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SEASONAL DYNAMICS IN THE PREVALENCE OF *Baylisascaris transfuga* OVA IN THE FAECES OF THE BROWN BEAR (*Ursus arctos)* IN SLOVAKIA

Diploma Thesis

Kosice, April 2009 Submitted by: Simon Finnegan

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Ďakujem☺

Declaration

This is to declare that the following paper hereby submitted is the work of Simon Finnegan, carried out at the Department of Parasitology and Infectious Diseases, Institute of Parasitology, Košice, Slovak Republic. This study was supervised by Assoc. Prof. Mária Goldová, PhD. The text is the result of experimental work supplemented by literature studies.

Date: April 2009. Signature: …….

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USED TERMS AND ABBREVIATIONS

Antihelmintic: Drug with efficiency against parasitic worms

Scat: Faeces

Spp: Species

SWS: Slovak Wildlife Society

2 ANNOTATION

A: Simon Finnegan, 5th year student (GVM) at the University of Veterinary Medicine, Košice, Slovak Republic.

B: Seasonal dynamics in the prevalence of *Baylisascaris transfuga* ova in the faeces of the brown bear *(Ursus arctos)* in Slovakia.

C: Assoc. Prof. Mária Goldová, PhD.

D: Department of Epizootology and Parasitiology.

E: 55 Pages, 18 Figures, 11 Tables, 107 References.

F: Abstract

Standardized flotation techniques were used to survey 188 brown bear (*Ursus arctos*) fecal samples for the intestinal nematode *Baylisascaris transfuga*. Scats were collected from free ranging bears in the Carpathian mountain regions in northern and central Slovakia. The samples analysed cover bear activity from March until November each year, spanning across 3 individual years, 2002, 2007, and 2008. This study revealed that that the prevalence of eggs of *Baylisascaris transfuga* varied seasonally, low prevalence was recorded in the Spring, an increase was evident during the Summer, with egg prevalence finally peaking during the Autumn. This phenomenon is not yet fully understood, but is possibly associated with the metabolic changes of the bear during winter hibernation. These findings are discussed in conjunction with findings from similar studies primarily from the USA. This is one of the first recordings of *Baylisascaris transfuga* in free ranging wild bear populations in Europe.

Keywords: Brown bears, faecal samples, *Baylisascaris transfuga*, seasonal prevalence.

3 INTRODUCTION

Members of the ascaridoid nematode *Baylisascaris* are common in bears worldwide. Sprent (1968) reclassified ursine members of the genera *Ascaris* and *Toxascaris* into a new genus, *Baylisascasis*. The bear roundworm *Baylisascaris transfuga,* is an ascaroid parasite that has been reported in all species of bears (Polar bear, *Ursus maritimus*; American black bear, *Ursus americanus;* Asiatic black bear, *Ursus thibetanus*; Sun bear, *Helarctos malayanus*; Sloth bear, *Melursus ursinus*; Spectacled bear, *tremarctos ornatus*; Giant panda, *Ailuropoda melanoleuca*; and Brown bears, *Ursus arctos*), (Schaul, 2006).

The brown bear is the most numerous large carnivore in Slovakia, population estimates suggest that there is a total of 770 - 870 individuals inhabiting a range of 13,000 km² in the Western Carpathians (Rigg & Adamec, 2007). Very little research has been carried out on this population. With the kind co-operation of Robin Rigg of the Slovak Wildlife Society, 133 scat samples were obtained from bears in the wild for parasite analysis from the 2007 and 2008 seasons. For analytical purposes this number was further expanded by 55 additional samples from 2002, which had been previously analysed by Goldova *et al.* (2003). This resulted in an overall total of 188 samples across 3 different years.

From the literature review it can be seen that there has been previous attempts to study *Baylisascaris transfuga* prevalence in wild bear populations (King *et al.,* 1960; Rogers, 1975; Worley *et al.,* 1976; Frechette & Rau, 1977, 1978; Manville, 1978; Crum *et al.,* 1978; Dies 1979; Barnes & Rogers, 1980; Gau *et al.,* 1999; Foster *et al.,* 2004; Joyner *et al.,* 2004). Only a handful of these studies have attempted to evaluate the seasonal dynamics of this parasite. Most studies on this subject are from the American and Canadian populations of both brown and black bears in the 1960's and 1970's. Recent literature on parasites in free-ranging populations is lacking (Gau *et al.,* 1999).

A selection of these studies lead towards a seasonal trend in *Baylisascaris transfuga* prevalence. Very little investigative work has been done with respect to *Baylisascaris transfuga* prevalence in the free-ranging brown bear populations in Europe. A definite seasonal trend was found to be evident in this study.

4 LITERATURE REVIEW

(A) *Baylisascaris transfuga*

Fig. 1. *Baylisascaris transfuga* adults.

4.1 Taxonomic Classification (Order: Ascaridida)

The superfamily Ascaridoidea (Nematoda: Secernentea) is represented by four families of the order Ascaradida. Ascaridodea is characterized by large roundworms with three lips, occasionally separated by interlabia (Gibson, 1983). Some ascaridoid species are heteroxenous parasites of the enteric tract of vertebrates, and can utilise intermediate hosts for larval development. Two subfamilies of Ascaridae, Toxacarinae and Ascaradinae are common helminth parasites of terrestrial mammals and of great veterinary importance (Gibson, 1983). More than 50 genera are represented by these two subfamilies (Nadler, 1992).

Phylogenetic study of the superfamily ascaridoidea is somewhat hindered by the lack of well characterized morphometric documentation and the reliance on life history patterns and host specificity (Nadler, 1992). Structural characteristics that have been evaluated in ascaridoid phylogenetic studies include lip features, excretory systems, the esophago-intestinal complex, and secondary sexual characteristics of males. At the intergeneric and generic levels, evolutionary relationships have been proposed on the basis of some recognized morphological and life history characteristics (Schaul, 2006).

Baylisascaris species are ascaridoid nematodes of the subfamily Ascaridinae and can exhibit both monxenous and heteroxenous life history patterns (Sprent, 1983; Adamson, 1986). The Superfamily Ascaridinae includes *Baylisascaris, Ascaris, Toxocaris, Parascaris, and Lagochilascaris*. The subfamily toxicarinae is represented by the genera *Toxocara* and *Porrocaecum* (Schaul, 2006). Sprent (1968) renamed members of the genera *Ascaris* and *Toxacaris* as *Baylisascaris* species. The genus contains 10 species, two of which are recognized as provisional (Sprent, 1968; 1970).

General Morphology

Ascarids are large white worms of the small intestine. The mouth lacks a buccal capsule but is surrounded by three lips. The eggs are thick shelled and infection is generally by ingestion of the egg containing an L2 larva (Urquhart *et al*., 1987).

Eggs and larvae

Egg size was measured in a number of studies. Wallach and Boever (1983) found a size range from 78.3-88.0 x 66.3-74.7µm. This is in line with an earlier studies where egg sizes ranged from 83 x 75 µm (Okoshi *et al*., 1962) and also with Davison (1972) whom found that ova were globular to sub-globular, measuring 73-83 x 57-73 µm, with a finely pitted surface. Khera (1951) found that the eggs were oval, 75-85 by 61-70 µm, with a thick transparent shell and outer yellowish irregular albuminous coat, developing to larvae in five days at room temperature (65-80 °F). Larvae were found to be 0.3-0.35 mm long, with a diameter of 0.025 mm.

4.2.2 Adult worms

Many studies have published data on the length of the adult intestinal worm. Fowler (1986) found that the adult worm ranges in length from 150 mm plus, whilst Moran *et al*. (1994) stated that males were up to 100 mm in length, the females being longer, measuring up to 250 mm (see Fig. 1). Okoshi *et al*. (1962) found that males measured 76.5 - 129 mm long and 1.6 - 2.35 mm wide, with females measuring 153.5 - 228.4 mm long and 3.1 - 4.0 mm wide. In a study by Davison (1972) adult males measured 108 - 110 mm, females 228-232 mm. Khera (1951) found males measuring from 115-208 mm, with a maximum thickness 2.5 mm, and a head diameter of 0.55-0.65 mm. Females ranged from 128-284 mm in length, with a maximum thickness 4 mm, the diameter of the head was 0.6-0.8 mm, with the vulva 82 mm from the anterior end in a worm of 281 mm.

Morphological characteristics include the presence of cervical alae, absence of an interlabium, and the absence of striation around the body at the site of the vulva (Okoshi *et al*., 1962). Davison (1972) found that the cervical alae may be reduced with rough areas peri-cloacally, having no subdorsal post-cloacal papillae. Khera (1951) described the adults as large stout worms with a thick striated cuticle, having three well-defined semicircular lips surrounding the mouth, and two symmetrical papillae on the dorsal lip. They possess two asymmetric papillae on each of the ventro-lateral lips, the lateral papilla small and the ventral papilla large, with well developed cervical alae, 1.5-4.5 mm long. The spicules are short and stout, 0.85-1.02 mm long and covered with small granulations.

The males of *Baylisascaris* spp. Possess pericloacal roughened areas known as area rugosa. The cervical alae of adult worms posses cuticular bars which reach the surface of the cuticle (McIntosh, 1939; Sprent, 1952; 1970). Labiae papillae (dorsal and subventral) are distinctly double. In males, spicules are stout and uniform, less than a millimeter in length. Males also possess pre-and post-cloacal groups of papillae on their tails (cited in Kazacos, 2001).

4.3 Life cycle

The complete life cycle of most *Baylisascaris* spp. is unknown (Gutiérrez, 2000). However, the life cycle of other related ascarids has been investigated, and the life cycle of the racoon roundworm, *Baylisascaris procyonis* will be used as an example. The adult male and female worms live in the racoon's large intestine, they mate and produce millions of eggs a day which are passed in feces. The eggs mature and are infective in two to four weeks if the temperature is warm enough.

When a racoon accidentally swallows the eggs, they hatch in the intestine, the larvae mature in the intestinal tissues and return to lie free in the intestine, the cycle beginning again. In other hosts larvae migrate through the tissues, a condition known as 'visceral larva migrans' and can invade the eyes and the brain causing serious damage. The racoon can also become infected by ingesting these sick animals (Garcia & Bruckner, 1997).

4.3.1 Information on susceptibility & transmission of *Baylisascaris transfuga*

With respect to *Baylisascaris transfuga,* Moran *et al*. (1994) stated that infection could be direct or via a paratenic host such as a rodent. Abdelrasoul and Fowler (1979) also found that the life cycle was direct, but also could be indirect via encysted larvae using various rodents, birds or insects as intermediate hosts. Transmammary and transplacental transmission are thought of as unlikely to occur (Kazacos, 2001).

Infection increases with age; a study of grizzly bears (Brown bears, *Ursus arctos*) in Montana and Wyoming found 40% of cubs to be infected with ascarids but 92% of six- to nine-year-old bears were infected. (Worley *et al*., 1976). Although Partridge (1992) and Moran *et al*. (1994) reported that young and immature bears appeared more susceptible. Gutiérrez (2000) stated that the complete life cycle of most *Baylisascaris* spp. is unknown, and as there was no diagram found in the literature, the author attempted to compile an illustration of the *Baylisascaris transfuga* lifecycle, see Figure 3 overleaf.

