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Authors: Howerth, E. W., Reeves, A. J., McElveen, M. R., and Austin, F. W.

Source: Journal of Wildlife Diseases, 30(3) : 450-453

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-30.3.450

Journal of Wildlife Diseases, 30(3), 1994, pp. 450–453

Survey for Selected Diseases in Nutria (*Myocastor coypus*) from Louisiana

E. W. Howerth,[1,2] A. J. Reeves,[1,3] M. R. McElveen,[4] and F. W. Austin,[1,5] [1] Louisiana Veterinary Medical Diagnostic Laboratory, School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana 70803, USA; [2] Present address: Department of Pathology, College of Veterinary Medicine, The University of Georgia, Athens, Georgia 30602, USA; [3] Present address: Louisiana Reference Laboratories, 6746 Goya Ave., Baton Rouge, Louisiana 70806, USA; [4] 8778 Goodwood Boulevard, Baton Rouge, Louisiana 70806, USA; [5] Present address: Diagnostic Laboratory Services, College of Veterinary Medicine, Mississippi State University, Mississippi State, Mississippi 39762, USA

ABSTRACT: Thirty-two trapper-caught nutria (*Myocastor coypus*) from East Baton Rouge, Iberville, Tangipahoa, and St. Helena Parishes in Louisiana (USA) were sampled for several disease agents. Antibodies against *Toxoplasma gondii*, *Chlamydia psittaci*, *Francisella tularensis*, *Leptospira* spp., and encephalomyocarditis virus were detected in 7%, 14%, 0%, 7%, and 0% of nutria, respectively. Both animals seropositive for leptospirae were positive for *L. interrogans* serovar *canicola*. No *Salmonella* spp. were isolated from feces, and no *Giardia* spp. were seen in trichrome-stained fecal preparations.

Key words: Nutria, *Myocastor coypus*, serology, *Toxoplasma gondii*, *Chlamydia psittaci*, *Francisella tularensis*, *Leptospira*, encephalomyocarditis virus, *Salmonella*, *Giardia*.

The nutria (*Myocastor coypus*) is a large semiaquatic rodent indigenous to South America. In 1937, naturalist E. A. McIlhenny imported nutria to be held in captivity at Avery Island, Louisiana (USA); during subsequent years numerous escapes occurred, resulting in establishment of a feral Gulf Coast population (Kinler et al., 1987). Several states and federal agencies subsequently moved nutria to Alabama, Arkansas, Georgia, Kentucky, Maryland, Mississippi, Oklahoma, and Texas (USA). Currently, nutria are reported in 15 U.S. states; free-ranging populations also are present in Europe, the Soviet Union, the Middle East, Africa, and Japan, largely as a result of escapes and releases from fur farming operations (Kinler et al., 1987).

Nutria are an important furbearing species in Louisiana but, despite the economic importance of nutria in Louisiana, relatively little is known about their diseases. Our objective was to determine the antibody prevalence for five disease agents, and to determine the prevalence of *Salmonella* spp. and *Giardia* spp., in the Louisiana nutria population. We were particularly interested in disease agents associated with other rodent species, especially other aquatic rodents, or those of public health concern.

Between 23 December 1988 and 24 March 1989, 32 trapper-caught nutria were sampled. Animals were killed in the trap and blood immediately was collected via cardiac puncture using a 16 gauge needle. Condition of the carcasses was noted and their sex determined. Animals were separated into adult and juvenile age classes based on genital development (Kinler et al., 1987). A sterile culturette was used to obtain a sample of feces from the rectum for bacterial culture. Approximately 5 g of feces were preserved in vials of polyvinyl alcohol fixative (Trend FeKal, Trend Scientific Inc., St. Paul, Minnesota, USA) for subsequent trichrome staining for *Giardia*. The vials were shaken vigorously and stored at 24 C until evaluation. A transverse section of kidney was fixed in 10% neutral buffered formalin. Nine of 32 skinned carcasses were transported to the laboratory for gross pathological examination.

