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EXPERIMENTAL INFECTION OF SOME NORTH AMERICAN WILD RUMINANTS AND DOMESTIC SHEEP WITH *MYCOBACTERIUM PARATUBERCULOSIS:* CLINICAL AND BACTERIOLOGICAL FINDINGS

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ABSTRACT: *Mycobacterium paratuberculosis* originally isolated from bighorn sheep (*Ovis canadensis*) with spontaneous paratuberculosis was used to orally inoculate Rocky Mountain elk (*Cervus elaphus nelsoni*) calves, mule deer (*Odocoileus hemionus*) fawns, white-tailed deer (*Odocoileus virginianus*) fawns, bighorn × mouflon (*Ovis musimon*) hybrid lambs, and domestic lambs. All experimentally exposed animals became infected. During the first year of infection, hybrid and domestic sheep were able to control the infection but infection was progressive in elk and deer. Clinical paratuberculosis occurred only in mule deer.

INTRODUCTION

Paratuberculosis, caused by *Mycobacterium paratuberculosis*, is an important chronic enteric disease of domestic cattle and occasionally of domestic sheep and goats. Clinically, the disease in these species is characterized by progressive weight loss leading to emaciation and often intermittent or constant diarrhea. Paratuberculosis has been diagnosed in many captive wild species (Katic, 1961; Vance, 1961; Soltys et al., 1967; Libke and Walton, 1975; Boever and Peters, 1974; Temple et al., 1979) and in a few free-ranging species, including bighorn sheep and Rocky Mountain goats (*Oreamnos americanus*) in Colorado and Wyoming (Williams et al., 1979), mouflon in France (Thiery, 1953), and Tule elk (*Cervus elaphus nannodes*) (Jessup et al., 1981), axis (*Axis axis*), and fallow deer (*Dama dama*) in California (Riemann et al., 1979). The presence of paratuberculosis in free-ranging populations raises questions about the susceptibility of other wild ruminants to *M. paratuberculosis*, the susceptibility of domestic livestock to strains of *M. paratuberculosis* isolated from wild species, and the possibility that free-ranging ruminants might act as reservoirs of the bacteria. This study was undertaken, in part, to answer some of these questions.

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MATERIALS AND METHODS

Rocky Mountain elk calves, raised by their dams until weaning, were obtained from a captive herd maintained at the Sybille Wildlife Research Unit, Wyoming Game and Fish Department; mule deer fawns were "orphans" donated by the Colorado Division of Wildlife; white-tailed deer fawns were obtained from Oklahoma State University; bighorn hybrid lambs were born at the Wild Animal Disease Center or were from a game farm in Nebraska; domestic lambs were obtained from local commercial sources. Deer fawns and bighorn hybrid and domestic lambs were hand-raised on cow's milk supplemented with vitamins and were weaned onto alfalfa hay, commercial grain mixture, and mineralized salt.

When 4 to 5 mo of age, eight elk, eight mule deer, two white-tailed deer, nine bighorn hybrid sheep, and nine domestic sheep were each orally inoculated with 50 mg wet weight of *M. paratuberculosis*. The organism was originally isolated in 1977 from a clinically affected bighorn sheep (inoculum was prepared by Dr. C. O. Thoen, Iowa State University, Ames, Iowa 50011, USA). Bacteria were suspended in 1 ml Hanks' balanced salt solution and introduced into the mouth and pharynx via 3 ml syringe. Four each elk and mule deer, three domestic sheep, and two bighorn hybrid sheep were maintained in separate facilities as unexposed controls. In addition, three uninoculated elk were housed with experimental elk and three uninoculated bighorn hybrid sheep held in separate outdoor quarters were naturally exposed to run-off from experimental animal pens.

Animals were handled monthly to obtain fecal samples from the rectum and blood samples from the jugular vein for diagnostic tests. Half of the animals of each species were killed 6 mo postinoculation (PI) and the remainder 12 mo PI by electrocution or overdose of succinyl choline chloride (Sucostrin®, E. R. Squibb and Sons, Inc., Princeton, New Jersey 08540, USA) after immobilization by intramuscular xylazine hydrochloride (Rompun®, Haver-Lockhart, Bayvet Division, Cutter Laboratories, Inc., Shawnee, Kansas 66201, USA). Contact elk were killed after 12 mo exposure to experimentally

TABLE 1. Culture of *Mycobacterium paratuberculosis* from tissues and feces of Rocky Mountain elk after oral inoculation or contact exposure.