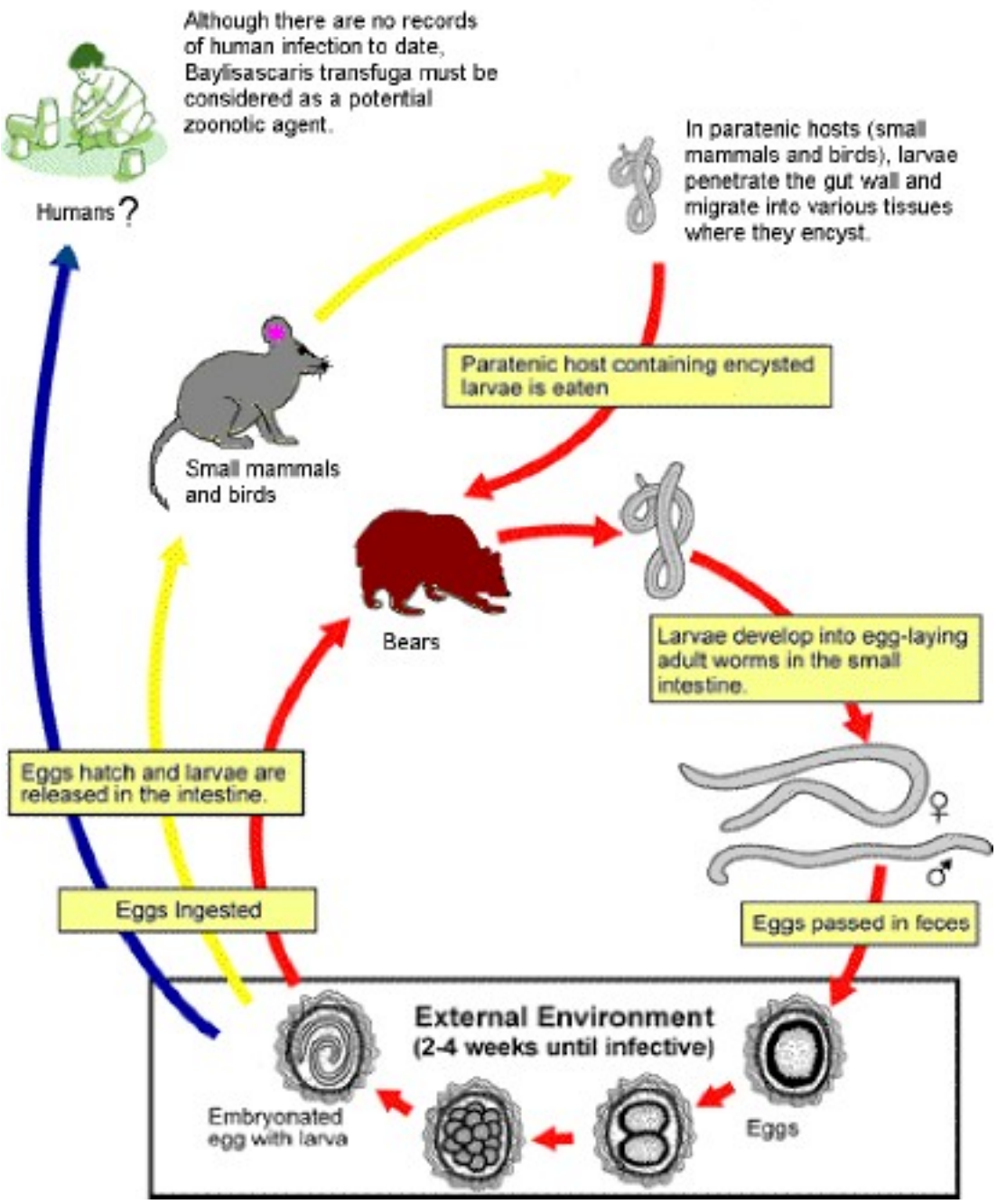

Fig. 3. *Baylisascaris transfuga* lifecycle.

4.3.2 Egg stability and infectious characteristics of *Baylisascaris transfuga*

Moran *et al*. (1994) carried out various investigations into the infectious nature and stability of the eggs. Their finding indicated that 93% of shed eggs were found to be viable. A coproculture test ran at a temperature of 25 °C and 95% humidity resulted in 10% of eggs being embryonated at day 15, 74% at day 30 and 82% at day 45. Eggs were found to be highly resistant, having the ability to remain infective for as long as 7-10 years in the environment and can survive for at least two months at -20 °C. They can develop to the infective stage when sunk in 10% formalin solution, at low oxygen levels and also in zinc sulphate solution.

4.3.3 Metabolism of helminths

Parasites of bears from northern regions are apparently well adapted to the hibernation habits of their hosts. Several authors have presented evidence that intestinal parasites which derive nourishment directly from the ingesta of their host pass out of the alimentary canal prior to the beginning of hibernation (Rush 1932; Rausch, 1954, 1961; Bromlei, 1965; Choquette *et al*., 1969; Rodgers, 1975).

The major metabolic activity in *Ascaris lumbricoides* was found to involve the glycolytic pathway (Entner & Gonzalez, 1959). Carbohydrates are the major, if not the exclusive source of energy for many parasitic helminths and are used commonly as an essential constituent of the media employed to maintain helminths *in vitro* (Von Brand, 1973). Most helminth parasites become immotile when they are kept in a sugar free medium. During starvation, however, the worms can survive at the expense of their body glycogen which is apparently synthesised from hexoses, hence glycogen plays an important role in providing energy to the worms (Afzal *et al*., 1975). Adult *Ascaris lumbricoides* were found to have a high glycogen uptake of 1.3g/100 g of ascarid (Jíra, 1998).

4.4 *Baylisacarsis* spp., in free-ranging bear populations

Baylisascaris transfuga is a widespread pathogen, found in bears in many areas of the world (Soulsby, 1982). This parasite occurs throughout the black bear's range in Canada and the northern USA (Rogers, 1975). There is very little information known about *Baylisascaris transfuga* in European wild bear populations. Averin (1948) noted that in Russia, almost all bears were infested with ascarids in Kamchatka regions.

In an overview carried out by King, (1960) two or more *Toxascaris multipapillata* were found in the intestines of 31% (17 out of 55) American black bears, *Ursus americanus* from New York (Adirondacks, plus five from Allegany State Park), from 1956-1959. One bear was host to 39 ascarids. In a separate investigation *Toxascaris multipapillata* were found in the intestines of two adult American black bears, *Ursus americanus* trapped in the Adirondacks, in 1956-1958.

Rogers (1975) found one to four adult *Baylisascaris transfuga* worms, in five of seven intestinal tracts from American black bears, *Ursus americanus* from Minnesota examined during the summer; additionally, two adult worms were passed by one bear and two others were found in droppings found 6-16 October. *Baylisascaris transfuga* was found in the small intestines of 53% of bears during a survey of American black bears, *Ursus americanus* from six states in the southeastern USA, July 1973-November 1976. This was the first report from the southeastern USA. There were no recorded associated pathogenic effects of the infections in these bears (Crum, 1978).

Eggs of *Baylisascaris transfuga* were detected in 64% (59 out of 92 faecal samples) of bears live-trapped in northern Wisconsin during summer 1974 and summer 1975. *Baylisascaris* were found in 89% (25 out of 29) intestinal tracts from hunter-killed American black bears, *Ursus americanus* from the same area during 1974-1975, with 1 to 132 worms per bear (hunting season is in the autumn). In two bears parts of the duodenum were totally occluded by nematodes. Additionally, one *Baylisascaris*

transfuga was taken from the anus of a female black bear captured July 1975 (Manville, 1978).

Baylisascaris transfuga were found in the small and large intestines of 62% (56 out of 91 black bears) during a study in northwestern Alberta, Canada, May 1976-September 1977 (Dies, 1979). In a review by Worley *et al*. (1976) *Baylisascaris transfuga* were found in 75.7% (53 out of 70) of bears during a study of grizzly bears (Brown bear, *Ursus arctos*) from Montana and Wyoming. Average intensity of infection was 33.8 parasites per infected bear (range 1-480). In a similar study *Baylisascaris transfuga* were found in the small intestines of 80% (24 out of 30 bears) of American black bears, *Ursus americanus* from Montana and Wyoming. Average intensity of infection was 22.7 worms/bear (range 1-177) (Worley *et al*., 1976).

Frechette and Rau (1977) found an infection rate of 21% in a study of helminths of the black bear, *Ursus americanus,* in Quebec, with as many as 15 worms found in one bear. An analysis of faecal samples from black bears in a subsequent study in Quebec showed that the prevalence of eggs of *Baylisascaris transfuga* was low in autumn (October and November) prior to denning (found in 13% of samples) but higher (42%) in spring (May) (Frechette & Rau, 1978).

B. transfuga was found in 23% of small intestines during a survey of 22 Florida black bears *Ursus americanus floridanus* (*Ursus americanus,* American black bears) cubs (up to 12 months old) between 1998 and 2003. This parasite had not been reported previously in *Ursus americanus* in Florida (Foster *et al*., 2004). *Baylisascaris* sp. eggs were detected in 5% of 56 faecal samples from grizzly bears (Brown bear, *Ursus arctos*) collected from the central Canadian Arctic spring and autumn 1995 and 1996 (Gau *et al*., 1999).

Baylisascaris transfuga infection was confirmed based on measurements and morphology of eggs detected in faeces in a juvenile female American black bear, *Ursus americanus*, from Virginia; the bear also had gastrointestinal infections with coccidia, strongyloides and pinworms (Joyner *et al*., 2004). *Baylisascaris transfuga* were present

in the small intestine of a wild 14-year-old female American black bear, *Ursus americanus* in northeastern Minnesota (Barnes & Rogers, 1980).

4.4.1 *Baylisascaris* spp., in captive bears

B. transfuga has also been reported in many zoo's and wildlife enclosures throughout the world (Rogers & Rogers 1976, Schaul 2006) with infection rates between 50-100% (Abdelrasoul & Fowler, 1979). In Europe, two *Toxascaris (Baylisascaris) transfuga* were found at necropsy in the stomach of a 30-year-old Sloth bear, *Melurusus ursinus* at Amsterdam Zoo (Kompanje *et al*., 2000). Jaros (1966) reported finding *Toxascaris transfuga* in the jejunum of the Brown bear, *Ursus arctos* from the Zoological Garden of Prague, 1954-64. Casarosa (1990) found *B. transfuga* in captive polar bears (*Thalarctos maritimus*) in Italy.

4.5 Seasonal Dynamics

One of the earliest records of helminths seasonality in bears came from Averin (1948) whom noted that in the Kamchatka regions (Russia) almost all bears were infested with ascarids, apart from during the spring, however, when the ascarids were absent.

