# Survey of *Baylisascaris sp.* in zoo-animals in the Netherlands.

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## ABSTRACT

**Objectives:** *Baylisascaris* species are large zoonotic roundworms of raccoons, bears and skunks. This study investigates the presence of *Baylisascaris* species in Dutch zoo animals.

**Method**: From eight public zoos 28 fecal samples were collected from 20 different animal populations susceptible for *Baylisascaris* spp. For 14 populations information on roundworm infections was provided using questionnaires.

**Results**: *Baylisascaris* spp. ova were recovered from 3 samples. All positive stool samples derived from one zoo and a total of 9 *Baylisascaris* eggs were isolated from both Ursus arctus (brown bear) and Nasua nasua (coati) feces. The eggs were identified as *B.transfuga* by molecular characterization. The clinical history revealed that *Baylisascaris* infections in zoo animals was documented earlier.

**Conclusion**: *Baylisascaris* parasites are prevalent in Dutch zoos and because of the zoonotic potential, constant vigilance by animal care takers and veterinarians is advised.

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## 1. INTRODUCTION

*Baylisascaris* parasites are large roundworms or ascarids and adults stage are found in the small intestine of the definive host. The parasites have a large resemblance with *Toxocara* spp. and occur primarily in carnivores. *Baylisascaris* species include *B. procyonis* in raccoons, *B. columnaris* in skunks, *B. melis* in badgers, *B. devosi* in martens and fishers, *B. transfuga* in bears and *B. tasmaniensis, B. ailuri B. schroederi* and *B. laevis* in Tasmanian devils, red pandas, giant pandas and marmots respectively. (1,7)

The life cycle of *Baylisascaris species* is that of facultative heteroxenous parasites.
Transmission involves either ingestion of infectious eggs or tissue larvae in prey animals by predation or scavenging, such as small birds and mammals, that act as paratenic host (7) The larvae in visceral and somatic tissue in the intermediate host are unleashed by the definite host digestive system and subsequently mature, mate and produce eggs (21). Adult (female) worms in the intestinal tract shed large numbers of eggs into the environment via the feces. For example, a single *B. procyonis* worm can produce an estimated 115,000 to 179,000 eggs per day, so a heavily contaminated raccoon may shed over a billion eggs. (7,10,21) Once the eggs are defecated they have to mature or embryonate during 2-4 weeks, depending on soil type and climatic conditions, to become infectious and may stay infectious for months to several years. The eggs are very persistent that they will remain viable in the hardest environmental conditions because they are highly resistant to desiccation, extended freezing and freeze-thaw. The eggs are also resistant to most common disinfectants, such as undiluted bleach, and only heat (>62°C), seems to be effective to deactivate infectious eggs. (1,6)

Diseases in the definite host have rarely been reported, but when birds or mammals, including men, ingest embryonated eggs, larvae can migrate actively throughout the body penetrating a wide variety of tissues (liver, heart, lungs, brain, and eyes), for some like *B. procyonis* with a preference for the central nervous system (CNS). This extraintestinal migration causes inflammation and tissue damage in several sites (6,7,11,18,22). In contrast to *Toxocara* larvae, *Baylisascaris* larvae continue to grow during their dormant stage in the intermediate host. Tissue damage, the signs and symptoms of baylisascariosis are often severe because of the size of *Baylisascaris* larvae, their tendency to wander widely, and the fact that they do not readily die. (6,7,18,21)

*Baylisascaris procyonis* is the only well documented and most frequently cause of human and animal baylisascariosis. Even though there is no unequivocal evidence of naturally occurring of other *Baylisacaris* sp. in humans, all *Baylisascaris* species are potentially zoonotic. Concerns also raised for the more unknown potentially zoonotic *Baylisascaris* species, such as *B. transfuga* (bear roundworm). (20)
Studies showed that *B. transfuga* larvae are able to migrate in chickens, rabbits, mice and Mongolion jirds, although clinical manifestations differ from *B. procyonis*. (3)
According to Papini and Casarosa (1994) *B. transfuga* larvae are less pathogenic, compared to *B. procyonis*, *B. columnaris*, and *B. melis*, due to their smaller size during migration. (12) Although distinct pathogenecity of *B. transfuga* compared to *B. procyonis* was demonstrated by Kazacos, Papini and Sato, larvae migrans syndromes in mammals are associated with *B. transfuga*, and therefore suggest the possibility of human infection. (3,7,10,14,16,20)

