reinforce spread and maintenance of infectious organisms.

Today, a portion of the turtle eggs are still brought in from the wild, from Surinam only, and numbers of the young turtle stock are now obtained from eggs that have been laid on the farm by large breeders. It is projected that the turtle farm will become self-sufficient by 1980 and that all new

Table 1.—Diseases commonly seen in commercially raised green sea turtles.

Disease	Tissues affected	Causative agent
Gray-patch	Skin, shell	Herpesvirus
Floppy-flipper	Skeletal muscles	Virus? Clostridium?
L.E.T.	Lungs, eyes, throat, ear canals	Herpesvirus?
Coccidiosis	Gastrointestinal tract	Coccidia (<i>Caryospora chelonia</i>)
Skin lesions	Skin	Fungi, Bacteria

¹Reference to trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

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hatchlings will come from farm-laid eggs. Since the breeding pond and the beach upon which nests are built are located at the same site as the stock farm, a situation exists in which disease organisms can spread to the breeders, and perhaps to the eggs, from stock animals, either by aerosol sprays produced from the circulating seawater, or perhaps by insects, wild birds, or rodents.

A young hatchling turtle is very small, only a few ounces in weight. In 3-3½ years, the baby turtles reach the size of approximately 45 kg (100 pounds); then they are usually slaughtered. There is generally a large mortality during the first year of life. Much of this loss is apparently caused by a herpesvirus infection to which we

have given the name of gray-patch disease (Table 1).

GRAY-PATCH DISEASE

Clinical and Histological Picture

Details of the clinical and histological pictures of gray-patch disease, the discovery of a herpesvirus in the lesions, and the artificial transmission of the disease have been published (Rebell et al., 1975). Gray-patch disease occurs in two forms. One form is a pustular-like lesion which is found on the skin of the neck and flippers of young turtles (Fig. 1). The disease in this form is benign, and does not seem to harm the turtle. Generally, these lesions resolve spontaneously. The second form of the

disease is manifested in a more extensive lesion in which obvious grayish patches are seen (Fig. 2). There may be spreading of these gray patches to large areas of the epidermal surface of the turtle, and in the most severely affected turtles, the complete epidermal surface may be covered. Often, these animals die from the disease.

The histological appearance of these lesions is typically one of a herpesvirus infection (Fig. 3) in which there are large cells containing nuclear inclusion bodies in the epidermis of the affected animals (Fig. 4). One receives the impression that if these animals were not living in an aquatic environment, the lesions would develop into distinct blisters, similar to those seen in the common herpesvirus infections of man,

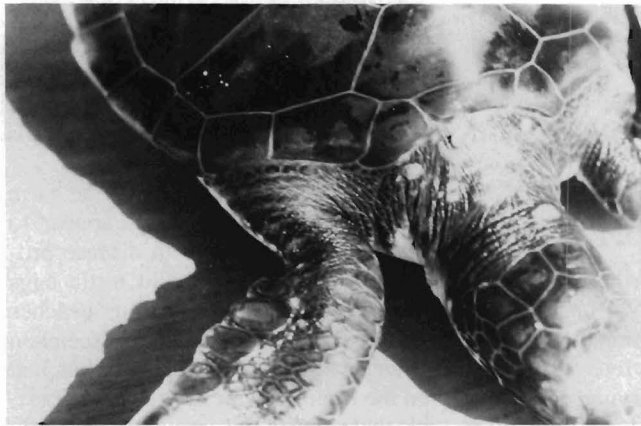


Figure 1.—Gray-patch disease in young sea turtle showing "pustular" lesions on neck and flippers.

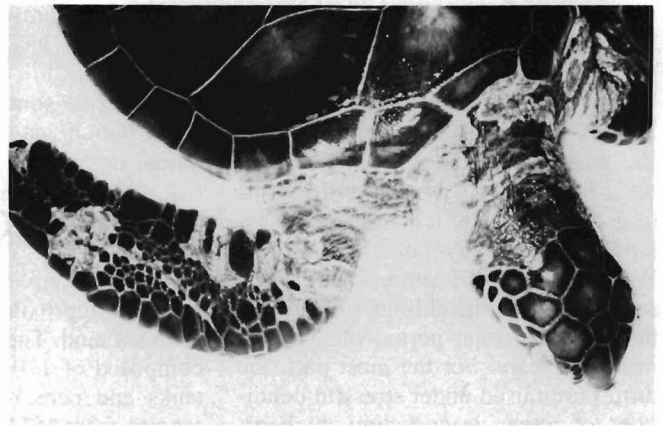


Figure 2.—Gray-patch disease in young green sea turtles showing spreading lesions on flippers and neck.