Rausch (1954, 1961) and Choquette *et al* (1969) indicate that helminths known to derive nourishment from chyme are lost prior to denning. Rodgers (1975) observed a wild bear pass two adult *B. Transfuga* on the 9th of September, 10 days before it went into hibernation, he also found specimens of *B. Transfuga* in bear scats found in October of the same year. However, these were the only helminths found in the macroscopic examination of 962 droppings examined between April and November.

Eggs of *Baylisascaris transfuga* were detected in 64% (59 out of 92 faecal samples) of bears live-trapped in northern Wisconsin during summer 1974 and summer 1975 (Manville 1978). Intestinal tracts from bears shot on the 19th and 20th of October 1974, and another from a bear shot on September 19th, 1975 were found to be free of *B. Transfuga*. In the same study (Manville, 1978), *Baylisascaris* were found in 89% (25 out

of 29) intestinal tracts from hunter-killed American black bears, *Ursus americanus* in northern Wisconsin during the autumn of 1974-1975, with 1 to 132 worms per bear.

Incidence of infection peaked June to August and was reduced over fall (autumn) and winter. (Clark *et al*., 1969). An analysis of faecal samples from black bears in Quebec showed that the prevalence of eggs of *Baylisascaris transfuga* was low in autumn (October and November) prior to denning (found in 13% of samples) but higher (42%) in spring (May). This unexpected finding may be an indication of maturation of overwintering larvae (Frechette & Rau, 1978).

The prevalence of gastrointestinal parasites (*Diphylobthrium* sp*.,* coccidia*,* strongyles*,* and *Baylisascaris* combined) in 56 faecal samples from grizzly bears (Brown bear, *Ursus arctos*) collected from the central Canadian Arctic spring and autumn 1995 and 1996 (Gau *et al*., 1999), showed a 31% prevalence in spring, and a 58% prevalence in autumn. Serial samples were available from four individual bears; gastrointestinal parasites were present in three of the four samples from the autumn 1995, whilst only one of the same four bear samples proved positive in the spring of 1996. However the overall prevalence of *Baylisascaris transfuga* in this study was only 5%.

4.6 Clinical characteristics, morbidity and mortality in natural hosts

Definitive hosts rarely show clinical signs unless heavy infections of adult worms block the enteric tract, this is more of a concern for zoo animals habitually exposed to parasite contaminated environments. Furthermore, baylisascariosis in paratenic or incidental hosts associated with somatic larval migrans may not induce clinical signs unless an infectious dose of nematode eggs is high enough to induce hemorrhagic pneumonitis from extensive pulmonary migrations (Schaul, 1996).

Nevertheless, a range of clinical signs have been reported over the years. Both Wallach and Boever (1983), and Fowler (1978) have found signs ranging from diarrhoea, anorexia, a dry and rough coat, with the occasional presence of ascarids in the faeces.

They both agree that death may occur due to gut obstruction in overwhelming infections. Partridge (1992) and Fowler (1986) found that a severe infection could result in a poor body condition and gut obstruction. A number of studies where necropsy was preformed has found ascarids in both the small and/or large intestines, (King, 1960; Jaros, 1966; Rogers, 1975; Worley, 1976; Manville, 1978; Crum 1978; Dies 1979; Foster *et al*., 2004). Manville (1978) stated that ascarids may be found completely filling parts of the small intestines.

Infection is common in bears in captivity, but mortality is rare. (Klos & Lang, 1982). In a survey of zoo-kept Spectacled bears (*Tremarctos ornatus*), ascarids were the commonest internal parasite reported. (Wolff, 1988). Infection increases with age; a study of grizzly bears (Brown bears, *Ursus arctos*) in Montana and Wyoming found 40% of cubs to be infected with ascarids but 92% of six- to nine-year-old bears were infected. (Worley *et al*., 1976). Although Partridge (1992) and Moran *et al*. (1994) reported that young and immature bears appeared more susceptible. Heavy adult ascarid burdens cause moderate enteritis and, by interfering with digestion and nutrient absorption, reduced growth of the host (Bowman, 1999). Death of a bear from parasitism with *Baylisascaris transfuga* has been recorded (Crum, 1978).

4.6.1 Pathogenicity in natural and experimental infections (incidental hosts)

Migrating larva of certain species of baylisascaris (*B. procyonis, B. melis* and *B. Columnaris*) have characteristic tropisms for the central nervous system and share neurological disease producing capacities. In a comparison of pathogenicities among Baylisascaris species, Boyce *et al.* (1989) found *B. procyonis* and *B. melis* to be most pathogenic on the basis of larval migratory patterns, as well as the growth rate and overall size of their larval stages, with *B. columnaris* rated third most pathogenic ahead of other *Baylisascaris* species.

An index of pathogenicity has been established in a number of laboratory studies, whereby the number of ingested or parenterally administered infective egg inoculations

required to induce clinical disease has been determined for *Baylisascaris* species (Schaul, 2006). In addition to this, Kozacos (2001) defines central nervous system pathogenicity among different *Baylisascaris* species on the basis of migrations through somatic tissues, invasion of the central nervous system, aggressiveness within the central nervous system and host defense (i.e. encapsulation of larvae*). Baylisascaris procyonis, B. columnaris,* and *B. melis* produce relatively large larvae, which migrate through somatic tissues and frequently invade the CNS.

With reference to the bear helminth, *Baylisascaris transfuga*, in addition to *B. devosi*, and *B. tasmaniensis*, it was found that although they are etiological agents of larval migrans disease, their slower growth rate, smaller size, and less frequent invasion of the brain suggested that they are less pathogenic than *B. procyonis, B. columnaris*, and *B. melis*. *Baylisascaris* procyonis larval migrans and subsequent nematodiasis have been reported in natural and experimental infections of more than 90 species of wild birds and mammals (Kazacos, 1997, 2001).

In a number of laboratory studies, nevertheless, migrating *B. transfuga* have been found to invade the CNS producing visceral, neural, and ocular disease (Papini & Carosa, 1994; Papini et al., 1994; Papini et al., 1996; Sato *et al*., 2003). *Baylisascaris* larvae tend to invade the CNS of intermediate hosts, and can cause fatal disease in the intermediate host (Bowman, 1999).

Baylisascaris transfuga larvae have been shown to have neurotropic affinities in laboratory mice (Crum, 1978). *Baylisascaris transfuga* in other species, such as rodents, can result in migration of larvae to the viscera, eyes and brain. The extent of clinical signs and pathological lesions in the CNS of rodents varied between rodent species (severe generalised signs and death in Mongolian jirds, *Meriones unguiculatus*, with free larvae and extensive malacia of the brain, while in laboratory mice, *Mus domesticus*, signs were more limited in extent and duration, and larvae were found to be immobilised by surrounding granulomatous reactions in the CNS (Sato *et al*., 2004).

4.7 Human Health - Zoonoses associated with larval migrans syndromes

The clinical syndrome 'visceral larval migrans' as described by Beaver (1969) is caused by migrating larva of certain nematode species. The most common etiological agents of ascarid larval migration syndrome in humans have been *Toxacara canis* of domestic dogs, *Toxacara felis* of domestic felids, *Baylisascaris columnaris* of skunks and *Baylisascaris procyonis* of raccoons (Schaul, 2006).

Many helminth parasites of carnivores are potential etiological agents of ocular, visceral and neural larval migrans syndromes (Beaver, 1969; Beaver et al., 1984; Kazacos, 1991, 1996, 1997, 2000). Helminth zoonoses associated with larval migrans syndromes include hookworms, ascarids, and others, including gnathostomes, *Spirometra* and *Alaria* (Kazacos, 2001).

Larvae of *Baylisascaris procyonis*, a parasite of raccoons, cause a zoonotic infection (Sato *et al*., 2004). It is not clear whether in some circumstances larvae of *Baylisascaris transfuga* also could cause disease in humans. However, although *Baylisascaris procyonis* is most commonly implicated in larval migrans disease, all *Baylisascaris* species are considered potentially zoonotic (Samuels et al., 2001; Sangster, 2004). Kazacos (2001) maintains that although the primary agents implicated as zoonoses of concern are *Baylisascaris procyonis*, the raccoon roundworm and *Baylisascaris columnaris,* the skunk roundworm, yet if enough ova are ingested, then all *Baylisascaris* species can considered potential etiological agents of somatic larval migrans.

The emergence of larval migrans syndromes and baylisascariosis associated with the raccoon roundworm *Baylisascaris procyonis* has shown that *Baylisascaris procyonis* and their procyonid hosts pose significant threats to human health (Huff et *al*., 1984; Fox *et al*., 1985; Kuchle *et al*., 1993; Cunningham *et al*., 1994; Boschetti & Kasznica, 1995; Conrath's *et al*., 1996; Kazacos, 1997, 2001; Park *et al*., 2000; Rowley *et al*., 2000; Gavin et *al*., 2002). Along with neural larval migrans, *Baylisascaris procyonis* has also been implicated in diffuse unilateral subacute neuroretinits in humans (Goldberg et *al*., 1993; Mets et *al*., 2003). Samuel *et al.* (2001) claims that although *Baylisascaris*

transfuga larva can potentially cause a larval migrans infection in humans, it is much less pathogenic than *B. procyonis* and is not a major threat to human health.

Children are the most susceptible human cohort to zoonotic ascarid infection via exposure soil or sand substrates contaminated with embryonated ascarid ova passed in the excrement of domestic and wild carnivores. Ocular larval migrans is more commonly reported in older children infected with toxocara species, but is not attributed to geophagia or pica (Schaul, 2006).

4.7.1 Control Measures

Regular anthelmintic treatment can be used for control (Albelrasoul & Fowler, 1979) but it is difficult to prevent reinfection (Fowler 1986, 1993). A variety of different anthelmintics can be used. However, due to the difficulty of eliminating the parasite from the environment repeated treatment every 4-8 weeks may be required (Moran *et al*., 1994). Ova can remain viable in the environment for years (e.g. five years); they are resistant to drying, freezing and exposure to sunlight. Ova are not destroyed by normal cleaning with water and disinfectants. In captivity, the usage of high-pressure water for cleaning can assist in spreading ascarid ova around the enclosure (Albelrasoul & Fowler, 1979; Fowler, 1986, 1993).

Ova can be destroyed by direct heat, i.e. use of a blowtorch, on surfaces which can resist this treatment (obviously not on either wooden or painted structures) (Albelrasoul & Fowler, 1979; Fowler, 1986; Partridge, 1992). Removal of faeces and spot application of a flamethrower to the site where the faeces were lying, prior to hosing down and disinfecting, should reduce the number of infective ova present (Fowler, 1986).

(B) The brown bear (*Ursus arctos*)

Fig. 4. Two brown bear siblings photographed at Košice zoo. (Orig.)

Background to the brown bear (*Ursus arctos*) in Slovakia

The brown bear is the most numerous large carnivore in Slovakia. There has been only limited research on numbers, but the results available suggest a total of 770-870 individuals inhabiting a range of around 13,000 square kilometres. Genetic effective population size is unknown. The age-sex structure has not been sufficiently studied and there has been no detailed research to determine the age at which females produce their first litter, how often they breed, the average litter size and how many cubs survive. Social organisation, dispersal, habitat selection and home range are also poorly understood (Rigg & Adamec, 2007).

