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Anaerobic oral flora in the North American Black bear (*Ursus americanus*) in eastern North Carolina

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ABSTRACT

Microbial flora can provide insight into the ecology and natural history of wildlife in addition to improving understanding of health risks. This study examines the anaerobic oral flora of hunter killed black bears (*Ursus americanus*) in eastern North Carolina. Oral swabs from the buccal and lingual supragingival tooth surfaces of the first and second mandibular and maxillary molars of 22 black bears were inoculated onto Brucella Blood Agar plates supplemented with hemin and vitamin K after transport from the field using reduced oxoid nutrient broth. Sixteen anaerobic bacterial species, representing nine genera were identified using the RapID ANA II Micromethod Kit system and a number of organisms grown that could not be identified with the system. The most frequently identified anaerobes were *Peptostreptococcus prevotii*, *Streptococcus constellatus*, and *Porphyromonas gingivalis*. The diversity in the anaerobic oral flora of black bear in eastern North Carolina suggests the importance of including these organisms in basic health risk assessment protocols and suggests a potential tool for assessment of bear/habitat interactions.

1. Introduction

The study of commensal and parasitic organisms to gain insight into the ecology, behavior and movements of wildlife is a rapidly growing field. The complexity and dynamics of the compositions of oral flora communities offers important potential to assist in these lines of inquiry. The large, omnivorous bears, with their opportunistic and seasonally and locationally diverse diets are good candidates for such studies, but the existing data, particularly on the anaerobic oral floral communities of wildlife are extremely sparse [1,2]. Research describing the bacterial oral flora of the Ursidae in the western hemisphere focuses on the risk of bacterial infections for humans bitten by bears in western, and northern North America [1–4]. These studies document the presence of mixed populations of aerobic gram negative and gram positive bacteria in the oral cavity of grizzly (*Ursus arctos horribilis*) and black bears (*Ursus americanus*), but provide no insight into their anaerobic flora [1,2]. Knowledge of normal oral flora in bears can help direct appropriate antimicrobial therapy in cases of bear–human interactions and the veterinary management of conspecific traumatic wounds that occur in bears maintained in captivity, but anaerobic infections are also clinically important and can result in poor wound healing and sepsis [5,6]. The lack of knowledge of the anaerobic oral flora of bears is also a detriment to efforts to gain insight into their ecology and behavior. These studies benefit from careful assessment of the complete complement of oral flora of species found in diverse ecosystems and habitats. To facilitate these studies, we characterized the anaerobic oral flora of free ranging hunter killed black bears in eastern North Carolina.

2. Methods and materials

2.1. Sample collection

Oral cultures were taken within 4 h of death from 22 American black bears killed in an annual hunt in Hyde and Tyrrell counties in northeastern North Carolina on November 8 and 9, 2010. Sample collection occurred as hunters presented the animals at check stations throughout the day. Information collected for each bear included hunter identification, and location of and estimated time of death of the animal as reported by the hunter. Inclusion criteria for our study were a sampling time within 4 h of reported time of death, and lack of gross contamination with soil and ground debris in the area of the mouth where the cultures would be taken. The

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weight and sex of the animal and the time the oral culture was collected were recorded. The weights of the 22 animals (15 males, 7 females) included in our cohort ranged from 74.54 kg to 288.63 kg. Only two of these bears were sampled less than 2 h after their death. Twelve bears in the cohort were processed between 2 and 3 h, and the remaining eight were samples between 3 and 4 h postmortem.

Oral cultures were obtained by inserting a sterile cotton tipped swab (Puritan Medical Products Company LLC. Guilford, Maine 04443-0149, USA) into each bear's mouth to contact the buccal and lingual supragingival tooth surfaces of the first and second mandibular and maxillary molars. The swab was then aseptically placed into a sterile cryovial (Globe Scientific Paramus, New Jersey 07652, USA) containing thawed sterile transport medium, and the swab stem broken at the level of the vial top, to allow the vial's top to be sealed. Each sample was placed into a large insulated cooler containing dry ice and transported to the North Carolina State Center of Marine Sciences and Technology within 24–48 h of collection. They were placed in a −80 °C freezer for 60–61 days until the cultures could be processed.

2.2. Transport media

Transport media was prepared using Oxoid Nutrient broth (Oxoid Ltd. 19 Mercers Row, Cambridge, CBS 8BZ, UK) containing 15% glycerol [7]. Aliquots of 1.5 ml of broth were pipetted into 5 ml nonsterilized cryovials (Cryogen™ Globe Scientific Inc. Paramus, New Jersey 07652, USA). Both the media and cryovials were sterilized by autoclaving at 250° C for 30 min (Hydroclave MC8® MDT Biologic Co. Rancho Dominguez, California 90220-6039, USA). The media was then reduced by placing the open vials in a sealed anaerobic environment containing dry ice [8]. After 24 h in the reducing atmosphere, the vials were sealed, frozen on dry ice, and maintained on dry ice during transport to the field. Individual vials of transport media were thawed at room temperature as samples were taken in the field, and then re-frozen on dry ice after the sampling swabs were inserted.

