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SEROLOGY AND VIROLOGY OF THE BOWHEAD WHALE (*BALAENA MYSTICETUS* L.)

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ABSTRACT: Sera from four bowhead whales (*Balaena mysticetus* L.) were examined for the presence of specific antibodies, and tissue and swab samples from six and four animals respectively were processed for isolation of viruses and for initiation of bowhead whale cell cultures. All sera were negative for antibodies to nine serovars of *Leptospira interrogans* and to 21 orthomyxovirus subtypes and a paramyxovirus (Newcastle disease virus). All sera were positive, however, for neutralizing antibodies to one or more calicivirus serotypes. Two untyped adenoviruses were isolated from colon samples of two different whales, but neutralizing antibodies to the agents could not be demonstrated in any sera. Three primary bowhead whale cell cultures were derived from kidney (two cultures) and testis (one culture), from three individual whales.

INTRODUCTION

For more than 2,000 yr, coastal Alaskan Eskimos have hunted the bowhead whale (Marquette and Bockstoce, 1980). Known since early times as the "ice whale" (Scammon, 1874), bowheads most commonly inhabit the shifting margins of the arctic pack ice, the western arctic stock migrating each spring through the Bering Strait to feeding grounds in the Beaufort Sea (Lowry and Burns, 1980; Tillman, 1980). Until the middle of the last century, the western arctic bowhead population (variously estimated between 20,000 to 40,000 and 14,000 to 20,000 individuals) co-existed in apparent ecological stability with Eskimo hunters, who harvested relatively small numbers of animals during the seasonal, nearshore migration (Evans and Cuccarese, 1980; Marquette and Bockstoce, 1980; Int. Whaling Comm., 1985). With the advent of arctic commercial whaling in the 1840's, the bowhead whale was hunted to near-extinction. By 1915, commercial harvesting of bowheads had come to an end because of their declining numbers and the reduced world demand for baleen ("whalebone") (Marquette and Bockstoce, 1980; Tillman, 1980). Eskimo subsistence harvesting, however, has continued.

Recent concern over the size of the western arctic bowhead population has prompted intensive scientific investigation. After more than half a century of protection from commercial exploitation, this stock may, based on recent upward adjustments of current population estimates, be undergoing some reproductive response. In 1984 it was estimated that only about 3,900 of these whales remain in existence (Dronenburg et al., 1984) whereas other censusing techniques have resulted in an upward estimation of this number to 4,417 animals (Breiwick et al., 1984; Int. Whaling Comm., 1985). The only documented causes of bowhead mortality to date have been the Eskimo subsistence harvest, and rare accounts of whales trapped in the ice (Braham et al., 1979; Evans and Cuccarese, 1980). These

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latter occurrences conceivably can be precipitated by a number of abnormalities, i.e., an altered reaction to behavioral cues, disorientation, or depletion of energy reserves. Certainly, many infectious disease agents are known to produce a similar range of effects in other more well known species including man, and thus would be suspect of contributing both to natural mortality and to reduced reproductive efficiency.

This and several previous reports (Johnston and Shum, 1979, 1981; Smith, 1979; Migaki, 1981; Smith et al., 1981a) describe the first investigations of infectious disease agents of the bowhead whale. These studies were initiated as part of a research program conducted by the Naval Arctic Research Laboratory, Barrow, Alaska, for the Bureau of Land Management, United States Department of the Interior. The immediate objectives of the present study were: (1) to examine serum from harvested whales for evidence of exposure to selected marine and terrestrial mammalian pathogens, and (2) to attempt isolation in cell culture of viral agents possibly harbored by these animals.

MATERIALS AND METHODS

Serum, swab, and tissue samples were received from four individual bowhead whales, designated 80B1, 80B2, 80B7, and 80B8; tissue samples alone were received from two additional whales, 80B3 and 80B5. All six whales had been harvested by Eskimos at Barrow, Alaska, during the spring migration of 1980. The samples were of three general categories: (1) whole serum for specific antibody studies; (2) frozen swab and tissue samples for virus isolation attempts; and (3) fresh (chilled) tissues for preparation of bowhead whale cell cultures.