From having been almost exterminated 75 years ago by hunting and persecution, legal protection has allowed bears in the West Carpathians to recover naturally. Numbers still seem to be increasing despite limited hunting since 1958. The population is still recovering from a low of 20-60 individuals in the 1930s caused by trophy hunting and persecution. According to expert estimates, the average population growth rate during the period 1932-2005 was 4.5% annually, which is comparable to other expanding populations in Europe. The current size and trend of the population suggest that it is in little short-term danger. However, socio-economic changes are bringing new and growing threats while conflicting approaches to management have resulted in failure to implement important conservation recommendations (Rigg & Adamec, 2007).

Bear distribution is closely related to forest cover and elevation, which are both inversely correlated to human settlement and activity. Besides deciduous, mixed and coniferous montane forests, important habitats for bears include sub-alpine and alpine meadows as well as open areas with food sources at lower elevations. Habitat is rather fragmented due to topographic characteristics of the landscape resulting in mountain ranges with prime bear habitat separated by areas of denser human settlement in broad river valleys. As a consequence, bear distribution is patchy. If measures are not taken, fragmentation of habitat is likely to worsen due to highway construction, the expansion of tourism infrastructure and other development (Rigg & Adamec, 2007).

The brown bear in Slovakia is both a game species and protected by national and international legislation. Management is mainly at national level and is overseen by the Environment Ministry and the Agriculture Ministry, which have highly contrasting approaches. The former views hunting mainly as a tool to reduce conflicts and sets quotas as maximum limits, whilst the latter regards population control as essential and sees quotas as hunting plans to be filled. This division of management has led to strife and failure to implement important conservation recommendations. Valuable income from trophy hunting has also resulted in conflict over management (Rigg & Adamec, 2007).

4.9 Bear hibernation and its possible effect on parasites

Bears that live in northern latitudes hibernate during the winter when food becomes scarce (Folk *et al*., 1976). Denning in America and Canada can begin as early as September and last until May (Rausch 1961, Choquette *et al*., 1969). The use of dens and hibernation is thought to be an adaptation to the limited food availability and perhaps also due to giving birth to fragile cubs which are unable to maintain their own thermoregulation (Swenson *et al*., 2000). The mechanisms that bring about the onset of denning behaviour are not entirely understood, but its thought that several interacting stimuli are responsible, including less food availability, lower temperatures, snow, in conjunction with the physical condition of the bear, including its age, sex, and reproductive status (Manchi & Swenson, 2005).

Halak (1993) observed bears in the Western Tatras from 1977 to 1986. The average interval between the last signs of bear activity and first signs of bear reactivation was approximately 97 days, with a range from 73 to 113 days. The date of last tracks observed varied from 4th November to the 31st of December. The date of the first tracks found following denning varied from 15th February to the 11th of April. Spring emergence may be stimulated by increasing day length or temperatures (Friebe *et al*., 2001; Manchi & Swenson 2005). If temperatures remain high and if food is available, then bears may not hibernate. Pelikan (1983) found that some bears in central Slovakia remained active during the mild winter of 1982-1983, and some were active in Eastern Slovakia during

the 2006-2007 winter (Rigg & Adamec, 2007). Intensive feeding by hunters (Hell & Slamecka, 1999) and a good crop of rowan berries (Balaz, 2002) have also resulted in extended bear activity.

In a 10 year study carried out on the physiology of the European brown bear (*Ursus arctos arctos*) it was found that in late summer the feeding rate is two to three times above the normal level, resulting in a body weight increase of 30 – 35%. Bears become anorectic just before entering their dens, they stop feeding and empty their stomachs and intestines. Sleeping bears do not eat, drink, defecate or urinate. Their body temperature decreases during their winter sleep by 3-5°C below the normal level of 37.0-37.5°C. The loss of body weight after denning was 20 – 25% (Hissa, 1997). It was once thought that bears ate roughage prior to denning to scour their digestive tract and form a plug in the anus to prevent them from eating any more food that autumn, however Rogers (1981) concluded that the plug was formed during hibernation, not before denning. The plug is made up of feces, dead intestinal cells, hair, and bedding material (Rogers, 1981).

4.9.1 Bear metabolism during hibernation

The black bear (*Ursus americanus*) survives hibernation for up to five months whilst maintaining approximately normal blood levels of glucose, amino acids, and proteins. It accumulates no wastes of protein catabolism and does not become acidotic (Nelson *et al*., 1973, 1975). Waste products are produced, but instead of disposing of their metabolic waste, bears recycle it. The urea produced from fat metabolism (which can be fatal at high levels) is broken down and the resulting nitrogen is utilised to build protein (Rogers, 1981). During hibernation the urea formed is hydrolyzed and the nitrogen released is combined with glycerol to form amino acids, which then re-enter the protein synthetic pathways, body fat supplies the substrate for metabolism (Nelson, 1980).

14C-labeled glucose experiments revealed a very slow carbohydrate metabolism during denning, suggesting that in winter glycerol helps to prevent uremia by serving as a carbon source for amino acid formation. The excess nitrogen is thus diverted from urea synthesis into protein synthetic pathways. In addition to this, glycerol which is derived from the

bear's fat stores and readily mobilised for systemic use appears to serve as an active substrate for gluconeogenesis and lipogenesis during hibernation (Ahlquist *et al*., 1984). During hibernation the bear's glucose use was reduced, and conversely, lipolysis was increased (Nelson & Jones 1987). However, hibernation activates glyoxylate cycle enzymes that allow dormant *Ursus americanus* to convert the fatty acid carbons from brown adipose tissue to glucose (Davis *et al*., 1990).

4.9.2 Diet Analysis

Bears pass through 3 biochemical and physiological states during their active period, hypophagia (decreased food intake) in spring and hyperphagia (increased food intake) in autumn. In late summer and early autumn it is very important for bears to consume food with high energy content in order to accumulate the fatty tissue necessary for hibernation (Swenson *et al*., 2000). In Slovakia, bear diet has been studied in the Tatra and Fatra regions of the north, the same areas as used in this study. In 2001-03 it was found that plant material constituted c.91% of total scat volume and c.84% of dry matter consumed (Rigg 2004, Rigg & Gorman, 2006a). Grasses/sedges and herbs dominated in spring and early summer, with a shift to fruits in July-October (Rigg & Adamec, 2007).

Animal material comprised c.8% of total scat volume and c.15% of dry matter consumed. Although predation on sheep and cattle was known to have occurred in the study area (Rigg & Gorman, 2006b), no remains of livestock were identified in any of 373 bear scats analysed, indicating that livestock is not a major component of the diet. Juvenile Cervidae and wild boar were identified in scats from May-July, some of them probably obtained by predation. In early spring bears scavenged on carcasses of predated or winter-killed ungulates. The total proportion of wild ungulates in the diet was estimated as 6% of dry matter consumed. Insects (mostly ants and wasps), which are rich in protein and a source of essential amino acids, occurred significantly more frequently and in greater quantities than large mammals (Rigg, 2004, Rigg & Gorman, 2006a).

All anthropogenic food items combined were estimated to account for at least 23% of total scat volume and c.40% or more of dry matter consumed (Rigg, 2004, Rigg & Gorman, 2006a). Refuse was found in c.7% of scats, significantly more frequently in spring than in any other season. Use of anthropogenic food was least in June-August, when bears fed mainly on green vegetation, berries and Formicoidea. Fruit, mast and wasps were important food sources in September-November. However, overall autumnal diet of bears in the study area was found to be dominated by cultivated grains, obtained at hunters' ungulate feeding sites and in fields as pre-harvest crops (Rigg & Adamec, 2007).

Fig. 5. Collection of bear scat for parasite analysis. (Orig.)

5 GOAL OF THESIS

The primary goal of this work was to investigate the seasonal changes in the prevalence of the nematode *Baylisascaris transfuga* in the scats of the brown bear (*Ursus arctos*) in Slovakia. When a definite trend was discovered, a further investigation was carried out as to why this seasonal trend might occur, with particular emphasis on bear hibernation and its possible effect on intestinal nematodes.

6 MATERIAL AND METHODS

6.1 Study area

All scats were collected in the Western Carpathian Mountains of central and northern Slovakia (Figs. 6&7). The majority of the scats were collected from the Tatransky and Nízke Tatry National Parks, the rest were gathered from Malá Fatra and Veľká Fatra National Parks and Poľana Protected Landscape Area. It is believed that some individuals can pass between the various areas (R.Rigg, personal comment) and so all the scats were pooled to represent one population. Density appears to reach 5-11 bears per 100 square kilometres in core areas. However social organisation, dispersal, habitat selection and home range are in need of further study (Rigg & Adamec, 2007).

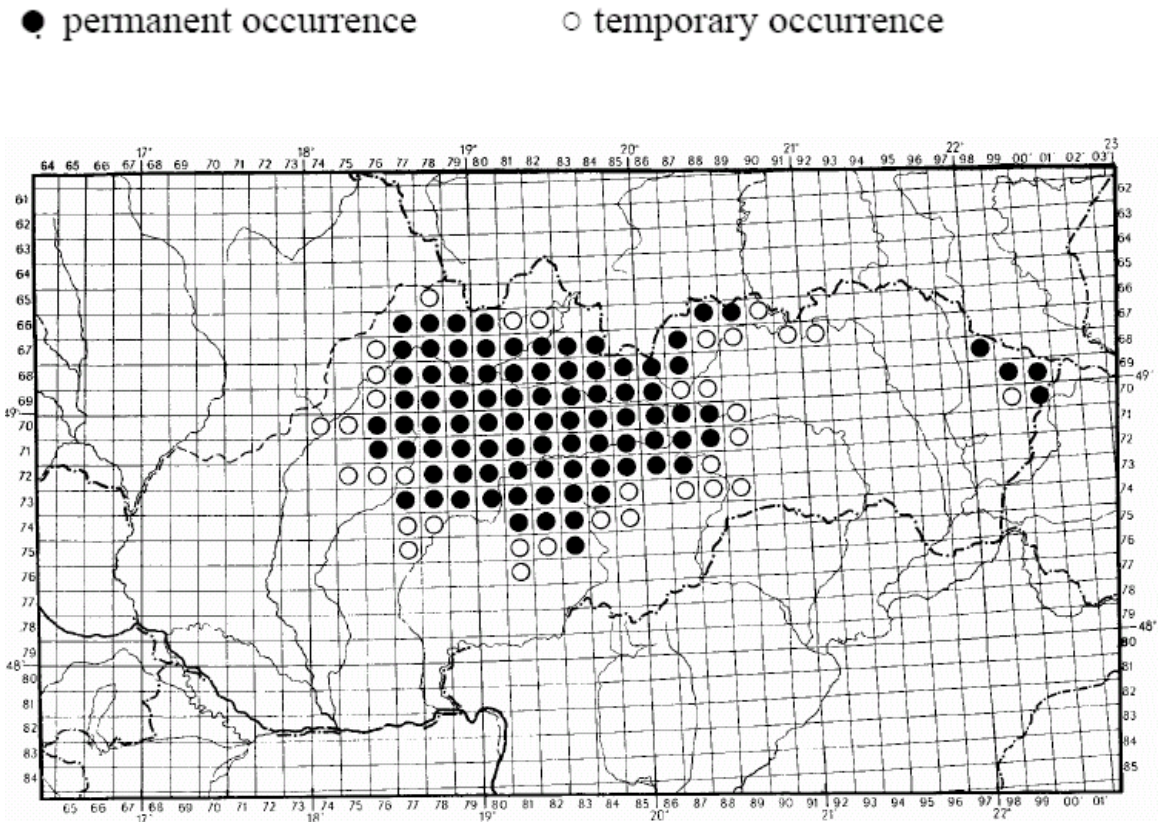

Fig. 6. Brown bear distribution in Slovakia in 2003. Source: State Nature Conservancy.