*Baylisascaris spp.* infections became of great awareness in zoo carnivore collections because of the potential to impact both human and animal health. (15,19) Captive animals are often kept in small and confined areas and following the introduction of *Baylisascaris* in an animal compound, the zoo enclosure becomes heavily contaminated because of the high loads and viability of the ova. The artificial environment has a reinforcing effect on the probability of transmission of communicable diseases, since the number of animals is high in a relatively small surface area (i) and the density of infectious materials as result of fecal contamination is high (ii). (16) The roundworm persists in its captive enclosure and precludes the eradication, causing permit awareness of the parasite burden. (16) These heavily contaminated enclosures contribute to conditions with repeated high

exposure to other animals and humans, especially zoo employees.

Little is known about the current situation of the prevalence of *Baylisascaris* type parasites in zoo-animals in the Netherlands. Prior to this study, a study in 1998 had failed to detect *B. procyonis* in raccoons of private owners, zoos and animal parks. (13) In spite the previous study only focused on *B. procyonis*, recent literature demonstrates that other *Baylisascaris* species have the same or similar substantially consequences in zoo animal collections and therefore demand equal attention. (12,14,16,20). In this study the prevalence of *Baylisascaris species* is investigated in Dutch zoos.

## 2. MATERIALS AND METHODS

### *2.1 Questionnaires*

Fifteen public zoos in the Netherlands, members of the Dutch Zoo Association (NVD), were requested to participate in the study if raccoons, skunks, bears, badgers, red pandas, martens and fishers were present. The zoo veterinarians were asked to fill in a questionnaire to get information about housing, animal care, deworming programs, clinical history and preventive measures, such as cleaning protocols and pest control. The questions were based on a former questionnaire from 1998. (13)

### 2.2 Sample collection

Fecal samples (fresh or from the outdoor pen) were collected and stored in the refrigerator at +4°C until analysis was performed by the laboratory of the National Institute of Public Health and the Environment in Bilthoven (RIVM). In case of groups of animals, the different droppings, were pooled to represent the majority of defecating animals.

### 2.3 Fecal examination

The microscopically examination of the feces was performed by the centrifugation flotation technique with a sugar solution with a specific gravity of 1.27 (7,13,20).
In a weighing tube of 50 ml, 3,0 gram of feces was mixed with 24 ml PBS/tween (1:8w/v) until all solid material was suspended. The suspension was flushed through a metal sieve (mesh width 200-300 µm) in a 50 ml tube and the solution was centrifuged at 1000 x g for 5 minutes at room temperature. The supernatant was removed and replaced by 12 ml sucrose solution and transferred into 15 ml tubes. The suspensions were centrifuged at 1000x g for 30 minutes at room temperature. Of each tube a top of 1 ml was collected and placed in 2.0 ml Eppendorf vials on to which 1ml water was suspended and centrifuged during 3 minutes at 14,000 RPM. The supernatant was discarded and the sediment (pellet) re-suspended in rest fluid flowing down from the walls (approximately 50 µl) and examined using a light microscope under 200-400x magnifications. *Baylisascaris* infections were diagnosed by finding the characteristic ascarid eggs in the feces.

### 2.4 Determination of *Baylisascaris* eggs

*Baylisascaris* eggs are round-oval (ellipsoidal) shaped, dark brown in color, contain large single-celled embryo surrounded by a thick shell and have a fine granular surface. The measurements are 62.5 – 70.0 µm x 52,5 – 57 µm (7,20,21). There is analogy between *Baylisascaris* eggs and *Toxocara* eggs, but the latter are lighter coloured, have a coarsely pitted shell and are slightly larger in size (respectively 85 – 75 µm)