Antibody determinations

All sera were examined for antibodies to *Leptospira interrogans* serovars *autumnalis, ballum, bataviae, canicola, grippotyphosa, icterohaemorrhagiae, pomona, pyrogenes,* and *wolffi* using the macroscopic agglutination test (Alexander, 1980). Sera were tested for antibodies to 14 serotypes of marine calicivirus (Smith, 1981; Barlough et al., 1985) and to 12 of the 13 extant serotypes of vesicular exanthema of swine virus (VESV, also a calicivirus) (Bankowski, 1981) using a microtiter serum-neutralization (SN) technique (Monto and Bryan, 1974; Smith et al., 1976). Sera were screened also for antibodies to orthomyxoviruses (18 influenza A viruses, including human, avian, porcine, and equine subtypes, and three influenza B viruses) and to a paramyxovirus (Newcastle disease virus, LaSota strain), by hemagglutination-inhibition (kindly performed by Dr. B. C. Easterday, School of Veterinary Medicine, University of Wisconsin–Madison) and/or agar gel immunodiffusion (kindly performed by Dr. A. Dardiri, Plum Island Animal Disease Center, Greenport, New York) (Palmer et al., 1975; Schild, 1981).

Virus isolations

Attempts to isolate viruses by use of cell culture were designed to detect both cell-associated and non-cell-associated viruses. Cell lines/cultures utilized included African green monkey kidney (Vero), a broad-spectrum cell line especially sensitive to calicivirus infection (Smith et al., 1977b); Madin-Darby bovine kidney (MDBK), for orthomyxovirus isolation; and Skilling-Smith *Balaena* testis (SSBT), a cell culture derived from bowhead whale 80B3 (see below). It was thought that the SSBT might prove a more suitable cell type for the isolation of cetacean viruses.

Swab and selected tissue samples from whales 80B1, 80B2, 80B7, and 80B8 were placed in 1-dram vials with cell culture medium and frozen immediately. To isolate non-cell-associated virus, samples were thawed, ground with sterile sand, and clarified by low-speed centrifugation (2,500 rpm for 15 min). Supernatant fluids were passed through 0.45-nm (APD) Millipore filters and 0.2 ml of each sample was adsorbed (37 C for 60 min) to the respective cell culture types grown in roller tubes. Tube cultures were then rinsed, fed, and incubated at 37 C on a roller drum. Cultures were examined daily for cytopathic effect; at least three blind-passages were performed before samples were considered negative.

Additional tissue samples from all six whales were processed for attempted isolation of cell-associated virus and for initiation of bowhead whale cell cultures. These were received as chilled (but not frozen) 1 to 5 g samples of chopped tissue in cell culture medium. Samples subsequently were minced and rinsed several times, then each was seeded into two or more polystyrene culture flasks, or overlaid onto Vero

or MDBK cell monolayers in roller tubes. In the latter case, tubes were held in a stationary position for some time and not always placed onto a roller drum. Cultures were incubated at 37 C, examined daily for cytopathic effect, and passaged directly, as necessary, at least three times, without freeze-thawing.

Bowhead whale cell cultures

The tissues seeded on polystyrene (described above) were observed for cell replication at 37 C. Tissues that grew to confluency were trypsinized, washed, and transferred to fresh flasks. The 80B3 testis (SSBT) cells were then used for virus isolation attempts, as described above.

RESULTS AND DISCUSSION

Antibody determinations

All whale sera were negative for antibodies to *Leptospira interrogans* serovars, and to the orthomyxovirus and paramyxovirus types tested. However, virus-neutralizing activity specific for three of the 14 marine calicivirus serotypes and two of the 12 VESV serotypes was detected (Table 1). All three marine calicivirus serotypes neutralized by bowhead whale sera had been isolated originally from northern fur seals (*Callorhinus ursinus* Linné) in the Bering Sea (Smith et al., 1977c, 1981b). Two other Bering Sea isolates, one from fur seals and one from Pacific walrus (*Odobenus rosmarus divergens* Illiger) (Smith et al., 1974, 1983a), were not neutralized by the four whale sera.

