

Aus dem Forschungsinstitut für Wildtierkunde und Ökologie
der Veterinärmedizinischen Universität Wien
(Leitung: O.Univ.Prof. Dr.rer.nat. Walter ARNOLD)

COPROSCOPY OF FRESH FECES SAMPLES OF FERAL HORSES
(EQUUS CABALLUS) IN THE DANUBE DELTA, ROMANIA

DIPLOMARBEIT

Zur Erlangung der Würde einer

MAGISTRA MEDICINAE VETERINARIAE

Der Veterinärmedizinischen Universität Wien

Vorgelegt von

Susanne Küker

Wien, im Juli 2013

Betreuer:

Univ. Prof. Dr. med. vet. habil., Christian Walzer
Arbeitsgruppe: Wildtiermedizin
Forschungsinstitut für Wildtierkunde und Ökologie

Gutacher:

Univ.-Prof. Dr.med.vet. Rene Van Den Hoven
Interne Medizin Pferde
Department/Universitätsklinik für Kleintiere und Pferde

Inhaltsverzeichnis

	Seite
1) Einleitung	04
2) Abstract	07
3) Introduction	08
4) Material and Methods	10
5) Results	12
6) Discussion	14
7) Conclusion	17
8) Deutsche Zusammenfassung	18
9) Acknowledgements	19
10) References	20

Einleitung

Equiden können mit einer Vielzahl an Parasiten im Magen- Darm- Trakt befallen sein, z. B. von einfachen Protozoen oder den komplexeren Würmer (Helminthen). Zu den häufigsten Vertreter der Helminthen bei Pferden gehört die Familie der Nematoden und Cestoden. Am meisten zu finden sind kleine Strongiliden (cyasthotomins); Ascariden (*Parascaris equorum*); Bandwürmer (*Anplocephala* sp.); große Strongiliden (*Strongylus vulgaris, edentatus and equinus*), und Madenwürmer (*Oxyuris equi*) (Rehbein et al., 2013; Nielsen, 2012; Matthews, 2011; Gasser et al., 2005). Weiter können *Halicephalobus gingivalis* und *Habronema spp.* und die zum Arthropoden gehörenden Dasseln (*Gastrophilis* sp.) auch regionale Bedeutung haben. Über die Prävalenz dieser Parasiten wurde weltweit ausführlich berichtet, dennoch besteht nach wie vor Forschungsbedarf.

Zunächst bezieht sich der Großteil der erhobenen Studien auf gehaltenen und domestizierte Pferde (*Equus ferus caballus*) und es gibt nur wenige Informationen über verwilderte Tiere oder wilde Przewalskipferde, die zwar eine Subspezies darstellen aber von den gleichen Parasitenfamilien betroffen sind (e.g. Bray et al., 1998; Young et al., 1999; Painer et al., 2011; Krecek et al., 1998; Rubenstein and Hohmann, 1989). Obwohl einige verwilderten Populationen (*Equus ferus caballus*) existieren (Safeguard of Agricultural Varieties in Europe, SAVE), liegen auch aus Europa kaum Publikationen vor (Slivinska et al., 2006; Kuzmima et al., 2006). Zudem gibt es keinerlei Studien über die Population wildlebender Pferde im Donaudelta (Rumänien).

Da die meisten domestizierten Pferde selten in naturnahen Herdverbänden gehalten werden, wurde der Großteil der Untersuchungen an Tieren in speziellen Gruppen-Zusammenstellungen erhoben. Dabei kann jedoch gerade die Altersstruktur einer Herde einen erheblichen Einfluss auf die Epidemiologie von Parasiten haben (z. B. *Parascaris equorum*). Diese Zusammenhänge sind wichtig für das Management von Gruppenhaltungen oder größeren Zuchtbetrieben, in denen die Herdenstruktur eher einer natürlicheren Zusammenstellung entspricht.