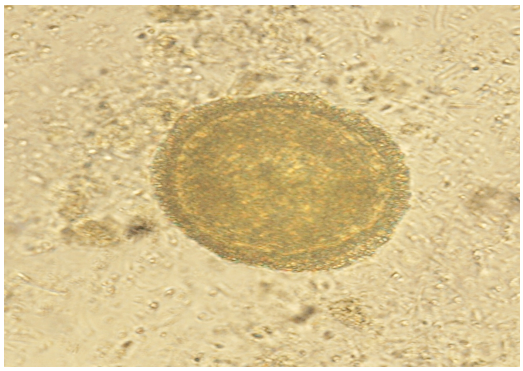

**Figure 2:** Underdeveloped *Baylisascaris*-type egg from fresh brown bear feces. Note the thick shell, fine granular surface and an ellipsoidal shape.

### 2.5 Molecular identification and characterization

Molecular characterization by PCR provides a strong alternative to overcome the limitations of the traditional identification. (4) Testini et al (2010) demonstrated that genomic DNA can be extracted from individual nematodes.(20) Subsequently molecular analysis was performed on

different target regions (respectively cox1, cox2, 28s rDNA an ITSs) after it was amplified by PCRs. The primers were derived from the GenBank database. (20) The sequence difference between the species is greater than the variation within each species, especially in target region ITS-1, therefore this is a good method to identify the different *Baylisascaris* species. (20)

### 2. 6 Soil sampling

Given the strong suspicion of a *Baylisascaris* burden in the enclosures of both the brown bears and coatis, soil samples were conducted from both enclosures to determine the contamination rate of ova in the environment and second to confirm our previous flotation findings. From both enclosures, three soil samples were scooped up with a sterile spoon (10 cm deep), where 45 to 164 g were collected. All six soil samples were placed in sterile containers. The samples were transported in cool boxes containing ice packs and stored at 4°C. All samples were analyzed within 24h after sampling.The contents were collected in 0,5 % Tween 20 (Interscience, St Nom La Bretêche, France) and transferred into plastic bags. This detergent detached worm eggs from the ground material. A sieve shaker was charged with 4 sieves, respectively, 63, 400, 1000 and 2000 microns pore size and a sample with detergent was added. The water flow and the sieve shaker were just been switched on for as long as until the run-off water was transparent. 2 full spatulas of material from the 2000 microns pore size sieve were transferred into 15ml tubes each. Sucrose solution with a specific gravity of 1.27 was added and covered with a coverslip. The suspensions were centrifuged at 3000 x g for 2 minutes at room temperature. After centrifugation, the four coverslips were placed on two slides and examined using a light microscope under 200-400 x magnifications.

## 3. RESULTS

### 3.1 Questionnaires

Eight zoos (Artis, Safaripark Beekse Bergen, Diergaarde Blijdorp, Dierenpark Emmen, Dierenrijk Europa, Ouwehand zoo, Burgers Zoo and Dierenpark Amersfoort) participated in the study. Individual profiles were obtained for 20 different animal populations susceptible for *Baylisascaris* spp. Information on roundworm infections was provided in health histories for 14 populations for which profiles were collected (Table 1).

The animals were housed in both indoor- and outdoor enclosures. In five zoos, animals share their environment with other animals, including porcupines (*Erethizontidae* spp.), ring-tailed cats (*Bassariscus astutus*), mandrills (*Mandrillus sphinx*) and rhesus macaque (*Macaca mulatta*).

| **Species** | ♂ | ♀ |
|---|---|---|
| Raccoons (*Procyon lotor)* | 7 | 12 |
| Polar bears (*Ursus maratimus)* | 3 | 3 |
| Malayan sun bears | | |
| (*Helarctos malayanus)* | 1 | 4 |
| Slothbears (*Melursus ursinus)* | 3 | |
| Asian black bear | | |
| (moonbear *Ursus thibetanus)* | 1 | 2 |
| Kodiakbear | | |
| (*Ursus arctos middendorffi)* | 1 | |
| Brown bear (*Ursus arctos)* | 3 | 1 |
| Speculated bear | | |
| (*Tremarctos ornatus)* | 1 | |
| Coatis (*Nasua nasua)* | 8 | 11 |
| Yellow throated marten | | |
| (*Martes flavigula)* | 1 | |
| Red panda (*Ailurs fulgens)* | 1 | 1 |
| Binturong *(Arcticits binturong)* | 2 | 2 |
| Skunks (*Mephitis mephitis)* | 2 | |
| Ground squirrels | | |
| (*Spermophilus variegatus)* | 1 | |