Virus isolations

Colon samples from two whales (80B1 and 80B7) yielded virus isolates in Vero cells. These were identified by physicochemical and morphological (electron microscopic) criteria as adenoviruses. All four whale sera (including sera from whales 80B1 and 80B7 themselves) were negative in subsequent microtiter SN tests for specific neutralizing antibodies to these agents. In addition, neither isolate was neutralized by antisera to bovine adenovirus serotypes 1 through 8, nor by antisera to a San Diego Zoo adenovirus isolate from gazelles and other hoofed species (Skilling, unpubl. data). Whether the two bowhead whale adenovirus isolates are identical has not been determined.

None of the tissues tested was shown to contain cell-associated virus.

Bowhead whale cell cultures

Tissues received from three of the six whales proved to be viable, and primary cell cultures were cultivated successfully from whale 80B1 and whale 80B8 kidneys (epithelial/mixed-cell type) and from whale 80B3 testis (fibroblast/mixed-cell type—SSBT cells) and subsequently frozen in liquid nitrogen. In general, all cell cultures grew quite slowly, requiring approximately 45 days to reach confluency, when passaged at a split ratio of 1:2. As has been the case with all marine mammal cell cultures we have initiated, with one exception (Smith, 1979), the bowhead whale cells quickly lost vitality and ceased dividing after six to nine passages *in vitro*. Others have cultured bowhead whale cells sufficiently to karyotype them (Jarrell, 1979).

The absence of leptospiral antibodies in the four bowhead whale sera examined compares to a similar negative finding for 64 great whale sera tested previously (Smith and Skilling, unpubl. data, 1977). By contrast, northern fur seals in the Bering Sea are infected routinely with leptospires, with these infections occurring presumably at sea (Smith et al., 1977a). In addition, Steller (northern) sea lions (*Eumetopias jubatus* Schreber) are known to carry leptospiral antibodies (Smith and Skilling, unpubl. data, 1977), and this pathogen (serovar *pomona*) has been isolated from coastal harbor seals (*Phoca vitulina* Linné) in Alaska (Ritter, pers. comm.). The absence of antibodies in the four bowhead whale sera examined may not necessarily indicate that these cetaceans do not harbor leptospires, however, since certain mammalian species have

been shown to acquire life-long infections without developing detectable agglutinating antibodies (Babudieri, 1958; Roth et al., 1963).

The discovery of neutralizing antibodies to three marine caliciviruses—San Miguel sea lion virus (SMSV) serotypes 5, 8, and 10 (all previously isolated from northern fur seals in the Bering Sea)—provides additional evidence of a Bering Sea cycle for these agents (Smith et al., 1983a; Barlough et al., 1985). In 1973, an SMSV-5 epizootic swept through the northern fur seal herds on the Pribilof Islands, apparently producing an increase in pelagic mortality among pups (Smith et al., 1976). The same SMSV serotype also appears to have infected both marine and terrestrial mammals as far south as southern California (Smith et al., 1976), suggesting that geographic or other barriers to disease spread among these populations are ineffective. To explore possible modes of transmission and spread of these agents, a calicivirus isolated from northern fur seals in the Bering Sea was used to experimentally infect a Southern California species of fish (*Girella nigricans*) which is a known calicivirus reservoir. These infected fish were used to feed and infect northern fur seals, thus suggesting that northern fur seals could acquire marine calicivirus infections from fish reservoirs in southern California waters and subsequently carry the viruses northward to the Bering Sea (Smith et al., 1980a, b). Additional evidence suggesting that bowhead whales could become infected with marine caliciviruses has been provided by a report of in vitro propagation of SMSV-2 (a southern California pinniped isolate) in bowhead whale cell cultures (Smith, 1979).