Fig. 7. View of Tatransky National Park. (Orig.)

6.2 Number of scat samples

133 scat samples were secured from bears in the wild for parasite analysis from 2007 and 2008 (thanks to Robin Rigg of the Slovak Wildlife Society). The scats are collected annually from March to November for an ongoing diet analysis programme (see Rigg, 2004, Rigg and Gorman 2006a, 2006b, Rigg & Adamec, 2007). The samples were refrigerated and frozen to -20°C until further analysis was possible. A small number of scats were also analysed fresh from the field. For analytical purposes, this number was further expanded by 55 additional samples from 2002 which had been previously analysed by Goldova *et al.* (2003). This gave an overall total of 188 samples across three different years.

6.3 Laboratory analysis of scats

All scats were examined qualitatively for helminth ova utilising standard flotation techniques according to Breza (1957). A limited number were also examined quantitatively utilising the McMaster technique (Gordon & Whitlock, 1939; Whitlock, 1948). Frozen samples were left to defrost for 24 hours prior to parasite analysis.

7 RESULTS

7.1 Intravital diagnosis of baylisascariosis of the brown bear (*Ursus arctos*)

All scats were examined qualitatively for helminth ova utilising standard flotation techniques according to Breza (1957). The ova found (Fig. 2) were compatible with those found in previous studies (see section 4.2.1 Eggs and larvae).

Morphological characteristics include;
Size: Medium, 70 x 54 µm
Shape: Globular to sub-globular
Shell Structure: Three thick shells
Internal Structure: Non embryonated, large protoplasmatic cell, homogenous structure
Colour: Dark brown

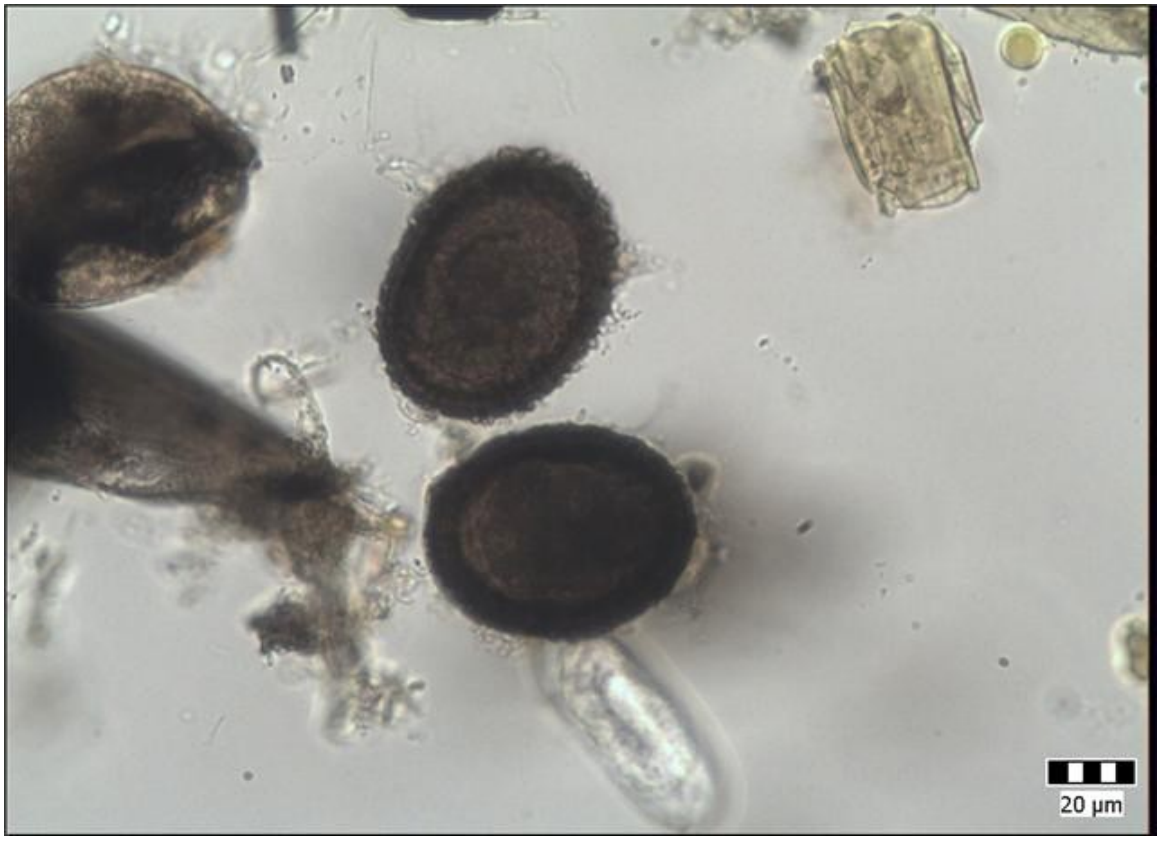

Fig. 2. *Baylisascaris transfuga* eggs, flotation method. (Orig.)

7.2 Overview of 2002

From the tables and graphs below (Figs. 8-9), it is evident that the prevalence increased throughout the year. The seasonal variation graph (Fig. 9) shows this most

clearly. When considering a month by month analysis (Fig. 8) some irregularities appear present, like the high prevalence in June. However upon further examination (Tab. 1) it is evident that there were only 4 scats available for analysis in June, which is too small of a sample size to generalise from. July and November also had very low sample numbers. The overall prevalence for the year was 25%.

Tab. 1. Monthly analysis for **2002**

2002	*Mar*	*April*	*May*	*June*	*July*	*Aug*	*Sept*	*Oct*	*Nov*	*Total*	*%*
Pos	0	1	0	2	0	0	3	6	2	14	25
Neg	0	12	14	2	2	0	8	3	0	41	75
Total	0	13	14	4	2	0	11	9	2	55	100

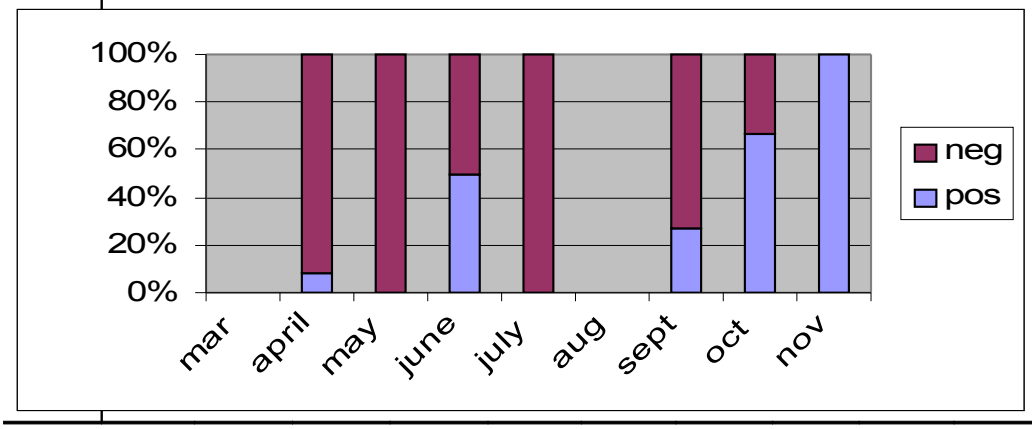

Fig. 8. Monthly analysis **2002**, see Table 1.

Tab. 2. Seasonal variation for **2002**

2002	*Spring*	*Summer*	*Autumn*	*Total*	*Percentage*
Positive	1 (3.7%)	2 (33.3%)	11 (50%)	14	25 %
Negative	26 (96.3%)	4 (66.7%)	11 (50%)	41	75 %
Total	27	6	22	55	100

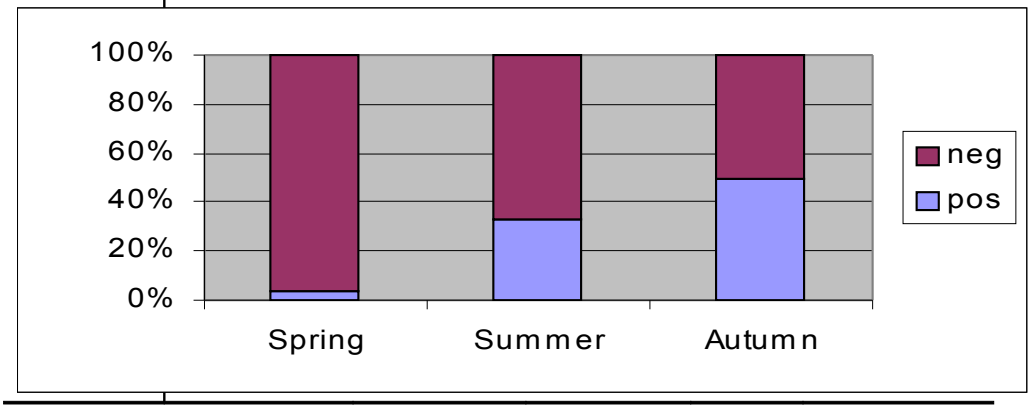

Fig. 9. Seasonal variation found in **2002**, see Table 2.

7.3 Overview of 2007

From the tables and graphs below (Figs. 10-11), it is evident that the prevalence increased steadily throughout the year. The seasonal variation graph (Fig. 11) shows this most clearly. When considering a month by month analysis (Fig. 10) a definite increase in the prevalence is evident. Upon further examination, however, (Tab. 3) it is evident that there were a low number of scats available for analysis in March, May,

and October, and none for April and November. The overall prevalence for the year was 42%.