**Table 1. Animals participating in the study**

Five zoos had histories of roundworm infections. Two zoos had two incidental findings of ascarids infections in their polar bears population (during anno 2007 and an unknown date) and one zoo stated that they frequently detected roundworms in their population of polar bears. One zoo detected in 2007 and 2008 roundworms in their skunk population. Of the mentioned ascarids infections, one polar bear and one skunk population were considered *Baylisascaris* positive. One zoo was also familiar with *Baylisascaris* in their captive raccoons a decade ago. Despite the thoroughly decontamination of the raccoon-

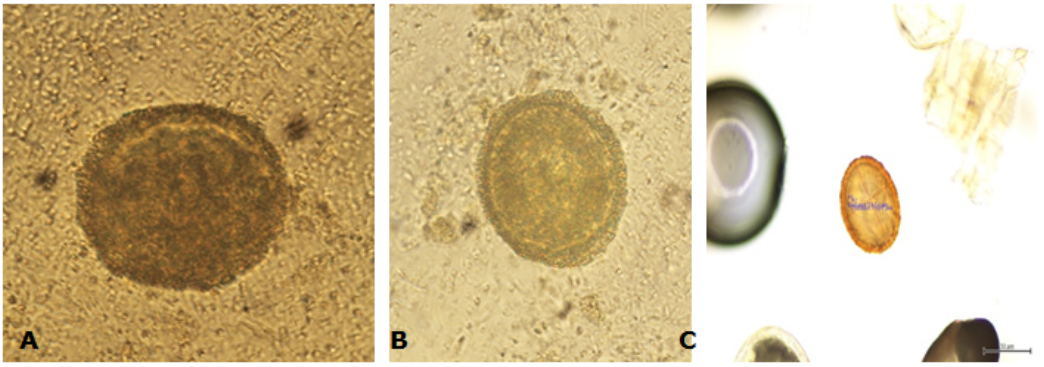


Figure 1. Microscopically examination of stoolsamples conducted in a public zoo, identified as of *Baylisascaris* type. ***A*** *Baylisacaris egg,* isolated from the *NasuaNasua* samples. ***B*** *Baylisascaris* egg, isolated from the Brownbear samples. **C** Picture of eggs found in soil samples. Derived from the department of I&I of the Veterinary Faculty of the University of Utrecht.

residence and treatment animals, the ascarids remained a recurring problem. Eventually the zoo therefore decided to shut down the raccoon-residence and relocate the remaining raccoons to other zoos.

The majority of zoo animal collections (14/20) were annually once to eight times dewormed as preventive control. In the remaining six zoos, deworming was performed only when faecal samples were positive for nematode eggs. Antihelmintics that were used are ivermectin, moxidectin, praziquantel, doramectin, fenbendazole and mebendazole.

Six zoos use a disinfectant (Halamid®) after cleaning the animal facilities with soap. All zoos clean mechanically with a pressure washer, some on daily basis and some once or twice annually. All zoo personnel provide multiple species.

The degree of attention to disinfection and prevention of infection is very diverse and varies considerably from one zoo to another. Three zoos insist that zoo employees should thoroughly clean their hands and boots with a disinfectants bath before entering the animal enclosure. Three other zoos only insist that personal hygiene is maintained. And in 2 zoos, personnel only require work clothes as preventive measure.

None of the conducted zoos have reported free-living raccoons. To prevent wild animals introducing a zoo, one zoo is fenced and, if necessary, performs pest control. The other remaining zoos indicated that preventive measures to ward off wild animals (including pest-animals) do not apply to them or did not take preventive measures.

### 3.2 Fecal analysis

In total 28 fecal samples, originated from 74 animals, (Table 2) were examined. *Baylisascaris* eggs were found in three samples (11%), originating from a *Ursus arctus* (brown bear) and two *Nasua nasua* (coati). All positive stool samples derived from one zoo.

