ISOLATION OF Pasteurella multocida FROM WILD RACCOONS AND FOXES: PRELIMINARY REPORT 1

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ISOLATION OF *Pasteurella multocida* FROM WILD RACCOONS AND FOXES: PRELIMINARY REPORT[1]

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Abstract: Pasteurella multocida was isolated from the tonsillar fossa of five of twelve wild raccoons *(Procyon lotor)* and one of two red foxes *(Vulpes vulpes)* which were collected with humane wire-cage traps near turkey farms. In one raccoon, the organism was recovered from the mouth in addition to the tonsillar fossa. The fermentation pattern of these isolates was the same as that for 84% of the 214 isolates of *P. multocida* recovered from turkeys in Missouri veterinary medical diagnostic laboratories during the last 5 years. Although these isolates were pathogenic for turkeys, the organism was not transmitted from raccoons to turkeys.

INTRODUCTION

Pasteurella multocida can survive as a saprophyte in the respiratory system of many wild and domestic animals[9,11,12,18]. Its existence in a carrier animal has caused infections in man through animal bites and scratches[4,7,8,9,10].

In reviewing our diagnostic laboratory case reports of turkey cholera, it was noted that wild raccoons *(Procyon lotor)* and red foxes *(Vulpes vulpes)* are frequent predators of turkeys on range. Since predator attacks often precede outbreaks of fowl cholera in turkeys by 7-10 days, there exists the possibility that these predators may be carriers or a reservoir for *P. multocida*.

The purpose of this study was first, to determine if wild raccoons and foxes could carry *P. multocida* and second, to determine the site for isolating this organism from live predators.

MATERIALS AND METHODS

Isolation of P. multocida: All raccoons and foxes were captured with humane wire-cage traps on or near turkey farms and brought to the Veterinary Diagnostic Laboratory, University of Missouri for study and evaluation. The raccoons were physically restrained for collecting swab samples, whereas the foxes were tranquilized due to their size and viciousness.

The buccal cavity including the teeth, the pharynx, and the tonsillar fossae were swabbed and the swabs streaked on dextrose-starch agar (DSA)*. After incubation for 18 hours at 37 C, the growth was examined for *P. multocida* using a stereomicroscope with an oblique source of light[5]. The mouths of most animals were swabbed at least three times.

Biochemic Tests of P. multocida: Suspected isolates of *P. multocida* were tested for their fermentative reactions and gas production in eight carbohydrates and for motility, hydrogen sulfide production and indole production in SIM medium*.

Fermentation reactions were determined in dextrose, lactose, sucrose, maltose, arabinose, dulcitol, xylose and

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mannitol. Purple broth base and phenol red broth base* containing 1.0% of one of the carbohydrates were used for determining acid production. Durham gas tubes were placed in the tubes containing dextrose, lactose, sucrose and maltose to test for gas production. The tubes were examined daily for 6 days.

Pathogenicity of P. multocida in Turkeys: Six groups of three clinically healthy turkeys, 21 weeks of age, were inoculated with the six confirmed isolates of *P. multocida* to check for pathogenicity. The isolates were grown in tryptose phosphate broth for 24 hours at 37 C. One ml containing approximately 500 organisms was injected into the breast muscle of each turkey. The colony-forming units per dose were determined by plate count on DSA. One group of six clinically healthy turkeys were used as controls. These turkeys received identical treatment as the test birds except they were not inoculated. All turkeys were observed for 21 days postinoculation and all survivors necropsied.

RESULTS

Pasteurella multocida was isolated from five of twelve raccoons and one of two red foxes (Table 1). All of the isolates were obtained from the tonsillar fossa (Fig. 1). In addition, *P. multocida* was isolated from the naso-pharynx, tongue, teeth, and buccal mucosa in one adult male raccoon.

Each of the six isolates fermented dextrose, sucrose, xylose, and mannitol with no gas production. They produced indole, were nonmotile and did not produce hydrogen sulfide in SIM medium (Table 2).

Two of the isolated colonies from the raccoons were iridescent and three were intermediate. The colony isolated from the red fox was iridescent.

Classification of the raccoon isolates was made using Dorsey's[11] system of classification which is based on the fermentation of xylose, arabinose and dulcitol. All of the isolates in this study were classified in Dorsey's group II which fermented xylose and were negative with dulcitol and arabinose.

Eighteen of the 21 exposed turkeys became dull, depressed and rough appearing in 3 days postinoculation (PI). Food and water consumption was also decreased. One turkey became lame in 5 days PI with death occurring at 9 days PI. Ten days PI, the turkeys inoculated with *P. multocida* became more alert with feed and water consumption being increased. From this time until necropsy at 21 days PI, these turkeys appeared the same as the uninoculated controls.