In Anbetracht der globalen Untersuchungen, gibt es nur wenige Informationen über die Prävalenz von Parasiten aus Gebieten, in denen Parasitenbehandlungen nicht üblich sind. So wurden z. B. in Teilen von Osturopa nur wenige Studien erhoben: in der ehemaligen

Sowjetunion (Petrov et al., 1954) und später im heutigen Osteuropa (Széll et al., 1999; Kuzmina et al., 2005, 2006 & 2012; Gawor, 1995; Uslu et al., 2007; Papazahariadou et al., 2009; Königova et al., 2001). Um jedoch vor Ort zukünftig ein selektives und spezifisches Parasitenmanagement entwickeln zu können und mögliche Anthelmintica Resistenzen hinauszuzögern, ist ein erweitertes Wissen über die parasitäre Fauna aus den betreffenden Regionen notwendig. Dabei müssen auch die jeweiligen klimatischen Bedingungen berücksichtigt werden, welche einen Entscheidenden Einfluss auf die Dynamik zwischen Wirt und Parasit haben (Cornell et al., 2008; Martin et al., 2008; Turner et al., 2010; Nielsen et al., 2007). So unterscheidet sich z. B. die Entwicklung von parasitären Stadien in gemäßigten Zonen deutlich von aridem Klima. Im Gegensatz zum gemäßigten Klima, weisen in trockenen Gebieten Strongiliden bereits mit Beginn des Frühlings einen Anstieg der Entwicklung auf, welcher aufgrund der trockenen Verhältnisse im Sommer rückläufig ist (Nielsen et al., 2007).

In den letzten fünf Jahrzehnten hat der regelmäßige Gebrauch von Anthelmintica in einigen Ländern die Prävalenz von Parasiten deutlich beeinflusst. So war zum Beispiel in Nordamerika bis in die neunziger Jahre *Strongylus vulgaris* zu 90-100% prävalent, während heute hauptsächlich nur noch kleine Strongiliden vorkommen (Kaplan et al., 2010; Chapman et al., 2002). In diesem Zusammenhang können Untersuchungen von wild lebenden, nicht behandelten Tieren hilfreich sein, um die Parasiten-Wirts Beziehung besser zu verstehen und gegebenenfalls die Behandlungsstrategien anzupassen.

Das Ziel dieser Studie war es, Daten über Ei ausscheidende gastrointestinale Parasiten bei wildlebenden und natürlich infizierten Pferden aus dem Donaudelta in Rumänien zu gewinnen. Um den Kot auf parasitäre Eier zu untersuchen und diese zu zählen, wurde die McMaster Technik mit der kombinierten Sedimentation-Flotationsmethode angewendet. Dies ist bisher die sensitivste Methode insbesondere für geringe Eizahlen (<50 Eier/g) und wird häufig im Parasitenmanagement genutzt (Stratford et al., 2011). Pferde variieren stark in der Ausscheidungsfrequenz von parasitären Eiern, ca. 20% einer Population kontaminiert die Weiden mit 80% der gesamten Eiausscheidung (Wood et al., 2013). Mit Zählmethode des McMasterverfahrens können signifikante Eiausscheider zur parasitären Behandlung auffindig

gemacht werden, Tiere mit einer Ausscheidungsrate von 200-500 Eiern/g werden in der Regel behandelt. Es sollte an dieser Stelle beachtet werden, dass die Ausscheidungsrate nicht die reale Wurmbelastung widerspiegelt (Startford et al., 2011).

Es wird erwartet, dass die Tiere mit Strongiliden, Bandwürmern, Ascariden, Madenwürmern und mit Leberegel (*Fasciola hepatica*) infiziert sind. Von ähnliche klimatischen Einflüssen auf die parasitäre Entwicklung gemäß arider Regionen wird ausgegangen. Da bisher keine Publikation in anerkannten Fachzeitschriften veröffentlicht wurde, ist dies die erste Studie über die Prävalenz von Parasiten bei Pferden aus der genannten Region.

Abstract

To investigate the prevalence of endoparasite infestations in a feral horse population roaming in the Danube Delta in Rumania, 96 fresh fecal samples were collected in May and November 2012. The combined sedimentation flotation technique was used to find worm eggs, additionally a bulk sample were examined with the Benedek`s method. The number of Strongyle eggs were determined by e modified Mc Master method. In this study, only eggs from strongyles and acarids were found in the feces. All horses were infected with strongyles, whereas the mean prevalence of *Parascaris equorum* infestation was 22%. Fecal egg counts showed variability in strongyle egg excretion and differed significantly ($p= 0.05$) between May and November, which might have been influenced from the previous winter. The present study underlines the phenomenon of a potentially over-dispersed worm burdens and demonstrates the high prevalence of strongyles in naturally infected horses