Organ	6 mo postinoculation		12 mo postinoculation		Contact elk[a]	
	No. infected/ no. cultured	Average bacteriologic score[b]	No. infected/ no. cultured	Average bacteriologic score[b]	No. infected/ no. cultured	Average bacteriologic score[b]
Tonsil	1/3	0.7	2/3	2.0	2/3	1.3
Suprapharyngeal LN[c]	4/4	3.0	3/4	1.5	2/3	1.3
Mediastinal LN	NC[d]	—	1/1	1.0	NC	—
Proximal mesenteric LN	NC	—	1/1	1.0	NC	—
Midmesenteric LN	NC	—	3/3	2.0	2/3	2.0
Distal mesenteric LN	NC	—	4/4	2.5	3/3	2.0
Ileocecal LN	4/4	1.75	4/4	2.5	3/3	2.3
Duodenum	NC	—	2/4	0.5	0/1	0.0
Jejunum	NC	—	1/1	1.0	NC	—
Ileum	NC	—	0/2	0.0	0/2	0.0
Ileocecal junction	3/4	1.3	2/2	1.0	1/2	1.5
Spleen	NC	—	NC	—	0/1	0.0
Liver	0/4	0.0	NC	—	1/3	0.3
Kidney	NC	—	0/3	0.0	1/2	0.5
Testicle	1/2	0.5	0/3	0.0	0/3	0.0
Uterus	0/1	0.0	0/1	0.0	NC	—
Mammary gland	0/1	0.0	NC	—	NC	—
Accessory sex gland	0/3	0.0	0/2	0.0	NC	—
Feces[e]	0/24	0.0	0/48	0.0	1/36	ND[f]

[a] Elk exposed to experimentally infected elk for 12 mo prior to necropsy.
[b] Each tissue cultured was assigned a bacteriologic score of 0 to 3 depending on the average number of colonies on three slants: 0 = no growth; 1 = ≤10 colonies, 2 = 11–50 colonies, 3 = >50 colonies.
[c] Lymph node.
[d] Not cultured.
[e] Feces from all elk were cultured monthly.
[f] Not determined.

inoculated elk; bighorn hybrid contacts were killed 3 and 25 mo after exposure to runoff from contaminated animal pens.

Postmortem examinations generally were conducted within 0.5 hr of death. Sections of distal small intestine, mesenteric lymph nodes, and feces were collected for mycobacteriologic culture from all animals. In addition, a variety of other tissues were cultured from most animals. Fecal and tissue samples were placed in sterile plastic bags at the time of collection. Most samples from the bighorn hybrid sheep were frozen at −70 C for up to 2 yr prior to culture; samples from the elk, deer, and domestic sheep were refrigerated and cultured within 2 days of collection. Fecal and tissue samples were processed and cultured on Herrold's egg yolk medium for *M. paratuberculosis* (United States Department of Agriculture, 1974). Fresh, antibiotic-free chicken eggs were obtained from local private flocks. Ferric mycobactins derived from *M. phlei* or *M. paratuberculosis* were incorporated into the media (mycobactins were supplied by Drs. R. S. Merkal, R. D. Angus, and G. M. Brown, National Animal Disease Center, Ames, Iowa 50010, USA). Inoculum prepared from each fecal or tissue sample was layered onto three slants of medium with and one without mycobactin and incubated at 37 C for 16 wk. Identification of *M. paratuberculosis* was based on slow growth, acid-fast staining properties, and mycobactin dependence. Number of colonies per slant were recorded as: 1+ for one to 10 colonies; 2+ for 11 to 50 colonies; and 3+ for greater than 50 colonies. These values were averaged for each tissue to obtain a bacteriologic score. Fecal samples from each group were examined at 6 and 12 mo by flotation for evidence of intestinal parasitism.

RESULTS

Rocky Mountain elk: Overt signs of paratuberculosis were not observed in elk. Soft feces occasionally were noted but diarrhea was not present. Control elk were subjectively larger with greater antler development than infected elk. Unfortunately, it was not possible to weigh the animals.