Figure 3.—Histology of gray-patch disease is typical of a herpesvirus infection. H & E stain.

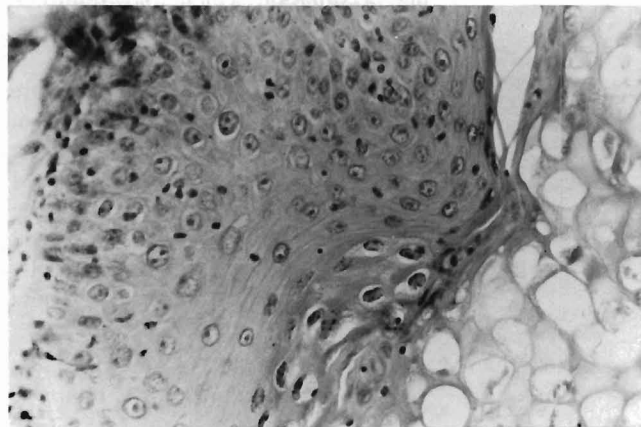
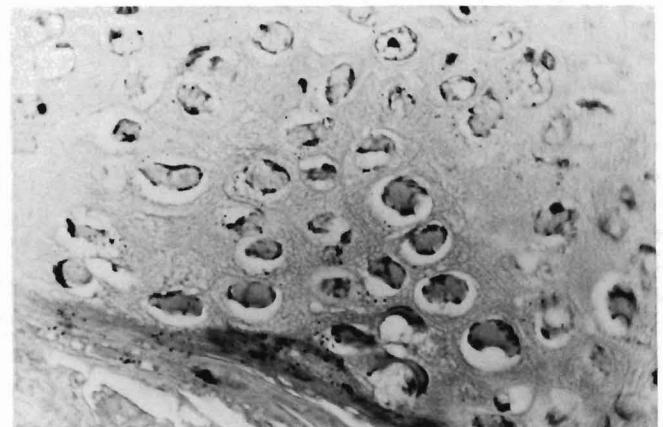


Figure 4.—Histology of gray-patch disease. Note large cells and intranuclear inclusion bodies. H & E stain.



such as herpes simplex or chicken pox. However, because of the aquatic environment, there is no epidermal roof to the gray-patch lesion, and any fluid which might form into a blister is quickly washed away. It is interesting to note that these lesions are truly epidermal in nature, and do not involve the dermal areas. No scars are formed on the skin of animals in which the lesions spontaneously resolve, unless as occasionally happens, the lesions become infected with bacteria or fungi which may invade the dermal area and deeper tissue.

Presence of Virus in the Lesions

Scrapings obtained from the gray-patch lesions, and examined by elec-

tron microscopy, show numerous herpes-type viruses. Figures 5 and 6 are electron photomicrographs which show the typical appearance of the gray-patch disease herpesvirus. Figure 6 is a higher magnification. As the electron photomicrograph illustrates, the viruses have a distinct capsid structure containing a nucleoid. The nucleocapsid is surrounded by an envelope. There also appear to be numerous empty capsids in lesion scrapings.

Because of the contaminated nature of the aquatic environment, bacteria also appear in gray-patch lesions. The bacterial flora of the lesions appears to change, depending upon the surrounding conditions, and we have never been able to show that a given strain of bacterium is a consistent inhabitant of the

gray-patch lesions. Apparently, the lesions provide a suitable tissue for secondary infection and new bacteria come in as old ones are displaced. As mentioned, an occasional secondary bacterial infection does become so severe as to initiate deep scarring.