Tab. 3. Monthly analysis for **2007**

2007	*Mar*	*April*	*May*	*June*	*July*	*Aug*	*Sept*	*Oct*	*Nov*	*Total*	*%*
Pos	0	0	0	1	3	11	7	5	0	27	42
Neg	5	0	2	13	10	7	1	0	0	38	58
Total	5	0	2	14	13	18	8	5	0	65	100

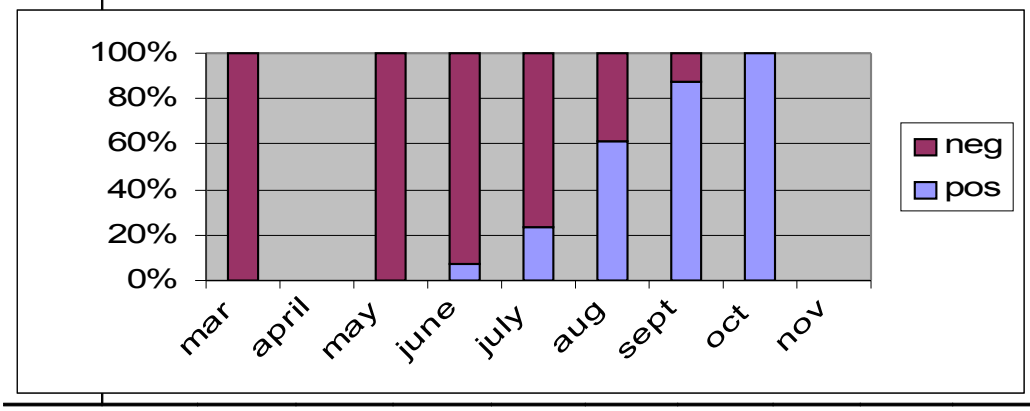

Fig. 10. Monthly analysis **2007**, see Table 3.

Tab. 4. Seasonal variation for **2007**

2007	*Spring*	*Summer*	*Autumn*	*Total*	*Percentage*
Positive	0 (0%)	15 (33.3%)	12 (92.3%)	27	42 %
Negative	7 (100%)	30 (66.7%)	1 (7.7%)	38	58 %
Total	7	45	13	65	100

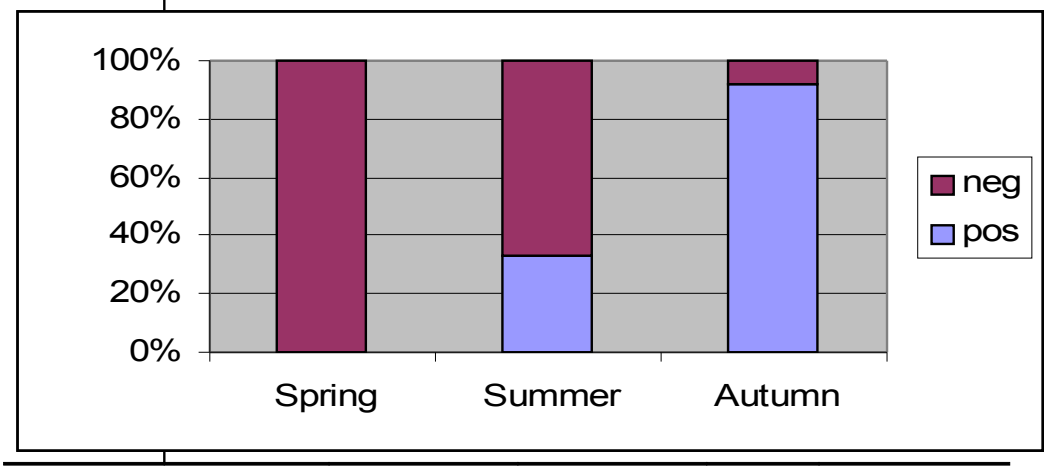

Fig. 11. Seasonal variation found in **2007**, see Table 4.

7.4 Overview of 2008

From the tables and graphs below (Figs. 12-13), it is evident that the prevalence increased throughout the year. When considering a month by month analysis (Fig. 12) some irregularities appear, like the high prevalence in March and April, and the complete lack of ova in April and July. However upon further examination (Tab. 5) it is evident that there were too few scats available for analysis in these months to generalise from. The summer prevalence appears quite high (Fig. 13) as most scats

were from August which is usually highly infected (see Fig. 10). The overall prevalence for the year was 69%.

Tab. 5. Monthly analysis for **2008**

2008	*Mar*	*April*	*May*	*June*	*July*	*Aug*	*Sept*	*Oct*	*Nov*	*Total*	*%*
Pos	2	0	1	0	0	16	12	7	9	47	69
Neg	3	1	1	0	1	6	3	4	2	21	31
Total	5	1	2	0	1	22	15	11	11	68	100

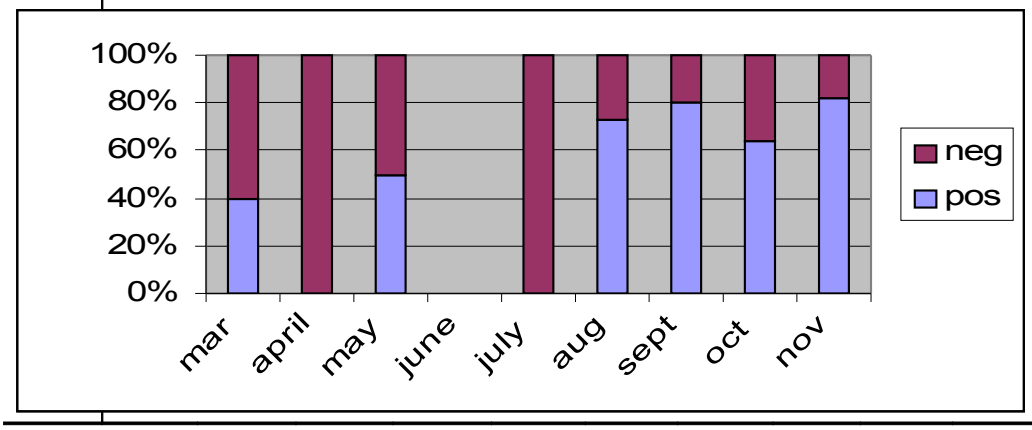

Fig. 12. Monthly analysis **2008**, see Table 5.

Tab. 6. Seasonal variation for **2008**

2007	*Spring*	*Summer*	*Autumn*	*Total*	*Percentage*
Positive	3 (37.5%)	16 (69.6%)	28 (75.7%)	47	69 %
Negative	5 (62.5%)	7 (30.4%)	9 (24.3%)	21	31 %
Total	8	23	37	68	100

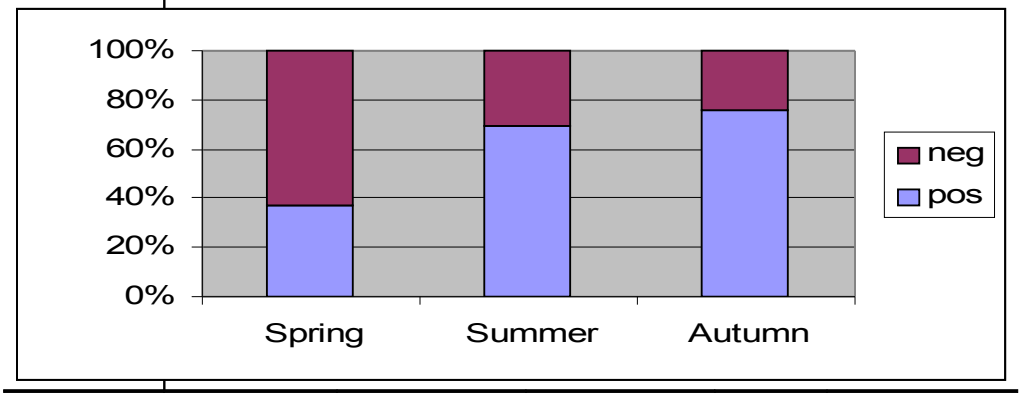

Fig. 13. Seasonal variation found in **2008**, see Table 6.

7.5 Combined Analysis

The monthly and seasonal trends continue to remain strong when all three years samples are combined. This is perhaps the best representation of the true prevalence of *Baylisascaris transfuga.* The minimum monthly scat count is now 10 (Tab. 7), which gives a more complete overall representation from a statistical viewpoint. From looking at the monthly analysis (Fig. 14) it is quite clear that the prevalence increases as the year progresses. This increase in prevalence is even more strikingly evident in the seasonal analysis graph (Fig.15). The overall average prevalence for all 3 years combined is 47%.

Tab. 7. Monthly analysis of 2002, 2007, and 2008 combined

3 yrs	*Mar*	*April*	*May*	*June*	*July*	*Aug*	*Sept*	*Oct*	*Nov*	*Total*	*%*
Pos	2	1	1	3	3	27	22	18	11	88	47
Neg	8	13	17	15	13	13	12	7	2	100	53
Total	10	14	18	18	16	40	34	25	13	188	100

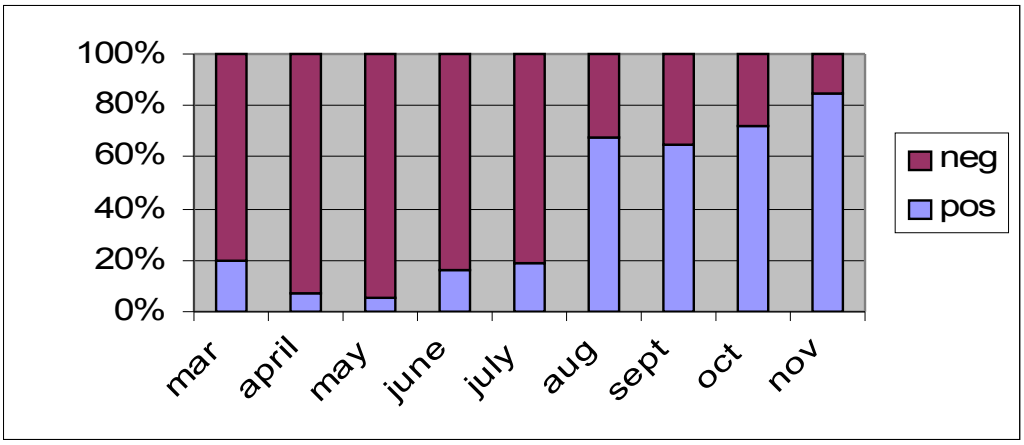

Fig. 14. Monthly analysis of 2002, 2007, and 2008 combined, see Table 7.