At necropsy, all animals were in good to excellent body condition. Mesenteric lymph nodes of inoculated and contact elk were slightly enlarged and most contained small foci of necrosis and mineralization.

Mycobacterium paratuberculosis was isolated from tissues of all experimentally inoculated and contact elk, but not control elk (Table 1). Six mo PI, organisms were isolated in greatest number from lymphoid tissues; many bacteria were cultured from suprapharyngeal and ileocecal lymph nodes. The organism was iso-

TABLE 2. Culture of *Mycobacterium paratuberculosis* from tissues and feces of mule deer and white-tailed deer after oral inoculation.

Organ	Mule deer				White-tailed deer	
	3–5 mo postinoculation		11–12 mo postinoculation		2–5 mo postinoculation	
	No. infected/ no. cultured	Average bacteriologic score[a]	No. infected no. cultured	Average bacteriologic score[a]	No. infected no. cultured	Average bacteriologic score[a]
Tonsil	1/4	0.3	0/2	0.0	1/1	1.0
Suprapharyngeal LN[b]	3/4	1.5	0/2	0.0	1/2	1.5
Proximal mesenteric LN	2/2	2.0	1/1	1.0	1/1	3.0
Midmesenteric LN	NC[c]	—	2/2	2.5	1/1	3.0
Distal mesenteric LN	5/5	2.0	2/2	1.5	1/1	3.0
Ileocecal LN	NC	—	2/2	2.5	1/1	1.0
Proximal ileum	0/2	0.0	2/2	1.0	0/1	0.0
Ileocecal junction	0/3	0.0	1/1	1.0	1/1	1.0
Liver	0/4	0.0	NC	—	0/1	0.0
Spleen	0/4	0.0	NC	—	0/1	0.0
Testicle	0/2	0.0	0/2	0.0	0/1	0.0
Uterus	0/2	0.0	NC	—	NC	—
Feces[d]	2/32	ND[e]	2/23	ND	0/7	0.0

[a] Each tissue cultured was assigned a bacteriologic score of 0 to 3 depending on the average number of colonies on three slants: 0 = no growth, 1 = ≤10 colonies; 2 = 11–50 colonies; 3 = >50 colonies.

[b] Lymph node.

[c] Not cultured.

[d] Feces from all deer were cultured monthly.

[e] Not determined.

lated from a testicle of one animal. By 12 mo PI, bacteria were cultured in greatest number from the mesenteric lymph nodes, but tonsil and suprapharyngeal nodes were also infected. All contact elk became infected and bacteria were isolated from lymphoid tissue of the head and mesenteric lymph nodes. Liver in one contact elk and kidney in another animal were infected. *Mycobacterium paratuberculosis* was cultured from only one of 108 fecal samples from infected animals. The positive sample was obtained from a contact elk 11 mo after exposure to inoculated elk. Eggs of strongyles and *Trichuris* sp. were in fecal flotations of most elk.

Deer: Clinical paratuberculosis occurred in experimentally infected deer. All deer had soft feces occasionally, however, infected deer frequently had soft or diarrheic feces. Infected deer were poorer in body condition than control deer; these differences became apparent by 4 to 5 mo PI. Infected deer shed their winter hair coat several months later than control deer and remained in fair to poor body condition as determined by lack of subcutaneous and visceral fat. Control deer had abundant body fat. Mesenteric lymph nodes of infected deer were mildly enlarged; white gritty foci frequently were visible in the cortex of mesenteric lymph nodes and were in the tracheobronchial and mediastinal lymph nodes of one deer 12 mo PI. During the study 11 deer died of causes presumably unrelated to paratuberculosis; two of malignant catarrhal fever; one of septicemia; one of hypothermia; five with history, clinical signs, and lesions suggestive of mycotoxicosis; one of trauma; and one of malignant edema.

Mycobacterium paratuberculosis was cultured from tissues of all infected deer but not from control deer (Table 2). Isolation most frequently was from the mesenteric lymph nodes; but in a few deer, *M. paratuberculosis* was also isolated from tonsils and suprapharyngeal lymph nodes. Four of 55 fecal samples were culture positive, one each at 4, 5, 8, and 11 mo PI; two were from the same animal. A few unidentified coccidian oocysts were in fecal flotations.