There was a necrotic area at the site of injection and a caseous exudate in the joints of the turkey dying 9 days PI. Cultures of the liver, joints, and injection site of this turkey were positive for *P. multocida.*

All surviving turkeys necropsied at 21 days PI had a large area of necrosis at the injection site with no other gross lesions observed .Cultures of the liver and injection site of each bird were negative for *P. multocida.*

Efforts to transmit *Pasteurella* directly from the raccoon to the turkey in common housing were unsuccessful. The raccoons did not attempt to attack the turkeys. One raccoon died from a secondary infection of an old injury unrelated to the present study. One escaped through a screen covering an open window, after 3 days confinement with eight turkey poults.

TABLE 1. Incidence of **Pasteurella multocida** in tonsillar crypts of wild raccoons and foxes.

Animal Species	No. Positive/No. Tested
Raccoon *(Procyon lotor)*	5/12
Red Fox *(Vulpes vulpes)*	1/2

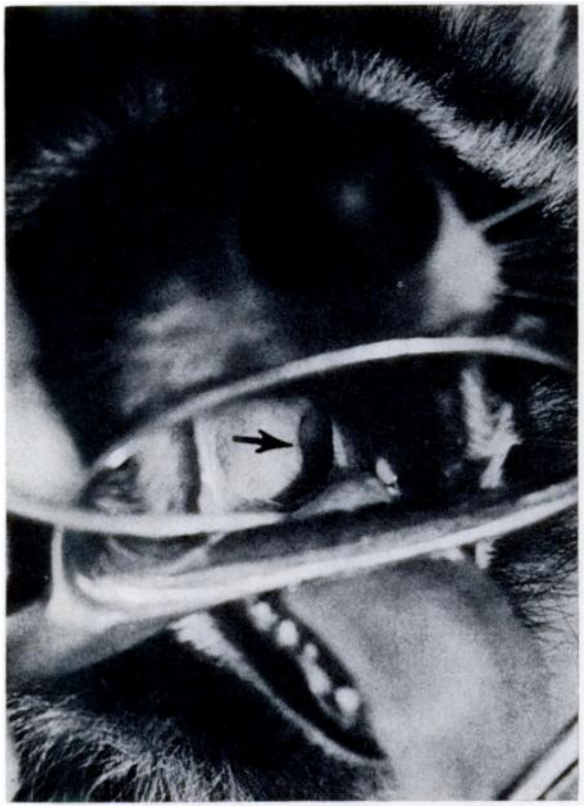

Figure 1. Technique used to swab Tonsillar fossa of raccoon.

DISCUSSION

The fermentation patterns of the raccoon and fox isolates were comparable with the results of a study by Donahue[5] who found that 83.6% of 214 turkey isolates from Missouri could be placed in Dorsey's group II. From results of this study, it is suggested that the *P. multocida* isolated from the raccoons and fox were the same as that causing fowl cholera in turkeys.

Since only one death occurred and all groups of turkeys were clinically ill for a period of 7 days after inoculation, the isolates used in this study were probably of low virulence and similar to the isolates from naturally diseased turkey flocks in Missouri.

It was found that *P. multocida* could be isolated consistently from the tonsillar fossae with little contamination. Swabs taken of other areas of the oral cavity were frequently contaminated with a *Proteus* swarmer that made impossible the identification of any *P. multocida* present.

Bergerud[1] has reported the transmission of *P. multocida* by predation of lynx on caribou calves. Therefore, the theory that the transmission of *P. multocida* in turkeys on range may be caused by the predation of wild carnivores was not rejected, even though attempts to demonstrate the phenomenon in this study were unsuccessful.

TABLE 2. Bacteriological characteristics of isolates of **Pasteurella multocida** from raccoons and foxes.

Culture No.	2876	2940	4001	4015	4179	4384
Source Date	Raccoon 6-2-71	Raccoon 6-15-71	Raccoon 6-24-71	Raccoon 6-29-71	Raccoon 8-2-71	Red Fox 9-7-71
Dextrose	A*	A	A	A	A	A
Lactose	—	—	—	—	—	—
Sucrose	A	A	A	A	A	A
Maltose	—	—	—	—	—	—
Arabinose	—	—	—	—	—	—
Dulcitol	—	—	—	—	—	—
Xylose	A	A	A	A	A	A
Mannitol	A	A	A	A	A	A
Motility	—	—	—	—	—	—
Gram Stain	—	—	—	—	—	—
H_2S-SIM	—	—	—	—	—	—
Indol-SIM	+	+	+	+	+	+
Type: Colony						
Iridescent	+	+				+
Intermediate			+	+	+	
Blue						

A* = acid produced; — = negative observation; + = positive observation.

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