### 3.3 Molecular identification and characterization

Genotyping of this small number of eggs is not reliable and therefore an antihelmintic treatment was used to collect adult worms from the infected animals. One adult worm was collected from the bear feces and genomic DNA could be extracted from this nematode. It was amplified by PCR for cytochrome oxidase 1(cox1) and sequence analysis was performed. The cox1 sequences of *Bayliascaris transfuga*, derived from the GenBank database, did correspond with the isolate.

### 3.4 Soil samples

From these soil samples, 5 *Baylisascaris* eggs (Figure 1C) were isolated after flotation. Noteworthy, eggs were only found in the brownbear enclosure. (Table 3)

| Animal species | Baylisascaris | Number of eggs | Size |
|---|---|---|---|
| Urcus arctus | negative | - | - |
| Urcus arctus | positive | 1 | 62.5 x 75.0* |
| Nasua nasua | positive | 5 | 67.5 x 82.5* |
| Nasua nasua | positive | 3 | -** |

**Table 1.** Fecal analysis of both Urcus arctus and Nasua nasua enclosures.
* is not mean size of detected eggs, but a random selected individual.
**sizes have not been obtained, however eggs were considered of Baylisascaris type.

| Sample nr. | Origin | Soil amount (gr) | Number of *Baylisascaris* spp. eggs |
|---|---|---|---|
| 1 | Nasua nasua enclosure | 98 | - |
| 2 | Nasua nasua enclosure | 69 | - |
| 3 | Nasua nasua enclosure | 130 | - |
| 4 | Urcus arctus enclosure | 45 | 3 eggs |
| 5 | Urcus arctus enclosure | 123 | 2 eggs |
| 6 | Urcus arctus enclosure | 164 | - |

**Table 3.** Egg count in conducted soil samples from both Urcus arctus and Nasua nasua enclosures.

## 5. DISCUSSIE

The previously survey on *Baylisascaris* in Dutch zoos has been published in 1998. (13) The prevalence of *B. procyonis* in raccoons was investigated in zoos, animal parks and private owners in the Netherlands. No infections with *B. procyonis* from raccoons housed in zoos and shelters were recorded and questionnaires revealed that deworming-programs within zoos were sufficient enough to interrupt the transmission of this nematode. It was therefore concluded that there were no risks of *B. procyonis* for the public health in the Netherlands. (13)

*Baylisascaris spp.* have emerged in several Dutch zoos since according to our findings.
Despite the former survey was only focused on *B.procyonis*, recent literature demonstrate that other *Baylisascaris spp.* have the same or similar substantially consequences in zoo animal collections and therefore demand equal attention. (12,14,16,20) Hence, this survey examined the prevalence in Dutch zoos of all *Baylisascaris* species, related to raccoons, skunks, bears and badgers.

We successfully gathered *Baylisascaris* eggs by the sugar-sedimentation-flotation- centrifugation methods in faeces from both brown bears and coati. Yet, due to the lack of data from non-participating zoos and the use of several pooled samples, it was not possible to determine the exact prevalence of *Baylisascaris* in Dutch zoo animals.
The soil-sampling findings did not entirely suit with our positive faeces findings in both populations. However, it confirmed our suspicion of Baylisascaris presence, as morphological features match with those of Baylisascaris eggs. In a later stage, we were able to confirm this burden, as *B. transfuga* was identified by molecular characterization.

It is unclear where the found infections retrieved from as no new animals have been introduced since 2006. Once introduced *Baylisascaris* persist in these enclosures despite repeated deworming treatments, which is remarkable because intermediate host are a key factor in the establishment of an infection in adult bears and coatis. (7,16) These intermediate hosts in the wild most often consist of birds and small mammals, such as rodents and mice. (16) However, there was no indication of a problem in the animal boundaries, which could have resulted in the opportunity for these captive animals to consume intermediate hosts. This suggest that the route of contamination is not evaluated thoroughly enough, as it is not known that coatis nor brown bears, in contrast to polar bears, may act as host in the direct life cycle. (16) Further investigations of the route of

contaminations are needed to prevent reintroduction of *Baylisascaris* after treatment.