Bighorn hybrid sheep: Experimentally exposed bighorn × mouflon hybrid sheep did not show clinical signs of paratuberculosis. All animals were in moderate to excellent body condition when killed. Mesenteric lymph nodes were enlarged mildly 6 mo PI but were essentially normal 12 mo PI. Focal necrosis and mineralization occurred in lymph nodes of a few animals.

Mycobacterium paratuberculosis was isolated from tissues and/or feces of all experi-

TABLE 3. Culture of *Mycobacterium paratuberculosis* from tissues and feces of bighorn × mouflon hybrid sheep after oral inoculation or exposure.

Organ[a]	6 mo postinoculation		12 mo postinoculation		Exposed sheep[b]	
	No. infected / no. cultured	Average bacteriologic score[c]	No. infected / no. cultured	Average bacteriologic score[c]	No. infected / no. cultured	Average bacteriologic score[c]
Tonsil	0/3	0.0	0/5	0.0	0/3	0.0
Suprapharyngeal LN[d]	0/3	0.0	0/5	0.0	0/3	0.0
Proximal mesenteric LN	NC[e]	—	1/5	1.0	0/2	0.0
Midmesenteric LN	0/3	0.0	1/3	0.3	0/2	0.0
Distal mesenteric LN	4/4	ND[f]	3/4	0.8	1/3	ND
Ileocecal LN	0/3	0.0	0/1	0.0	0/2	0.0
Proximal ileum	0/4	0.0	1/5	0.4	0/2	0.0
Ileocecal junction	4/4	ND	1/1	1.0	1/3	ND
Liver	0/3	0.0	0/4	0.0	0/1	0.0
Spleen	0/3	0.0	0/2	0.0	0/1	0.0
Testicle	0/2	0.0	0/2	0.0	0/2	0.0
Uterus	0/1	0.0	0/1	0.0	0/1	0.0
Ovary	NC	—	0/1	0.0	NC	—
Feces[g]	0/24	0.0	3/60	ND[h]	1/56	ND[h]

[a] Some tissues frozen (−70 C) ≤2 yr prior to culture.
[b] Naturally exposed to runoff from infected animal pens. One sheep was killed 3 mo and two were killed 25 mo postexposure.
[c] Each tissue cultured was assigned a bacteriologic score of 0 to 3 depending on the average number of colonies on three slants: 0 = no growth; 1 = ≤10 colonies; 2 = 11–50 colonies; 3 = >50 colonies.
[d] Lymph node.
[e] Not cultured.
[f] Tissues cultured at Veterinary Services Laboratory, National Animal Disease Center, Ames, Iowa 50010, USA. Bacteriologic score not determined.
[g] Feces from all sheep were cultured monthly.
[h] Not determined.

mentally infected animals, from two of three sheep exposed to runoff from contaminated animal pens, and was not isolated from tissues of control animals (Table 3). One hundred forty fecal samples from infected sheep were cultured. One positive fecal culture was obtained 6 mo PI and two 11 mo PI from three experimentally infected sheep; there was one positive sample from a sheep 9 mo postexposure to runoff. Fecal flotations contained few oocysts of *Eimeria ovinoidalis*, *E. ahsata*, *E. parva*, *E. ovina* and eggs of strongyles and *Nematodirus* sp.

Domestic sheep: Clinical paratuberculosis was not observed in domestic sheep experimentally infected with *M. paratuberculosis* and all were in excellent body condition when killed. Only slight enlargement of mesenteric lymph nodes was apparent in some sheep.

Mycobacterium paratuberculosis was cultured from only two of five sheep killed 6 mo PI. Twelve months PI the organism was isolated in low numbers from three of four inoculated sheep; but in two cases bacteria were only cultured from one organ (Table 4). Control animals were culture negative. All 80 fecal cultures of exposed animals were negative for *M. paratuberculosis*. Fecal flotations were negative for intestinal parasites.