Tab. 8. Seasonal variation of 2002, 2007, and 2008 combined

2002, 07 & 08	*Spring*	*Summer*	*Autumn*	*Totals*	*Percentage*
Positive	4 (9.5%)	33 (44.6%)	51 (70.8%)	88	47 %
Negative	38 (90.5%)	41 (55.4%)	21 (29.2%)	100	53 %
Totals	42	74	72	188	100

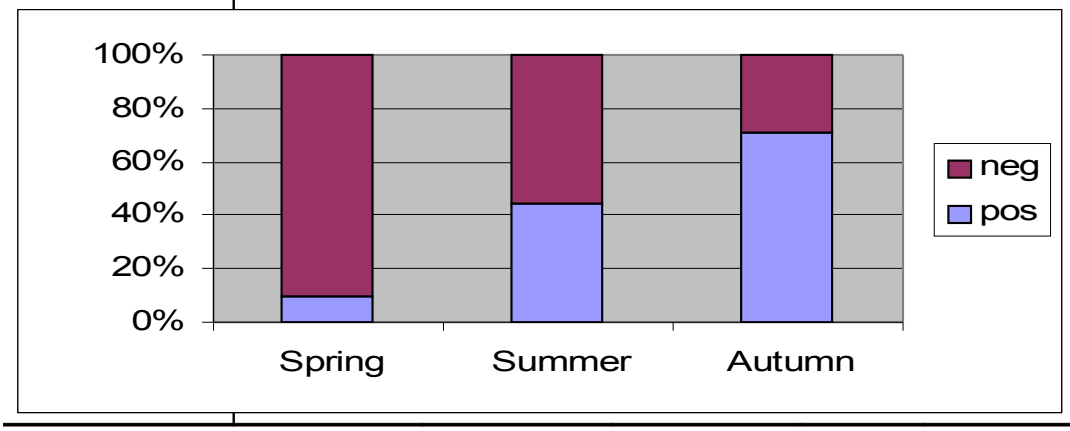

Fig. 15. Seasonal variation of 2002, 2007, and 2008 combined, see Table 8.

7.6 Prevalence and distribution

The prevalence appears to increase annually (Tab. 9, Fig. 16). However, this apparent increase can be explained by examining the number of scats collected per season over the three individual years (Tab. 10, Fig. 17). In 2002, where a particularly low overall prevalence was evident it is noted that more scats were analysed from spring, which would naturally herald more negative scats. In 2007, the majority of scats were available for analysis from the summer, which resulted in a close to average result. Whilst in 2008, the majority of the scats were collected in the autumn, which would explain the high prevalence evident. Average prevalence was 47%.

Tab. 9. Annual prevalence expressed as a percentage

Year	*2002*	*2007*	*2008*	*Average*
Prevalence %	25	42	69	47

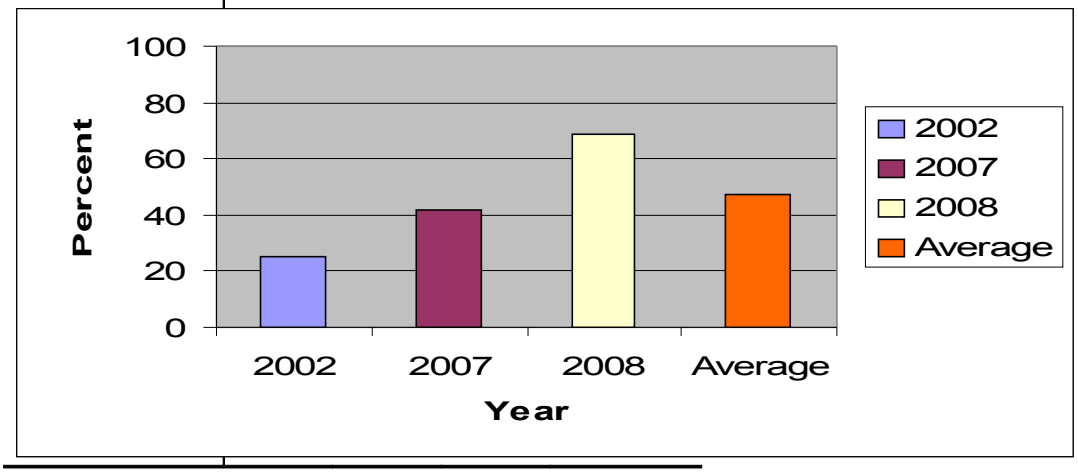

Fig. 16. Annual prevalence expressed as a percentage**,** see Table 9.

Tab. 10. Total number of samples collected each season

Year	*Spring*	*Summer*	*Autumn*	*Totals*
2002	27	6	22	55
2007	7	45	13	65
2008	8	23	37	68
Totals	42	74	72	188

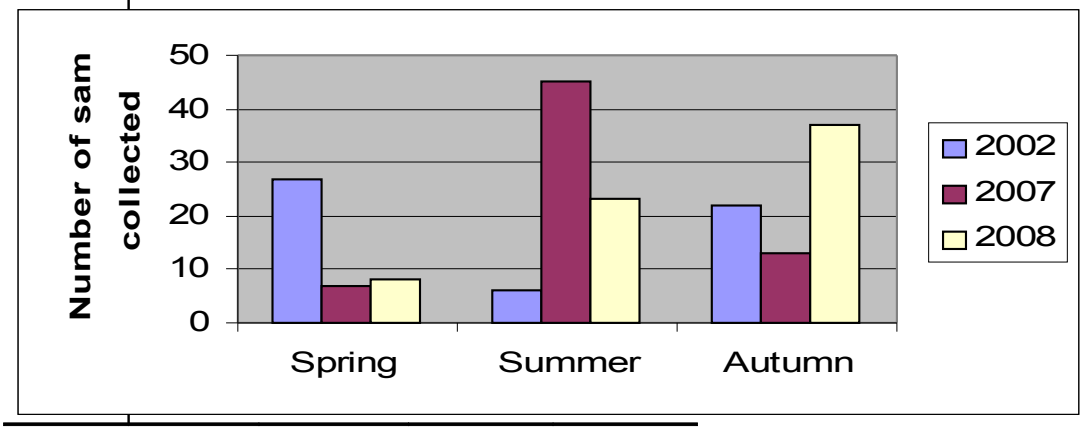

Fig. 17. Total number of samples collected each season, see Table 10

7.7 Intensity of infection

Although all samples were analysed qualitatively (flotation), only a sample number were analysed quantitatively (McMaster). This was due to constraints such as not always having enough scat available for both techniques. Table 11 and Figure 18 below show the relative findings. The decreased number found in October could be due to the low sample size available for analysis. The average number of eggs/gram of faeces was 480.

Tab. 11. Number of eggs/gram when analysed by McMaster technique

Month (2008)	*August*	*September*	*October*	*November*	*Average*
Pos. samples	16	12	7	9	44
No. analysed	6	12	3	9	30

Mean amount	467	679	133	561	480

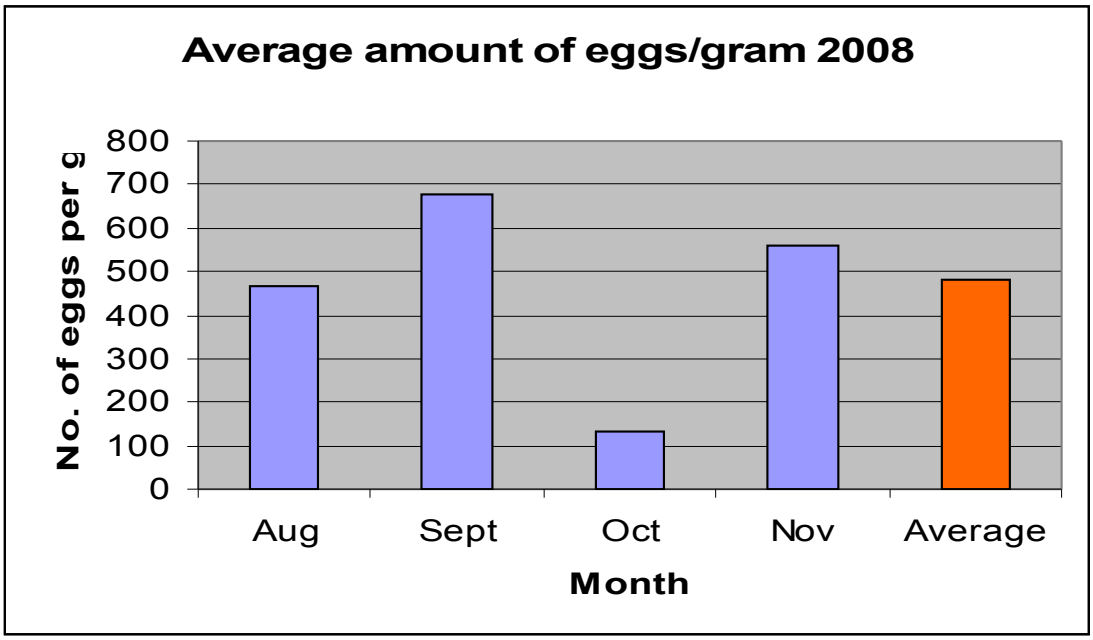

Fig. 18. Average amount of eggs/gram when analysed by McMaster technique.

8 DISCUSSION

Literature documenting the intestinal parasites of free-ranging populations of brown bears (*Ursus arctos*) in Europe is lacking. The majority of research in this field has been conducted in America and Canada (King, 1960; Rogers, 1975; Worley *et al*., 1976; Frechette & Rau, 1977,1978; Crum 1978; Manville 1978; Barnes & Rogers, 1980; Gau *et al*., 1999; Foster *et al*., 2004; Joyner *et al*., 2004). As bears in the wild are quite reclusive and have large home ranges, it is quite difficult to collect a large number of scats for parasite analysis. Many of the aforementioned studies from America and Canada were conducted during necropsy investigations from bears shot during the hunting season, and hence did not follow the yearly activity of the bears.

This study gives a unique insight into the seasonal variations of the intestinal nematode *Baylisascaris transfuga* across three individual years.

Some researchers have speculated that shortly before hibernation, bears void most of the adult endoparasites that derive nourishment from the host ingesta, Rush (1932), Rausch (1954, 1961) and Choquette *et al*. (1969). In these studies the presence of helminths in the feces was by no means a common occurrence, and may well have been an incidental finding. Gau *et al*., (1999) stipulates that the causal factors remain questionable.

Rodgers (1975) observed a wild bear pass two adult *B. transfuga* before it went into hibernation, he also found specimens of *B. Transfuga* in two scats found in October of the same year. However, it must be noted that these were the only helminths found in the macroscopic examination of 962 droppings examined between April and November of that year. Furthermore, over the last 8 years Robin Rigg of the Slovak Wildlife Society has examined over 700 bear scats during his work on diet analysis (personal comment, see also Rigg, 2004, Rigg & Gorman, 2006a, 2006b). He has never encountered a voided intestinal worm (personal comment). Hence evidence in support of bears 'ridding themselves' of intestinal worms is limited.

With these question in mind, a handful of studies have been carried out to assess the potential seasonal dynamics of the bear helminth *Baylisascaris transfuga*, and only limited evidence has been put forward in relation to the possible seasonality of this nematode (Clark *et al*., 1969; Rodgers, 1975; Frechette & Rau, 1978; Manville, 1978; Gau *et al*., 1999). Although by no means were all previous results clear cut or definitive, there was some evidence of a low prevalence in spring, and a higher prevalence in the autumn. None of the authors could come to a definite conclusion as to why this apparent seasonality occurred, indeed the complete life cycle of many *Baylisascaris* spp., is still unknown (Gutiérrez, 2000).