Keywords feral horses, parasites, Romania, prevalence, fecal egg count, Danube Delta

Introduction

Equines can host a variety of parasite species in their gastro intestinal tract, from simple protozoans to the very complex worms (helminthes) and arthropods (*Gastrophilus* sp.). The most ubiquitous helminthes are nematodes and cestodes. Mostly small strongyles (cyathotomins); ascarids (*Parascaris equorum*); tapeworms (*Anoplocephala* sp.) large strongyles (*Strongylus vulgaris* and *edenatus*) and pinworms (*Oxyuris equi*) are reported (Rehbein et al., 2013; Nielsen, 2012; Matthews, 2011; Gasser et al., 2005). The occurrence and often the prevalence of these parasites have been previously documented worldwide, but still more research is at a local level is needed.

Most of the studies on the prevalence of the named parasites have been performed in domestic horse populations (*Equus ferus caballus*) and only a few studies were carried out in feral populations or in wild Przewalski horses *Equus ferus przewalskii* (Bray et al., 1998; Young et al., 1999; Painer et al., 2011; Krecek et al., 1998; Rubenstein and Hohmann, 1989), which are in fact a separate subspecies, but hosting same parasite families.

While several feral groups exist in Europe (Safeguard of Agricultural Varieties in Europe, SAVE), limited investigations have been performed on them (Slivinska et al., 2006; Kuzmina et al., 2006) and nothing is known about the parasite status of the feral horse population in the Danube Delta, Romania.

Because most domestic horses are rarely kept in natural herds, previous studies are constrained by specific (artificial) group compositions and the usual treatment recommendations are basing on these results. However, especially age structure in herds can affect the epidemiology of parasites (e.g. *Parasacaris equorum*) differently. This might be of relevance for management systems with other group compositions like stud farms or wild equids in captive husbandry.

Compared to developed countries, there is a lack of information concerning the prevalence of parasites from areas, in which worm treatment is not regularly conducted. For instance, in parts of East Europe just few investigations have been performed in the Soviet Union (Petrov et al., 1954) and later in former Eastern Europe (Széll et al., 1999; Kuzmina et al., 2005, 2006 & 2012; Gawor, 1995; Uslu et al., 2007; Papazahariadou et al., 2009; Königova et al., 2001). To establish selective and specific endoparasite management program

and delay probable future antihelminthic drug resistance, more information about the parasitic fauna and its biology of the respective region is necessary. Thereby, the climatic circumstances must be considered as this strongly affects the host-parasites dynamics (Cornell et al., 2008; Martin et al., 2008; Turner et al., 2010; Nielsen et al 2007). For example, the development of parasitic stages in temperate climate differs markedly from arid zones. In contrast to temperate areas, L3 larvae of strongyles in hot climates survive well in winter and spring but are killed by dry heat in the summer due to the dry conditions (Nielsen et al., 2007).

In the last 5 decades regular usage of anthelmintics in many countries has changed the prevalence and diversity of some parasites in domestic horses (Kaplan and Nielsen, 2010; Nielsen et al., 2010; Gasser et al., 2005). For instance, in North America, *Strongylus vulgaris* have been 90-100% prevalent in the nineties (Lyons et al., 1999), whereas today cyathotomins (small Strongyles) are the principle parasitic pathogen (Kaplan et al., 2010; Chapman et al., 2002). In this context, studies on feral populations can bring better understanding of host-parasite relation and may affect the strategy of anti-parasite medication.

The aim of the present study was, to obtain data on the prevalence of fecal egg excreting, gastrointestinal parasites of naturally infected feral horses in the Danube Delta, Romania. We used the McMaster technique for fecal egg counts (FEC) with the combined sedimentation flotation technique for detecting and counting parasitic eggs. It is the most sensitive method, especially for low egg numbers (i.e. detection limits at <50 eggs/g), which is today commonly used for parasitic management (Stratford et al., 2011). Horses highly differ in egg shedding and about 20 % of a population is contaminating the pasture shedding the main amount of 80% of the eggs (Wood et al., 2013). With FEC significant shedders can be detected for anthelmintic treatment, individuals above a cut-off of 200–500 eggs/g are usually considered for treatment (Stratford et al., 2011). It should be noted that FECs do not correlate with the real worm burdens in individuals.