In this study the parasite loading of both positive faeces samples and soil samples were unexpectedly low, which may be as a result of the diagnostic test specifics, intermitted shedding and periodically deworming. The sensitivity and specificity of the used flotation-centrifugation method could not be accurate evaluated due to a lack of a golden standard. (3) Regarding the reliability of flotation, a previous study on the prevalence of *B. transfuga* in the wild population of European brown bears reported a rather low (only 60%) sensitivity of the flotation method, in comparison with PCR methods, and stated that using flotation alone could result in missing positive samples. (3) Unfortunately, some samples were too small for a repeated examination and therefore we were unable to perform a secondary flotation with the aim to reduce the risk of false-negative feces results.

The majority of animals were periodically dewormed, according to the questionnaire, which may also resulted in false-negative findings and lower eggs count. After a successful deworming it last at least 4 weeks before new adult worms mature and release new eggs in the feces. If during that time feces examination is carried out this could result in missing parasite burdens. (3) An insufficient deworming dose however, may be responsible for the small number of eggs that were collected, when not all adult worms were killed.

Another possible cause for the low number of *Balisascaris* eggs may be intermitted shedding of the eggs. Particularly raccoon and skunk juveniles are known to have a prolonged Baylisascaris development, during that time these individuals can be fecal negative for many weeks after which suddenly shedding starts of large numbers of eggs. (7) Adult *B. transfuga* are also known to shed intermittent. (16)In the future, more fecal examinations should be taken with a higher frequency throughout the year to obtain more nonbiased evaluation by eliminating confounding variables associated with flotation, anthelmintics and intermittent shedding of eggs.

Given the current recognition of *Baylisascaris* in Dutch zoo collections, we believe that precautions should be taken when working with Baylisascaris susceptible animals or in a area potentially contaminated with faeces. It is important to institute regular fecal examination and anthelmintic treatment in zoo animals.(9)
Fecal examination should be evaluated wisely considering the possible absence of eggs, despite worms may actually be present. (16) Repeated sampling measures both pre and post treatment would allow clinicians to stop further (re-)infections. Most anthelmintics proved to be effective for Baylisascaris, though clinicians need to foresee that resistance to anthelmintics may occur. (9,16) Decontamination of enclosures is difficult because of the marked resistance of *Bayliascaris* eggs to all disinfectants. (17) Only a thoroughly cleaning operation by discarding the soil and flaming up its entire interior would be effective to eradicate all eggs. (17,19) However, this is very costly and success is not guaranteed. In the absence of thorough cleaning the enclosures, isolating of the contaminated areas and daily removing as many of the contagious faeces is advised. Moreover, proper pest control as well as repeatedly deworming ,within the 4 weeks preparent period, is needed to interrupt the parasite lifecycle. (19)

Zoopersonnel understanding of the hazards of *Baylisascaris* spp. and baylisascariasis prevention are poor. Protective measures for care takers is in the majority of the zoos only limited to the provision of work clothing and the ability to hand washing. Only one zoo indicates work with gloves. In the case of an ongoing infection, it is evident that these measures are too limited. Cultivating awareness among staff should decrease parasite exposure and disease. (2,8)Therefore, employees, including the medical officer, should be educated on sources of infection, modes of transmission, manifestations of disease, and preventative measures for baylisascariasis. (2,8)Present guidelines have recently been summarized by Maas et al.(9) (2014).(9)

## 6. CONCLUSION

Despite few human cases have been reported, the severity of the aggressive larvae migrans and the lack of effective medication may consider baylisascariasis as an important zoonosis (6). The risk of human infection is present in any area where humans, especially zoo-staff and veterinarians, have direct or indirect contact with raccoons, skunks, bears and other natural host. (7,21) Several animals in one zoo in this study were infected with potential zoonotic parasites, *B. transfuga*. Despite no humans casualties have been recorded from *B. transfuga* infections, transmission of *Baylisascaris* spp. to the human may occur. (7,16) The difficult eradication of these persistent parasites and their zoonotic potential makes *Baylisascaris* in captive animals both of financially and public health importance. (5,7,16,19)

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