The suggestion that specific disease agents infecting marine and terrestrial mammals of the southern California coastal zone may also infect an endangered species of cetacean confined to far northern waters is a new concept deserving further scientific investigation. Similarly, the discovery of antibodies specific for two VESV serotypes (J_{56} and K_{56}) is remarkable in that these two agents have been isolated on only a single occasion, from infected domestic swine in New Jersey in 1956 (Holbrook et al., 1959). There is ample serologic evidence that both of these VESV serotypes continue to circulate today among marine and terrestrial mammalian populations along the southern California coast, the improbability of cross-reactivity or non-specificity of these antibody responses has been argued extensively (Smith and Latham, 1978). As was

TABLE 1. Calicivirus antibodies in bowhead whales.[a]

Virus	Whale no. 80B1	80B2	80B7	80B8
Marine caliciviruses				
SMSV-1[b]	–[c]	–	–	–
SMSV-2	–	–	–	–
SMSV-4	–	–	–	–
SMSV-5	1:20	1:20	1:10	–
SMSV-6	–	–	–	–
SMSV-7	–	–	–	–
SMSV-8	–	1:10	–	1:20
SMSV-9	–	–	–	–
SMSV-10	1:40	–	1:40	–
SMSV-11	–	–	–	–
SMSV-12	–	–	–	–
Walrus calicivirus	–	–	–	–
VESV[d] serotypes				
VESV-1-34	–	–	–	–
VESV-A_{48}[e]	–	–	–	–
VESV-B_{51}	–	–	–	–
VESV-C_{52}	–	–	–	–
VESV-D_{53}	–	–	–	–
VESV-E_{54}	–	–	–	–
VESV-F_{55}	–	–	–	–
VESV-G_{55}	–	–	–	–
VESV-H_{54}	–	–	–	–
VESV-I_{55}	–	–	–	–
VESV-J_{56}	–	1:71	–	–
VESV-K_{56}	1:22	1:110	–	–

[a] Values given are for terminal antibody titers.
[b] SMSV = San Miguel sea lion virus.
[c] Negative result.
[d] VESV = vesicular exanthema of swine virus.
[e] Subscript = year of isolation (48 = 1948, etc.).

the case with SMSV-5 (and, presumably, with both SMSV-8 and SMSV-10 as well), these agents apparently have developed effective natural mechanisms for transport to northern waters. In species wherein their effects have been examined, caliciviruses produce vesiculation and ulceration of the skin, lips, mouth, and tongue; aborted or weakened newborn; agalactia; pneumonia; enteritis; myocarditis; and encephalitis (Bankowski, 1981; Smith, 1983; Barlough et al., 1985). Among cetaceans, caliciviruses have been associated with vesicular lesions in Atlantic bottlenosed dolphins (*Tursiops truncatus* Montagu) (Smith et al., 1983b), while antibodies to caliciviruses have been found both in dolphins and in a number of species of great whales (Smith, 1983), in addition to the bowheads of this report.

None of the bowhead sera tested was found to contain antibodies to influenza viruses. In the past, migratory waterfowl and pelagic birds of arctic and subarctic regions have been shown to harbor these agents (Slepuskin et al., 1972; Zakstel'skaja et al., 1972; Easterday, 1981). It is now well established that influenza viruses are evolving dynamically, and that certain avian subtypes can infect mammalian species (Schild, 1981), including, perhaps, Atlantic harbor seals (Webster et al., 1981) and whales (Lvov et al., 1978). It thus seemed possible to us that similar influenza cycles may have developed within the bowhead population, but we could find no evidence of this, using the material at hand.

We were successful, however, in isolating two adenoviruses from bowhead samples. Since 1965, there have been many well planned research efforts to isolate viruses from cetaceans; almost all have failed. The two viruses reported here represent only the third and fourth known viruses isolated from great whales. The first, tentatively classified as an enterovirus, was recovered in 1968 from the rectum of a California gray whale (*Eschrichtius robustus* Lilljeborg) (Watkins et al., 1969). The second, an adenovirus, was isolated from the rectum of a sei whale (*Balaenoptera borealis* Lesson), sampled in Antarctic waters in 1977 by Dr. M. Dailey (Smith and Skilling, 1979). There were no detectable neutralizing antibodies to our adenovirus isolates in the bowhead whales from which the viruses were recovered. Although this was somewhat unexpected, it is not altogether uncommon for virus excretion to occur in the absence of a detectable serologic response, especially in regard to mucosal surface-related agents. Further work will be required to confirm the antigenic identity of these new isolates, and to determine their pathogenic effects (if any) in the bowhead whale.

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