Transmission Studies

To prove that gray-patch disease is caused by the herpesvirus found in the lesions, we carried out transmission studies in which antibiotic-treated or filtered gray-patch lesion scrapings were scratch-inoculated into the epidermis of young, susceptible turtles. In 100 percent of the animals inoculated, the disease was produced but not in mock-infected controls. The new lesions produced by transmitted virus

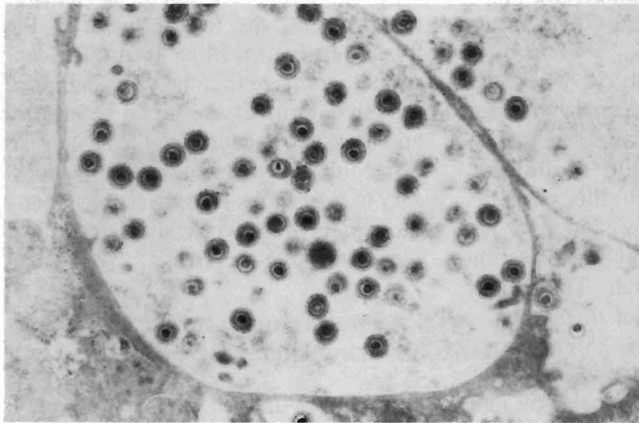


Figure 5.—Electron micrograph of a herpes-type virus in scraping from gray-patch lesion.

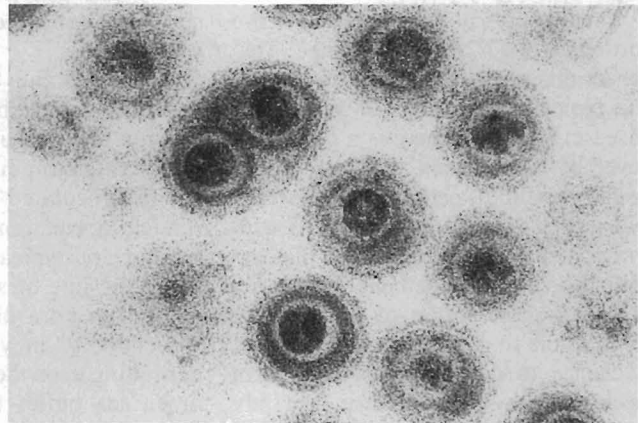


Figure 6.—Electron micrograph at higher magnification of herpes-type virus shown in Figure 5.

Figure 7.—Scratch-inoculated flipper showing gray-patch lesion along scratch line.

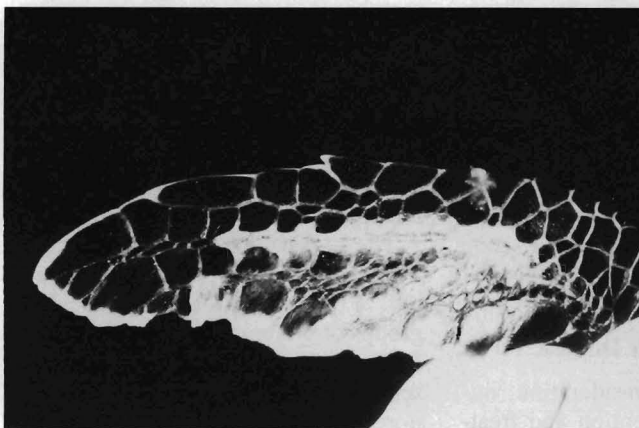
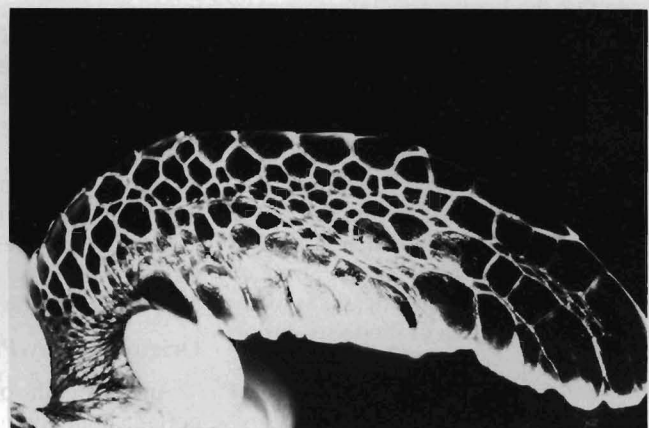


Figure 8.—Mock-infected scratch-inoculated control flipper. Note absence of lesion.



also contained large numbers of the herpes-type virus, as determined by electron microscopy.