In the present study, 133 scat samples were secured from bears in the wild for parasite analysis from 2007 and 2008. The scats are collected annually from March to November for an ongoing diet analysis programme (see Rigg, 2004; Rigg & Gorman, 2006a, 2006b). For analytical purposes, this number was further expanded by 55 additional samples from 2002 collected from the same study area, which had been previously analysed by Goldova *et al.* (2003). This gave an overall total of 188 samples across three different years. It cannot be assumed that each scat collected was from a different bear, in fact as the same trails were sometimes used, its presumed that scat from an individual bear could have been sampled more than once. However, with a large sample size of 188, the implications should be minimal, and the prevalence could be skewed both positively and negatively in an equal fashion.

8.1 Seasonality

When looking at the resultant data on a year by year basis (Figs. 8-13, Tabs. 1-6) a definite seasonal trend is persistently evident, there were some irregularities, although this can be mostly explained due to insufficient scat sample numbers. When all the data from the three individual years were combined however (Figs. 14-15, Tabs. 7-8), the seasonal trend is most evident. This combined result is the best example, given the large data set available. If we compare the combined seasonal prevalence of spring (9.5 %), summer (44.6 %) and autumn (70.8 %) with previous studies, some interesting comparisons can be made.

One of the earliest records of helminths seasonality in bears came from Averin (1948) whom noted that in the Kamchatka regions (Russia) almost all bears were infested with ascarids, apart from during the spring, however, when ascarids were absent. Rogers (1975) carried out a study in the Lake Superior region, USA, and found that during the summer, 71% (5 out of 7) of intestinal tracts harboured *Baylisascaris transfuga*. Later in the year 2 intestinal tracts from denning bears that had died on 24th of November and 20th of March were examined and appeared free of helminths.

The prevalence of gastrointestinal parasites (*Diphylobthrium sp., coccidia, strongyles, and Baylisascaris* combined) in 56 faecal samples from grizzly bears (Brown bear, *Ursus arctos*) collected from the central Canadian Arctic spring and autumn 1995 and 1996 showed a 31% prevalence in spring, and a 58% prevalence in autumn (Gau *et al*., 1999). Serial samples were available from four individual bears; gastrointestinal

parasites were present in three of the four samples from the autumn 1995, whilst only one of the same four bear samples proved positive in the spring of 1996. In this study the overall prevalence of *Baylisascaris transfuga* was only 5%.

Eggs of *Baylisascaris transfuga* were detected in 64% (59 out of 92 faecal samples) of bears live-trapped during summer 1974 and summer 1975 (Manville, 1978). In the same study Manville (1978) also inspected viscera from bears shot during the hunting season (autumn) from the same area, during 1974 and 1975 and found that 89% were infected, indicating the higher prevalence in the autumn. In the present study, the summer prevalence (combined analysis) was found to be 44.6%, increasing in the autumn to 70.8% (Figs. 14-15, Tabs. 7-8).

The opposite trend was found during an analysis of faecal samples from black bears in Quebec showing that the prevalence of eggs of *Baylisascaris transfuga* was low in autumn (October and November) prior to denning (13%) but higher (42%) in spring (May), this result seems at odds with the seasonality theory, the authors suggest that this finding may indicate the maturation of overwintering larvae (Frechette & Rau, 1978). It must be noted that in the same study they also investigated the seasonality of another bear helminth, *Diphyllobothrium ursi,* and found a low prevalence in spring followed by a higher prevalence in autumn. Clark *et al*. (1969) found that the incidence of infection peaked from June to August and was reduced over autumn and winter.

Very few year long studies of bear parasites have been carried out, as a result of this is hard to make comparisons with the average annual prevalence of 47% found in this study (fig 9, table 9). Even in the present study, markedly different results were apparent over the three years, yet this was due to the majority of the samples being collected in a different season in a particular year (Figs. 16-17, Tabs. 9-10). Many previous studies on bear endoparasites did not specifically investigate the seasonality of *Baylisascaris transfuga*, choosing instead to concentrate solely on the prevalence of the nematode, irrespective of the time period involved. However, upon closer examination of this research, although specific dates are not always given, certain conclusions can be drawn with regards to overall prevalence and seasonal dynamics.

Baylisascaris transfuga were found in the small and large intestines of 62% (56 out of 91 black bears) during a study in northwestern Alberta, Canada, May 1976-September 1977 (Dies, 1979). *Baylisascaris transfuga* was found in the small intestines of 53% of bears during a survey of American black bears, *Ursus americanus* from six states in the southeastern USA, July 1973-November 1976 (Crum, 1978). The average prevalence of 47% found in this present study may appear lower because of the analysis of spring scats, whilst in the aforementioned studies it could be inferred that they were mostly inspecting hunter carcasses which would skew the results to a higher prevalence.

In a review by Worley *et al.* (1976) *Baylisascaris transfuga* were found in 75.7% (53 out of 70) of bears during a study of grizzly bears from Montana and Wyoming during the period 1968 until 1973. Once again no specific dates are supplied, but it specifies that bear carcasses were obtained from National Park personnel, predator control agents, and hunters, which could mean that bears examined were most likely to have been killed in the autumn (hunting season). This finding would be comparable to the autumn prevalence found in this study, 70.8 %.

8.2 Quantitative analysis

To the best of my knowledge, no quantitative analyse of bear scat has been completed to date. The results in this study show an average intensity of infection to be 480 eggs/gram of feces. However this average is only from a limited number of samples over a four month period (Fig. 18, Tab. 11), and hence I will not extrapolate too much from these results. The low October average could be mistaken for giving strength to the theory that before denning the bear rids itself of intestinal parasites, yet the higher egg count/gram of faeces in November clearly shows that this was not the case. This goes to show that large data sets are very important in order to get a fair viewpoint of the situation.

8.3 Hibernation and its effect on parasites

Rush (1932) and Rausch (1954, 1961) speculated that loss before, and reinfestation of intestinal parasites after hibernation, was mainly facilitated through changes in dietary items. However, in line with the findings of Gau *et al.* (1999), the diet of the bears in

this study were somewhat similar for the late autumn and early spring seasons (Rigg 2004, Rigg & Gorman 2006a). Choquette *et al.* (1969) was also sceptical of using changes in dietary items to explain the alleged elimination of parasites before hibernation. Frenchette and Rau (1978) maintained that the seasonal changes in parasite prevalence were due to a cessation of feeding during hibernation. Gau *et al*. (1999) concluded that it remains unknown whether a change in diet, both quantitatively and qualitatively, or some biochemical or physiological changes in the bears intestinal tract caused the seasonality of gastrointestinal parasites.

With respect to the present study, a review of the relevant literature was undertaken as to the metabolic and biochemical status of the bear during hibernation, and to the metabolic needs of the intestinal helminth to sustain life. The hibernating bear has a unique series of metabolic pathways which allow it survive for extended periods. Although no specific study has been undertaken to decipher the metabolic needs of *Baylisascaris transfuga*, comparisons from previous experiments on other nematodes was attempted.

Helminths are heavily reliable on carbohydrates (Von Brand, 1973), and conversely, the bear maintains a very slow carbohydrate metabolism during denning (Ahlquist *et al*., 1984). Glycogen plays an important role in providing energy to the worms (Afzal *et al.,* 1975) yet during hibernation the bear's glucose use was reduced (Ahlquist *et al.,* 1984). Adult *Ascaris lumbricoides* were found to have a high glycogen uptake of 1.3g/100 g of ascarid (Jíra 1998). The major metabolic activity in *Ascaris lumbricoides* was found to involve the glycolytic pathway (Entner & Gonzalez, 1959). This all points to the conclusion that because of the changes in the bears metabolism, the helminth will not be able to survive for an extended period.

Yet the situation is not quite as clear cut as it would appear, as the black bear (*Ursus americanus*) survives hibernation for up to five months whilst maintaining approximately normal blood levels of glucose, amino acids, and proteins (Nelson *et al.,* 1973, 1975). Additionally, it was found that hibernation activates glyoxylate cycle enzymes that allows dormant *Ursus americanus* to convert the fatty acid carbons from brown adipose tissue to glucose (Davis *et al.,* 1990). Furthermore the glycerol which is derived from the bear's fat stores and readily mobilized for systemic use appears to

serve as an active substrate for gluconeogenesis and lipogenesis during hibernation (Ahlquist *et al.,* 1984).

Taking all the above information into account, it appears that certain metabolic changes associated with bear hibernation, particularly the suppression of the carbohydrate metabolism, may well be enough to disturb the metabolic needs of the intestinal nematode and prohibit its survival. The question then arises as to where this dead helminth ends up? Another query might be as to why do a certain amount of spring scats remain infected with *Baylisascaris* eggs? Frechette and Rau (1978) put forward the motion that such a finding may indicate the maturation of overwintering larvae. In addition to this, some bears are known to have a relatively short hibernation, some wake up early, and some might not hibernate at all if suitable conditions exist (Pelikan, 1983; Rigg & Adamec, 2007). These factors could help explain the low spring prevalence.

8.4 Human health considerations

Epidemiologic patterns and host ecology influence the potential for infection and disease producing capabilities of *Baylisascaris* species. The most common etiological agent of *Baylisascaris* neural larval migrans or cerebrospinal nematodiasis is the raccoon roundworm, *Baylisascaris procyonis*. Although bears can be feral inhabitants of urban areas, they are not nearly as abundant as raccoons, have characteristically different defecation habits, and their larva are not as pathogenic. Raccoons commonly aggregate in large numbers and can be found living within or near human dwellings, domestic animal facilities, and zoos. Their highly discriminate defecation habits lead to localized accumulations of heavy egg burdens which amass over time at latrine sites (Schaul 2006).

In reference to the bear roundworm, *Baylisascaris transfuga*, the possibility of natural infection to humans is minimal, owing to its relative rarity in the wild, extensive home range, and due to the fact that it does not utilise communal latrine sites. *Baylisascaris transfuga* has been shown to have a lower pathogenicity than other *Baylisascaris* spp. (Boyce *et al*.,1989). Furthermore, no cases of human infection have ever been recorded. Yet it is potentially a zoonotic agent, and hence special care must be taken

when working with bears in enclosures like in zoos where the possibility of infection is much higher than in their natural environment.

9 CONCLUSION

As can be seen from the literature review and discussion above, only a limited amount of work has been done previously to show the seasonal dynamics of *Baylisascaris transfuga*. Whilst a number of authors have alluded to this phenomenon, no previous study has presented data consistent over several years. The results presented clearly show that a seasonal trend exists. The question of why such a seasonality exists is without doubt inextricability linked with the fact that the bear goes into hibernation each winter, and in doing so completely alters its homeopathic state, which in turn could certainly have adverse effects on intestinal nematodes.

Although a few previous authors have found voided helminths in the faeces prior to denning, this appears to be a very rare occurrence indeed. I suggest that the *Baylisascaris transfuga* nematode disintegrates in the bears intestines during hibernation due to lack of carbohydrates, and is possibly absorbed, or more probably voided during the first faeces (or plug). Rogers (1981) found that an intestinal 'plug' was formed during hibernation, comprising of faeces, dead intestinal cells, hair, and bedding material. Could this plug also enshroud the remnants of *Baylisascaris transfuga*? Further investigation is recommended.

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