DISCUSSION

Elk, mule deer, white-tailed deer, bighorn × hybrid, and domestic sheep were susceptible to infection with *M. paratuberculosis* derived from paratuberculous bighorn sheep. During 1 yr of infection only deer developed clinical paratuberculosis characterized by poor body condition and diarrhea. These signs are similar to those described in spontaneous paratuberculosis in deer (Libke and Walton, 1975; Temple et al., 1979). Clinical paratuberculosis was not apparent in elk although adverse effects on growth in experimentally infected elk possibly occurred. It is not known if infected elk would have developed clinical paratuberculosis if allowed to live for a longer period though reports of clinical paratuberculosis in Tule elk (Jessup et al., 1981) and in red deer (*Cervus elaphus*) (Vance, 1961) suggest some elk might have become clinically diseased if given more time.

Mycobacterium paratuberculosis was cultured from all experimentally exposed deer and elk. Contact elk readily became infected and distribution of bacteria was similar to experi-

TABLE 4. Culture of *Mycobacterium paratuberculosis* from tissues and feces of domestic sheep after oral inoculation.

Organ	6, 8 mo postinoculation[a]		12 mo postinoculation	
	No. infected/ no. cultured	Average bacteriologic score[b]	No. infected/ no. cultured	Average bacteriologic score[b]
Tonsil	0/5	0.0	0/4	0.0
Suprapharyngeal LN[c]	1/5	0.2	1/4	0.3
Mediastinal LN	NC[d]	—	0/3	0.0
Midmesenteric LN	NC	—	1/3	0.3
Distal mesenteric LN	NC	—	1/4	0.3
Ileocecal LN	2/5	0.4	0/4	0.0
Proximal ileum	NC	—	2/4	0.5
Ileocecal junction	2/5	0.4	0/3	0.0
Liver	0/4	0.0	NC	—
Kidney	NC	—	0/1	0.0
Uterus	0/2	0.0	0/1	0.0
Feces[e]	0/32	0.0	0/48	0.0

[a] One sheep in this group was killed 8 mo PI.

[b] Each tissue cultured was assigned a bacteriologic score of 0 to 3 depending on the average number of colonies on three slants: 0 = no growth; 1 = ≤10 colonies; 2 = 11–50 colonies; 3 = >50 colonies.

[c] Lymph node.

[d] Not cultured.

[e] Feces from all sheep were cultured monthly.

mentally inoculated animals. In contrast, *M. paratuberculosis* was not isolated from tissues of one of nine hybrid sheep and four of nine experimentally exposed domestic sheep. Average bacteriologic scores were low in infected sheep. In the case of the hybrid sheep, low recovery could have been due in part to freezing the tissues because freezing is known to decrease the viability of *M. paratuberculosis* (Kim and Kubica, 1973; Richards, 1981).

Isolation of *M. paratuberculosis* from extraintestinal tissues was most frequent from the cervids. Tonsils and suprapharyngeal lymph nodes were often infected; involvement of this lymphoid tissue has been considered important in the pathogenesis of bovine paratuberculosis (Payne and Rankin, 1961a, b). Distribution of bacteria to other organs occurred as demonstrated by culture and presence of microscopic lesions (Williams et al., 1983). *Mycobacterium paratuberculosis* has been isolated from many extraintestinal organs of domestic ruminants (Levi, 1948; Goudswaard, 1971; Karpiński and Żórawski, 1975) and involvement of the reproductive tracts of paratuberculous cattle is well documented (Pearson and McClelland, 1955; Kopecky et al., 1967; Larsen and Kopecky, 1970; Larsen et al., 1981), thus the presence of *M. paratuberculosis* in the testicle of an elk was not surprising. The presence of the organism in semen is unlikely to lead to systemic infection of the cow (Merkal et al., 1982).

Bacterial isolation scores remained essentially static or slightly increased from 6 to 12 mo PI in both elk and deer; thus the cervids were not able to control the infection during 1 yr. These scores decreased from 6 to 12 mo PI in domestic sheep. Most hybrid and domestic sheep appeared to be able to control, and in some cases overcome the infection during 1 yr. Some sheep, however, would be expected to develop clinical paratuberculosis after exposure to the bacteria given adequate incubation times. Spontaneous cases in free-ranging bighorn indicated that clinical disease occurs (Williams et al., 1979).