Figure 7 is a flipper of an animal which has had the virus transmitted to the epidermis by scratch-inoculation along a line. This figure clearly shows the gray-patch lesion along the scratch-line. Figure 8 is a control flipper which was mock scratch-inoculated. There is no lesion along the scratch-line.

Development of Natural Gray-Patch Disease

There is an interesting feature to the artificial transmission of the turtle herpesvirus. It appears that the susceptibility of the inoculated turtle depends upon its age. This age dependency apparently parallels the critical times in the life of the turtle at which they naturally acquire gray-patch disease.

The first critical time in the life of the turtle is around 2-6 weeks of age. Turtles of this age do not appear to acquire the typical skin lesions of gray-patch disease; however, there is a consistent level of mortality in this age group, usually about 20 percent. If 2-6-week-old turtles are scratch-inoculated with gray-patch disease virus, or are injected with the virus, 100 percent of them die within a short period of time. These animals die so quickly they do not have a chance to develop the typical skin lesions of gray-patch disease. The only obvious clinical sign is impacted fecal material in the lower intestinal tract. Interestingly, this is the same clinical sign that appears in the animals that die naturally during the first 2-6 weeks of life. This observation appears to indicate that the herpesvirus infection may cause a severe disease and mortality in some of the young turtles. Possibly the animals initially acquire the herpesvirus at this stage in their life.

The second critical time in the life of the turtle is from 8 to 15 weeks of age, when the majority of turtles that have survived develop gray-patch lesions in one of its two clinical forms. Some of these turtles die, but not nearly as many as in the initial peak of mortality during the first 2-6 weeks of life. It is during this 8- to 15-week period that typical spreading gray-patch disease appears.

It is also during this period of time that we are able to obtain large amounts of skin scrapings containing the herpesvirus. This material is useful for preparation of vaccines, for transmission studies, and for laboratory work with the virus.

Because the virus is present on the surface of the turtle, it is very possible that it is transmitted throughout the farm by water with new virus being shed constantly from infected turtles.

It appears that the severity of gray-patch lesions and also the mortality rate depend upon stress factors to which the turtles are subjected. For example, if the animals develop gray-patch disease in the summer, a season in which the water temperature of the tanks is high (around 30°C), the lesions appear to be very severe, and there is also a somewhat higher mortality rate. The opposite seems to be true in the colder winter months.

Turtles in the 8- to 15-week age group are susceptible to artificial transmission of gray-patch disease virus by the scratch-inoculation technique. When inoculated with the virus by scratch-inoculation, they do not die, but develop typical gray-patch lesions along the line of scratch. Thus, there appears to be a distinct difference in susceptibility in young green turtles, depending upon their age. Very young, green sea turtles (2-6 weeks of age) appear to be highly susceptible, and die as a result of artificial inoculation, whereas somewhat older turtles (8-15 weeks of age) contract the disease in its epidermal form, and most of the time do not die as a result of the artificial inoculation. The reason for this difference in susceptibility is not known.

Turtles over 1 year of age do not have the natural lesions of gray-patch disease. Interestingly, we have not been able to artificially transmit gray-patch disease to this group of turtles by injection or by scratch-inoculation. This indicates that turtles which have gone through previous epizootics of this disease have become immune.

Origin of Gray-Patch Disease Virus

Another point of consideration, important both for prevention and treat-

ment of this herpesvirus disease, is the question of origin of the virus. Did it arrive with the original eggs from the beaches in the wild, or is it a virus that is maintained on the farm, and transmitted to each successive group of hatchlings?

To answer this, we have taken several groups of eggs, hatched them away from the contaminated environment of the farm, raised the hatchlings in different locations, and monitored them for naturally acquired gray-patch lesions. Different groups of turtles have been raised in Bimini, The Bahamas, in Arizona, and Virginia Key and Pigeon Key, Fla. Each group of turtles raised in these diverse locations, under different environmental conditions, has developed the lesions of gray-patch disease. In general, however, because these animals were not in a stressful environment, the lesions were of the less extensive, pustular form. This appears to indicate that the virus is indeed a natural virus of the green sea turtle, and possibly occurs in the wild. To prove this, however, it would be necessary to raise stocks of turtles from many more parts of the world and determine if they also have the virus.