We expected horses to be infested with strongyles, tapeworms, ascarids, pinworms and liver flukes (*Fasciola hepatica*). Previously described climate conditions in studies in arid regions (Nielsen et al., 2007) were expected to affect parasite development and L3 larvae survival. The current study is, to our knowledge, the first study on the prevalence of egg excreting gastro-intestinal parasites in horses from this region.

Material and Methods

Study Area

The Danube Delta is part of the largest continuous marshland after the Volga Delta in Europe as it covers more than 5400 km². It was declared a UNESCO- biosphere reserve in 1990 (IUCN, 1991) and is characterized by a number of lakes and reedbeds, which are constitute floating or fixed islands, as well as forested patches on sandy soil. The Delta is interconnected by a huge network of ditches and channels and is bordered by arable lands to the north, the Black Sea to the east, agricultural areas and steppes with small rocky hills to the west and by a brackish lagoon system to the south. Forest cover amounts to 6%, mainly seasonally flooded. Non-flooded, continental type hardwood forests are located on Letea (2,825 ha) and Caraorman islands, covering less than 1% of the whole delta whereas true wetland covers 92%. (First records of the pine marten (*Martes martes*) in the Danube Delta) Kiss et al., 2012). The main vegetation in this hydrological ecosystem are reeds (mostly *Phragmites australis*), *Juncus* sp. and *Carex* sp. vegetation in the small wetlands in north and northeast, as well as dry short grass steppe, scattered bushy vegetation and pine plantations (*Pinus nigra*) In non flooded areas (IUCN, 1991). The climate is dry-temperate, continental and the Delta itself is one of the driest areas in Romania with mean annual precipitation of 345mm (Vespremeanu-Storet et al., 2007).

Feral horses were pasturing in the community of Letea (N 45 20.629 E 29 35.331), a 4500 ha non-flooded island bordered by water channels. All horses had similar exterior and were colored from black to brown. The population was estimated up to 800/900 individuals. Other grazers were sheep (*Ovis orientalis aries*) and feral domestic cattle (*Bos primigenius taurus*).

The area seemed to be overgrazed, a problem already known since 1990 (IUCN, 1991). There is no direct human interaction with the animals besides singular capture attempts for slaughter.

Animals and parasitological examination

The feral horses showed shy behavior and were therefore observed from distance with binoculars. All animals were in a fair body condition and seemed to be in a good health status (Henneke et al., 1983) also no sign of diarrhea were found in the sampled area. Due to their similar exterior and the considerably population size, it was not possible to identify individuals, nor their sex or age. There was no evidence that the equids had received an anthelmintic treatment in the past 20 years.

In May and November 2012, a total of 100 fresh fecal samples from horses were collected from the ground. It was not possible to estimate the time between dropping and collecting, therefore only fresh feces were used. Pellets with moist surface were considered as fresh. To avoid contamination with soil nematodes, only the inner part of the pellets were used for the examinations. The samples were directly stored in a solution of sodium acetate - acetic acid and formalin until the analyzing procedure (15g sodium acetate, 20ml acet acid, 40ml of 37% formalin and 925 ml tap water).

Each individual sample was examined with the modified combined sedimentation-flotation technique (modified Stoll method, zinc-sulfate solution 331g/1l of aqua font; Schnieder et al. 2000). Fecal egg count (FEC) was determined with the McMaster technique (Schmidt, 1971). Additionally, a bulk sample of the feces was investigated with the Benedek's method for trematodes. Eggs were identified under light microscopy (100x magnification) based on morphologic characteristics (Thienepont, 1990). Samples were positive if at least one egg was present in one of two slides per sample. Using the student's t-test, means of FECs were compared between May and November. Daily temperature and precipitation from the year 2000- 2012 were analyzed to monthly means and compared (European Climate Assessment & Dataset, ECA&D) to investigate possible influences on the FEC. All statistical analysis was run in R version 2.15.2 (R Core Team, 2012), using the package Rcmdr (Fox, 2005), and a p value $\leq 0,05$ was considered as significant.