Few fecal samples were culture-positive even though *M. paratuberculosis* was readily isolated from tissues of exposed animals. Thus, fecal culture could not be recommended as a single diagnostic test for paratuberculosis. One explanation for few positive fecal cultures in infected animals is low sensitivity of the cultural procedure. Fecal culture has been widely used as a diagnostic test for paratuberculosis; however, animals shedding few organisms or shedding intermittently may go undetected (Johnson et al., 1977).

Deer appeared to be more susceptible to infection with *M. paratuberculosis* than other species. Clinical paratuberculosis in fawns has

been reported in white-tailed (Libke and Walton, 1975), sika (*Cervus nippon*), and fallow deer (Temple et al., 1979). In contrast, paratuberculosis in domestic species is considered a disease of adults requiring several years of incubation before appearance of clinical signs (Buergelt and Duncan, 1978).

These studies of susceptibility to paratuberculosis indicate that some wild ruminants could become infected by sharing range or pasture contaminated by subclinical or clinically affected domestic livestock or other wild ruminants. Dairy cattle were considered the source of *M. paratuberculosis* affecting axis and fallow deer in California (Riemann et al., 1979). Bighorn sheep appear to be able to maintain the disease within the population without reintroduction of the bacteria from domestic animals (Williams, 1981). Whether deer or elk could effectively maintain the disease in a free-ranging population is not known. It seems likely that given a high prevalence of paratuberculosis in cattle, deer and elk sharing pasture with livestock could become infected and then could act as disseminators or reservoirs of *M. paratuberculosis*.

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BOOK REVIEW . . .

Animal Disease in Relation to Animal Conservation, M. A. Edwards and U. McDonnell, eds. Academic Press, Inc., 111 Fifth Avenue, New York, New York, USA. 1981. 336 pp. $49.00 US.

This publication is the proceedings of a symposium held in 1981 by the Zoological Society of London. The symposium was designed to review the relationship of animal disease to animal conservation. It explored the direct effects of disease on wildlife populations, as well as the effects of human and domestic animal activities, including disease control, on wildlife populations. These activities often resulted in conflict between the moral need for animal conservation and the human need for disease control.

Each chapter of this publication is the contribution of an individual scientist(s); it includes a list of relevant references and a record of the discussion period which transpired. The latter includes remarks by his royal highness, The Prince Philip, President of the World Wildlife Fund. Appropriate figures and tables illustrate each presentation.

To present the subject, the chapters of this book are grouped into four sections. In the first section, specific diseases are discussed including rinderpest and its control in Africa, trypanosomes and its impact on wildlife, rabies in wildlife, myxomatosis and its evolution, and botulism in waterfowl. Interesting reviews on each of these diseases are provided with the emphasis on European, African, and/or Asian epidemiology. Too often we consider the boundaries of wildlife disease to be limited to North America. For example, trypanosomiasis control, including the tsetse fly, illustrates the importance and need for the integration of ecological, geographical, agricultural, and sociological factors in land use planning, and disease control.

The second section of the publication discusses animals as reservoirs of disease and provides examples including influenza, leishmaniasis, trichinosis and hydatid disease. It is fascinating, as well as enlightening, to explore the life cycle and epizootiology of *Trichinella spiralis* in leopards, hyaenas, jackals, and lions.

The third section stresses epidemiological principles and policies. The major methods of transmission are discussed and the epidemiology of plague in the United States is used to illustrate these principles.

The last section of the book discusses conservation in relation to animal disease in Africa and Asia and the control of disease in wildlife including its reduction when a threat to man or domestic animals occurs.

A major aim of the symposium was to make the scientific community aware of the complexity of the relationship between animal disease and animal conservation. This aim is met and excellent examples of this complexity are illustrated in the chapter on the badger/cattle, TB controversy in the United Kingdom, and the chapter on trypanosomiasis in Africa where control measures often directly or indirectly threaten wildlife.

This book will be an important addition to the library of anyone interested in wildlife and their diseases. It vividly documents that epidemiology is a world-wide subject and it uses excellent examples to illustrate this. As the teacher of a course in diseases of wildlife, I welcome this new information on diseases, hosts, and epidemiology in other parts of the world. It will provide new and broader concepts on wildlife disease and wildlife conservation. As the editors state, "This publication will be essential reading for all conservationists, veterinarians, and public health workers, and for those physicians and agriculturists concerned with this important and challenging subject."

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