Effect of Temperature on Gray-Patch Disease

It appears that the total stress load on the animals determines both the extent and outcome of gray-patch disease. As mentioned above gray-patch disease is more severe in summer months when the water temperature of the tanks is 4°-5°C higher than in the cooler, winter months. A series of experiments was carried out to determine the exact effect of water temperature upon the onset and severity of natural gray-patch disease in young turtles. We hatched a large number of turtles away from the farm and performed a heat induction experiment in an artificial tank environment.

The experiment was carried out in Tucson, Ariz., under carefully controlled, experimental conditions. Four groups of 8-week-old green sea turtles, randomly selected, were subjected to a series of temperature changes and monitored for subsequent gray-patch dis-

ease development (Haines and Kleese, 1977).

One group of 52 animals was held in water at a constant temperature of $25^{\circ}\pm 0.5^{\circ}\text{C}$. These were the control animals. A second group of animals was subjected to a water temperature increase from 25° to 30°C at a rate of 1° per day. They were then left for 3 days at 30°C and subjected to a decrease from 30° to 25°C at the same rate of 1° per day. A third group was subjected to the same gradual temperature increase of 1° per day from 25° to 30°C but maintained at 30°C for the duration of the experiment. A fourth group was taken immediately from water at 25°C and placed in water at 30°C , left for 4 days at 30°C , and then returned directly into water at 25°C . These animals were, in essence, temperature "shocked." Each group was observed for the development of gray-patch lesions over a period of 48 days.

The animals subjected to temperature shock treatment and those which were subjected to a gradual increase to 30°C , and maintained at 30°C , had an earlier onset of disease, with more severe lesions, than either the control group or the group of animals which were subjected to a gradual increase to 30°C and then brought back down to 25°C . This is good evidence that heat is one of the stress factors which induce gray-patch disease and that higher water temperatures tend to increase the severity of the lesions. It must be pointed out that this experiment was carried out under ideal conditions for the turtle, in terms of stress. There were no other stress factors that were applied to the animals other than water tempera-

ture changes. Under actual aquaculture conditions on the turtle farm, a variety of other stress factors may have a cumulative effect upon the severity of the disease and upon the mortality rate. It appears, however, that water temperature may be the major stress factor that triggers gray-patch disease.

Vaccination Attempts With Gray-Patch Disease Virus

We have also produced an inactivated vaccine from gray-patch disease virus (Haines et al. unpubl. manuscript.²). Scrapings from animals with active disease were semipurified and the virus was injected into young, susceptible animals which were subsequently challenged by the scratch-inoculation technique. Intramuscular injections of dead gray-patch virus protects against challenge with active gray-patch material in inoculated animals. These preliminary trials have shown that the inactivated virus vaccine is feasible for farm-wide use.

Attempts to Isolate Gray-Patch Disease Virus

We have carried out many attempts to isolate the turtle herpesvirus in cell culture. Primary green turtle kidney cells, primary green turtle lung cells, and primary green turtle skin cells have been used as potential susceptible cell types. We have also used a large number of other mammalian, reptilian,

and fish cell lines. The only cells in which we have been able to achieve a constant infection showing consistent cytopathic effect are green turtle skin cells which have been passed in the laboratory numerous times (Koment and Haines, in prep.). Very recently, my associate, Roger Koment, has succeeded in taking material from these turtle skin cultures, and successfully infected young turtles by the scratch-inoculation technique. The inoculation produced new gray-patch lesions in the animals. So, it appears that the virus does, in fact, replicate in some cell cultures, although the replication is difficult to detect by normal methods.

SUMMARY

In summary, an epizootic herpesvirus disease of farmed green sea turtles has been described. This disease, which has been named gray-patch disease, affects young sea turtles, with the highest mortality and most severe lesions seen in animals that have been subjected to higher water temperatures. Controlled laboratory experimentation proved that high water temperature affects both the onset and severity of the lesions. An inactivated virus vaccine appears to protect against challenge with the herpesvirus, and it is possible that the disease may be controllable by vaccination of young turtles.

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