Blood was kept at approximately 4 C and transported to the laboratory where serum was separated from the clot. All serum samples were kept frozen at −20 C until tested for antibodies to *Toxoplasma gondii*; *Chlamydia psittaci*; *Francisella tularensis*; *Leptospira interrogans* serovars *bataviae*, *canicola*, *grippotyphosa*,

hardjo, *icterohaemorrhagiae*, and *pomona*; and encephalomyocarditis virus (EMCV). Antibodies to *T. gondii* were detected by microtiter indirect latex agglutination (Toxotest-Mt "Eiken," Tanabe, USA, Inc., San Diego, California, USA); titers ≥1:32 were considered positive. A microtiter direct complement fixation test was used to detect antibodies to *C. psittaci* (Page and Bankowski, 1960) with titers ≥1:10 considered positive. Antibodies to *F. tularensis* were detected by microagglutination (Brown et al., 1980); titers ≥1:128 were considered positive. Microscopic agglutination (Cole et al., 1973) was used to detect antibodies to Leptospira. *Leptospira* serovars *bataviae* and *canicola* were screened at a serum dilution of 1:50 while the other serovars were screened at a serum dilution of 1:100. For determination of serum neutralizing antibody titers to EMCV, two-fold serial dilutions of heat-inactivated serum diluted 1:16 were prepared in 96-well plates. To each well an equal volume of media containing 500 tissue culture infective doses ($TCID_{50}$) of EMCV was added; wells then were inoculated with baby hamster kidney (BHK_{21}) cells (American Type Culture Collection, Rockville, Maryland, USA). Plates were examined daily for cytopathic effect. The reciprocal of the greatest dilution that inhibited 100% of EMCV cytopathology was expressed as the antibody titer. Antibody titers ≥1:32 were considered positive.

Fresh fecal samples were kept at 4 C until inoculated into selenite cysteine broth (Difco, Detroit, Michigan, USA) for *Salmonella* spp. enrichment. After 24 to 48 hr incubation, selenite cysteine enrichment cultures were transferred to Hektoen and MacConkey agars (Difco). Inoculated plates were incubated at 37 C and checked daily for growth. Nonlactose fermenting and H_2S producing isolates were inoculated into the following media: triple sugar iron; urea; lysine iron agar; sulfur indole motility; citrate; and phenylalanine deaminase (all from Difco). Isolates biochemically identified as *Salmonella* spp. were confirmed by rapid slide agglutination using *Salmonella* poly A to I and Vi O antiserum (Difco) in a rapid slide agglutination. Polyvinyl alcohol-fixed feces were centrifuged at 800 × *g* for 5 min. The fixative was decanted off, and the fecal sediment was streaked on a microscope slide, allowed to air dry, and stained with Trend Fekal Trichrome Staining Set (Trend Scientific, St. Paul, Minnesota). Trichrome-stained slides were examined for 15 min at 500× magnification for the presence of *Giardia* spp. cysts and trophozoites. Formalin-fixed kidneys were embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin. Kidneys with microscopic inflammatory lesions also were stained by the Warthin-Starry method for spirochetes (Luna, 1968).

Fifteen animals were trapped near free-flowing water. These included five adult males, three adult females, one adult of unrecorded sex, and two juvenile females from East Baton Rouge Parish trapped in and around Baton Rouge (30°25′N, 91°10′W) and one adult male and three adult females from Iberville Parish near Spanish Lake (30°18′N, 91°02′W). All were in good condition, and four of eight females were pregnant. Seventeen animals were trapped around oxidation ponds near Hillsdale (30°44′N, 90°37′W) in either St. Helena or Tangipahoa Parishes. Four were adult males, seven were adult females, and six were juvenile males. All were in good condition, and five were pregnant.

Both nutria testing positive for antibodies against *T. gondii* (Table 1) were adult females. One was trapped near an oxidation pond and the other near free-flowing water in Iberville Parish. Of three animals testing positive for antibodies to *C. psittaci*, one was an adult male trapped in East Baton Rouge Parish and the other two were adults (one male, one female) trapped around oxidation ponds. Both animals seropositive for *Leptospira* spp. had antibodies to serovar *canicola* and were juvenile females from East Baton Rouge Parish. Six adult nutria of the 32 animals

TABLE 1. Prevalence and reciprocal titers for antibodies to selected disease agents among nutria from Louisiana, 1988 to 1989.

Agent	Number of nutria		Percent positive	Reciprocal titer	
	Tested	Positive		Minimum	Maximum
Toxoplasma gondii	28	2	7 (1–25)*	32	32
Chlamydia psittaci	22	3	14 (4–34)	10	10
Francisella tularensis	28	0	0 (0–15)		
Leptospira serogroups	28	2	7 (1–25)	160	160
Encephalomyocarditis virus	25	0	0 (0–17)		

* Percentage positive (95% confidence interval). Confidence intervals determined by the method of Fleiss (1981).

evaluated had lymphocytic/plasmocytic interstitial nephritis compatible with leptospirosis, but no spirochetes were seen in sections stained by the Warthin-Starry technique and none of these six animals had antibodies against *Leptospira* spp. Therefore, this inflammation probably was not related to leptospirosis.

