NEW DEVELOPMENTS IN BOTULISM

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Abstract: Toxin was readily produced using aquatic or terrestrial invertebrates as a nutrient source. Failure to demonstrate toxin production using mud or marsh debris and water as nutrient sources indicates that the toxin of Clostridium botulinum Type C is produced, contained, and protected within invertebrate bodies.

The etiology of botulism and the role of these invertebrates is discussed.

INTRODUCTION

Each year many thousands of waterfowl die of botulism. An actual count of over 100,000 waterfowl died this year in California. Over twice that number would have died if we had not utilized the knowledge from our past botulism research. We know that botulism is an intoxication caused by the ingestion of toxin produced by the bacterium Clostridium botulinum Type C (Giltner and Couch, 1930). We know that this bacteria and its toxin can be isolated from many different sources of animal protein (Quortrup and Sudheimer, 1942). In our studies, we have demonstrated toxin in maggots from duck, fish, pheasant and mammal carcasses. Maggots that were migrating to land from free-floating carcasses were also toxic. Toxin has also been found in blowfly eggs from a duck carcass, adult blowflies and blowfly pupae. From our observations, we believe nearly all of the invertebrates in a marsh can be toxic if the botulism bacteria or spores are present in their digestive tract and the temperature and nutrient requirements are favorable to germinate the spores and support metabolism.

On the basis of our data and the data obtained by other researchers cited herein, we present the following concepts of the initiation, perpetuation and duration and eventual subsidence of a waterfowl botulism outbreak.

Initiation of an Outbreak

An outbreak can start as the result of one or more of the following conditions: (1) following flooding of dry land during warm weather; (2) following the withdrawal of water so that mud flats are formed during warm weather; (3) as the result of decaying animal carcasses that produce maggots; (4) when the weather conditions become unfit, and the environment is unable to support the aquatic invertebrates inhabiting it, causing their demise; (5) unknown factors during the winter--such factors have been investigated but no explanation can be given unless the outbreaks actually started during warm weather as the result of one of the above reasons.

When dry land is flooded, the terrestrial invertebrates, such as crickets, spiders, nematodes, and larvae are drowned. Many of these invertebrates contain botulism bacteria or spores that multiply as the invertebrates' carcasses decay. Because the toxin is contained within the invertebrate carcass, it is protected from the outside environment and cannot be denatured by adverse chemical and physical agents. Production of toxin by botulism bacteria under laboratory incubation conditions requires 3-5 days. Waterfowl deaths in a freshly flooded pond also begin to occur 3-5 days after flooding, indicating that the bacteria is utilizing a medium of good quality which is readily available at the time of flooding. It has been suggested that decaying vegetation would be a source of toxin production. However, we have tried producing toxin from barley straw collected from an area when botulism was actively in progress, but all tests, even when toxic cells were inoculated into the medium were negative for toxin production from invertebrates when incubated under the same conditions in the laboratory. Therefore, in the near vegetationless preirrigation land as found in the Tulare Lake bed, a source of toxin would most logically be the drowned terrestrial invertebrates or aquatic invertebrates brought in with the water and dying as a result of an abrupt transfer to hostile conditions. Waterfowl feeding on these discrete packages of toxin will begin to die. When the water level is reduced, feather edges and potholes are formed that are ecologically unfavorable to aquatic invertebrates causing their deaths. The same sequence of events happens to these aquatic invertebrates as happens to the drowned terrestrial invertebrates, that is, they become small packets of toxin. Frequently, outbreaks start as the result of decomposing carcasses that produce fly maggots. Botulism resulting from maggot ingestion may start regardless of the water condition.

We have investigated several outbreaks in which the water was either cool and flowing swiftly or contained in a deep, steep-banked pond. The original carcasses were not the result of botulism losses, but rather some completely unrelated factor, i.e. dead birds, or fish or mammals left to decompose on the shore. The effect of vegetation on botulism is indirect. The aquatic or decaying terrestrial vegetation is the primary producer of the herbivorous invertebrates which then serve as nutriment for C. botulinum bacteria.

The toxic invertebrates are ingested by the waterfowl and the toxin is absorbed through the gut wall where it travels to the nerve-muscle synapses. Progressive flaccid paralysis is apparent as more and more nerve endings are affected. The bird becomes flightless in the early stages of intoxication, and is unable to leave the toxic area. The leg muscles are next in the progression of paralysis. As more toxin is absorbed, the bird has difficulty holding up its head, hence the term "limberneck" as this disease has been called. If in open water, death is usually due to drowning; if the bird can reach shore its death is usually due to cardiac or respiratory failure.