2.3. Culture media and culture methods

Samples collected in the field were stored in a −80 °C freezer for 60–61 days until they could be further processed. Samples were rapidly thawed in a 37 °C water bath, mixed on a vortex mixer for 30 s and examined for lack of grossly visible ice crystals before plating [7,8]. Three quadrants of a Brucella Blood Agar plate (BBA) supplemented with hemin and vitamin K (Anaerobe Systems Morgan Hill, California 95037-5451, USA) were streaked with each sample using sterile 1 μL flexible loop and the quadrant streak method. All plates were labeled and incubated at 37 °C in an anaerobic chamber (Mitsubishi Gas Chemical America Inc. Atlanta, Georgia 30326, USA) using CO_2 generating gas packs (Anaero Packs® Thermo Fisher Scientific Remel Products Lenexa, Kansas 66215, USA) and an anaerobic indicator (Anaero-Indicator® Thermo Fisher Scientific Remel Products Lenexa, Kansas 66215, USA). To reduce the potential loss of samples to rapid overgrowth by a single organism, cultures were checked every 4–6 h during the day for up to 15 days after the initial plating. From the BBA culture plate from each bear, up to six bacterial colonies exhibiting good growth were further isolated and subcultured on fresh BBA plates incubated under anaerobic conditions. Colony color and growth characteristics were recorded and Gram stain (Protocol® Biochemical Sciences, Inc. Swedesboro, New Jersey 08085, USA) applied to slides made from samples of each subsampled colony to determine bacterial morphology and that colonies were morphologically homogeneous.

2.4. Testing and identification

Anaerobic isolates were identified using the pattern of metabolic end products and reactions in the RapID ANA II Micromethod Kit system (Innovative Diagnostic System Inc. Norcross, Georgia 30071-1802, USA). Bacterial isolates were prepared and tested according to the manufacturer's instructions and allowed to incubate for 4 h [9,10]. The confidence values provided when data were entered into the RapID-ANA II Code Compendium were noted as being either >95% or not [11].

Bacterial species isolation data were examined by inspection for patterns of bacterial growth associated with the time interval between sample collection and time of death, bear weight and bear sex. When appropriate, descriptive statistics were calculated to assist assessment of variability. Based on the initial results, further statistical analysis was not warranted.

3. Results

Anaerobic organisms were grown from the swabs taken from all 22 bears sampled. Sixteen different anaerobic bacterial species were identified by the RapID ANA II system with 95% confidence, representing nine genera (Table 1). These species represented 59% (49 of 82) of the colonies analyzed. The system also identified 5 colonies with less than 95% confidence, but failed to identify the remaining 28 colonies (34%) tested.

The most frequently isolated organisms that could be identified were *Streptococcus constellatus* (n = 10 different bears) and *Peptostreptococcus prevotii* (n = 10 different bears), followed by *Porphyromonas gingivalis* (n = 9 different bears), and *Capnocytophaga* sp. (n = 4 different bears). The five colonies reported at less than 95% confidence by the Rapid ANA II system included two isolates reported as *S. constellatus,* two of *P. prevotii*, and one isolate reported as *Peptostreptococcus tetradius.* Fourteen of the 82 isolates were taken from colonies noticed to arise after longer incubation of the original plates. These organisms included 4 isolates that could not be identified with the RapID ANA II system, 8 of the 9 *P. gingivalis* isolates obtained, and the only isolates of *Porphyromonas endodontalis* and *Bacteroides stercoris* cultured. All of these slower growing organisms were identified with greater than 95% confidence. *P. endodontalis*, and *B. stercoris* were each isolated only from one of the two bears sampled within 1 h of death and not from any

Table 1
Bacterial species identified with ≥95% confidence by the RapID ANA II Micromethod Kit system and the total number of bears positive for each organism.

Bacterial species identified	Number of Black bears (*Ursus americanus*) positive for the organism
Gram positive cocci	
Gemella morbillorum	2
Peptostreptococcus anaerobius	1
Peptostreptococcus assaccharolyticus	1
Peptostreptococcus micros	3
Peptostreptococcus prevotii	10
Peptostreptococcus tetradius	1
Staphlyococcus saccharolyticus	1
Streptococcus constellatus	10
Streptococcus intermedius	2
Gram negative cocci	
Veillonella sp.	1
Gram negative rods	
Bacteroides distasonis	1
Bacteroides stercoris	1
Capnocytophaga sp.	4
Fusobacterium mortiferum	1
Porphyromonas endodontalis	1
Porphyromonas gingivalis	9