No *Salmonella* spp. were isolated from fresh fecal samples and no *Giardia* spp. were seen on trichrome-stained preparations of feces. We necropsied nine skinned carcasses (five from East Baton Rouge or Iberville Parishes and four from oxidation ponds in St. Helena and Tangipahoa Parishes). One animal had a subcutaneous abscess at the point of the shoulder but no other significant gross lesions were seen.

Although the prevalence of seropositive animals to *T. gondii* in this study was low (7%), toxoplasmosis can be a common infection in both farm-raised and free-ranging nutria. Wenzel et al. (1983) found that 40% of farm-raised nutria had titers to *T. gondii* ranging from 1:4 to 1:2,048. In Great Britain, Holmes et al. (1976) trapped 111 nutria in Norfolk and East Suffolk and found that "many" nutria had titers to *T. gondii* between 1:16 and 1:32, and 30 had titers of 1:256 or more. Despite a low prevalence of seropositive animals to *T. gondii* in this study, it would seem prudent to thoroughly cook meat prior to human consumption. Moreover, caution should be taken in feeding uncooked nutria to animals susceptible to toxoplasmosis.

Because some nutria had antibodies to *C. psittaci*, nutria probably should be considered a possible source of zoonotic infection. Muskrats (*Ondatra zibethica*) were implicated in an epidemic of severe pneumonitis due to chlamydiosis that occurred in Louisiana in the 1940's (Olson and Larson, 1945). The index case of this epidemic was the wife of a trapper who helped her husband pelt animals, chiefly muskrats (Olson and Treuting, 1944). It is unknown whether nutria pelts also were handled.

Based on other reports of *Leptospira* spp. infection in nutria, we expected a higher prevalence of seropositive animals. Roth et al. (1962) isolated *Leptospira* from eight of 26 nutria collected in south-central Louisiana; 10 (38%) of the 26 animals had antibodies against *L. paidjan* (*bataviae* serovar). In a serologic survey in Great Britain, 24% of free-ranging nutria collected in Norfolk and Suffolk had serologic evidence of leptospiral infection and most of the seropositive animals reacted against *L. interrogans* serovar *icterohaemorrhagiae* (Waitkins et al., 1985). The prevalence in our study might have been higher had we screened for more serovars.

Although we found no evidence of *F. tularensis*, EMCV, *Giardia* spp. or *Salmonella* spp. exposure or infection, this may have been due to the fact that the sampled group contained a relatively high percentage (25%) of juveniles; juveniles would have had less chance of exposure to these agents. Five animals (three adult males, one adult female and one juvenile female) had titers (1:4 or 1:8) to *F. tularensis*, but were not considered positive by the laboratory performing the test. Per-

haps these very low titers were indicative of exposure. Failure to find *Giardia* spp. in this study could have been a technical problem stemming from the use of feces for examination. Erlandsen et al. (1990) have shown that higher prevalences in beaver and muskrats are detected when mucosal scrapings rather than feces are examined.

In retrospect, a different method of determining nutria age should have been used, such as measuring the foot or weighing eye lenses. Many nutria that appeared to be juveniles based on size were pregnant, and were thus classified as adults based on genital development. In Louisiana, nutria may reach sexual maturity at 4 to 5 mo of age, and determining age by genital development may not give a good indication of the age classes sampled (Kinler et al., 1987).

Although prevalence of seropositive animals to the agents screened in this study was relatively low, the disease agents of concern have the potential to cause epizootics in nutria populations or disease in domestic livestock or humans. Diseases of free-ranging nutria populations are poorly understood, but based on our findings, we believe they may warrant further study.

We thank Trend Scientific Inc., St. Paul, Minnesota, for donating the polyvinyl alcohol fixative vials and trichrome stain; the Diagnostic Virology Laboratory, NVSL, Ames, Iowa, for performing the *Chlamydia* and EMCV serology; the Bacterial Zoonoses Branch of CDC, especially Ronald Shriner, for performing the *Francisella tularensis* serology; and Wayne Roberts, Athens Diagnostic Laboratory, for assistance with *Leptospira* serology.

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Received for publication 17 September 1992.