Perpetuation and Duration

A carcass during warm weather will become fly-blown by Phaenicia, Phormia, or Sarcophaga species. Maggots develop rapidly and within three days reach their prepupal stage if the temperature is approximately 95°F. The maggots then begin migrating to find a spot to pupate. These migrating maggots are tempting morsels to waterfowl as they wiggle along the surface of the water. Only 6-10 of these toxic maggots are needed to cause the death of a bird. These maggots become extremely lethal as they absorb toxin from the tissues of the decaying carcass. Each carcass can produce enough maggots to theoretically kill 200 birds if each bird ingested 10 maggots from a toxic carcass. Then each of the 200 carcasses would produce maggots and if all of these maggots were eaten, 40,000 birds could die as the result of one bird dying. This situation does not proceed exactly as theoretically presented since many of the moribund birds seek shelter on the shoreline and the maggots are not as readily available.

Observations made of outbreaks occurring in the Tulare Basin and Sacramento Valley during the summer, fall and winter of 1969 indicate the importance of maggots. The Tulare Basin had far more flooded area and thus more potential to produce high botulism mortalities than the Sacramento Valley did. When outbreaks were detected in the Tulare Lake Basin carcass pick up was immediately instigated before maggots could be produced in great numbers.

In the Sacramento Valley two separate outbreaks escaped detection until thousands of ducks had died and produced maggots. Before the outbreaks were over, 65,000 or more ducks died in the two outbreaks in the Sacramento Valley. The outbreaks in the Tulare Basin only killed 40,000 birds even though there were many individual outbreaks. The difference in mortality was largely due to the availability of large numbers of toxic maggots in the Sacramento Valley because dead waterfowl were not promptly removed. The hot spots with heavier losses in the San Joaquin were also primarily due to maggot ingestion.

Numerous observations were made of freshly dead or sick ducks in small areas that had one or more carcasses heavily laden with maggots. One specific observation was of five fresh dead pintail ducklings in the immediate vicinity of an adult female pintail carcass covered with maggots. Examination of the five ducklings showed that each digestive tract contained one or more maggots.

It was obvious that the more severe losses occurred in areas where deaths were not detected until many carcasses had developed maggots. Losses could be stopped or significantly reduced if a complete carcass pick up was carried out before large numbers of maggots were produced. When losses were undetected for a time long enough to produce numerous decaying carcasses, the die-off would not cease immediately even after clean up operations becasuse of the large number of maggots already produced.

Such observations were most obvious when cold weather set in before the maggots developed into adult flies. The cold weather retarded or stopped the developmental stages, and larvae so arrested or protected from decay by the cold were ideal storage capsules for the toxin.

Jensen and Allen (1966) made some very interesting and enlightening observations about the persistence of toxin in adult blowfly (Phaeniacia sericata) carcasses. They used dead flies that had fed on a toxic duck carcass and divided them into two groups. Both groups were stored in open containers, one kept at 37 C. and the other at 8 C. These fly carcasses contained approximately as much toxin after 20 months as they did when first tested at one year.

Toxin stored within invertebrate carcasses would logically have a higher breakdown rate during warm weather in the marsh water, but might last for weeks or more during cold winter weather.

Subsidence of an Outbreak

We have observed outbreaks during the summer that have stopped rather abruptly. We suggest that the reasons are: 1) That during warm weather maggots developed rapidly into adults; and 2) that invertebrate populations may suddenly decline and carcasses rapidly decompose. Therefore, toxin is not continuously available due to lag on reproduction of new invertebrates.

Toxin production may be terminated by cold weather, but preformed toxin may persist and still be available through the winter, protected within invertebrate carcasses.

Outbreaks also end as the result of one or more of the following factors: 1) Toxin sources decay or develop so that they are no longer available as food sources; 2) Toxin sources are no longer available because of changed habitat or water levels; and 3) Waterfowl change feeding habits as different foods become available and because of migration.

Conclusion

During the 1967, 1968, 1969 outbreaks in California it became apparent that the

theories of water, mud and marsh debris as the primary toxin sources could not be reconciled with our laboratory investigations and observations.

Bell et al. (1955) theorized that animal protein and not vegetative material was the primary source of toxin. We now believe this is so and that terrestrial and aquatic invertebrates, flies, and maggots are largely, if not solely, responsible for serving as vehicles for the ingestion of toxin by aquatic birds and that it is in these channels that present and future research must be directed.

LITERATURE CITED

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