Results

One hundred samples were collected in May (n=46) and November (n=49) 2012. Four were discarded due to damage during transportation. Eggs of *Strongylus spp.* and *Parascaris sp.* were found. The main finding was *Strongylus sp.* with a prevalence of 100%. The prevalence of *Parascaris equorum* was 17,3 % in Mai and 26,5% in November. Eggs of *Anopolcephala sp.*, *Oxyuris equi*, and trematodes were not found. Also *Eimeria leuckarti* was not present. The individual horses showed a significant difference in fecal egg output (Fig. 1). The average number of eggs was 141 eggs per gram (epg) with a range of 0-422 epg. When comparing mean epg in May and November, fecal egg excretion was higher in November (p=0,05). The temperature and precipitation data showed frost and thaw and little snow cover from January to March 2012 (Fig. 3). Monthly mean precipitation and temperatures were calculated in Fig.4).

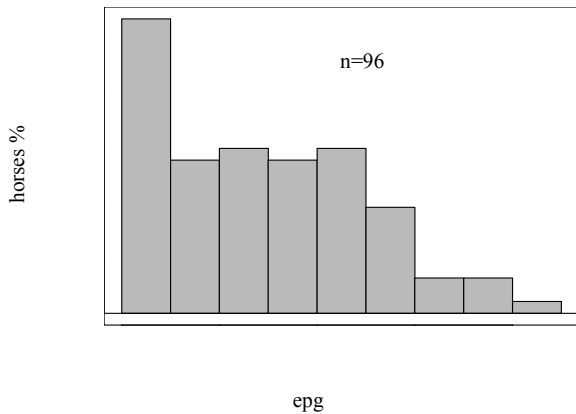


Fig. 1 Frequency distribution of feral horses (n=96) shedding strongyle egg (eggs per gram; epg) (n=96) during a spring and autumn in the Danube Delta, Romania 2012

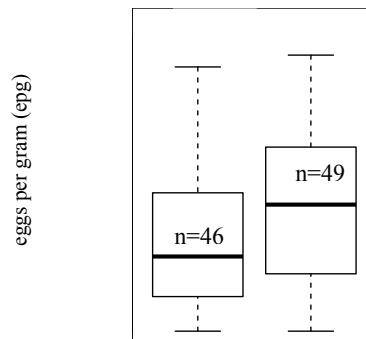


Fig. 2 Boxplot of fecal egg count from feral horses in the Danube Delta during May and November 2012. Median and 1st and 3rd percentile are shown

Fig. 3 Temperature and snow cover from the 1st of January to the 12th of March 2012, showing 10 frost - thaw alterations and a maximum snow cover of 4,4 mm

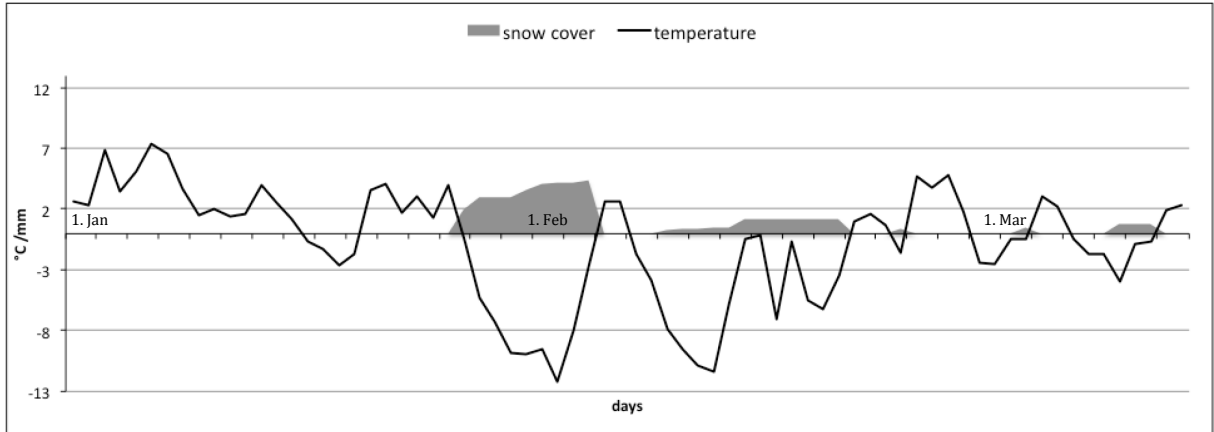
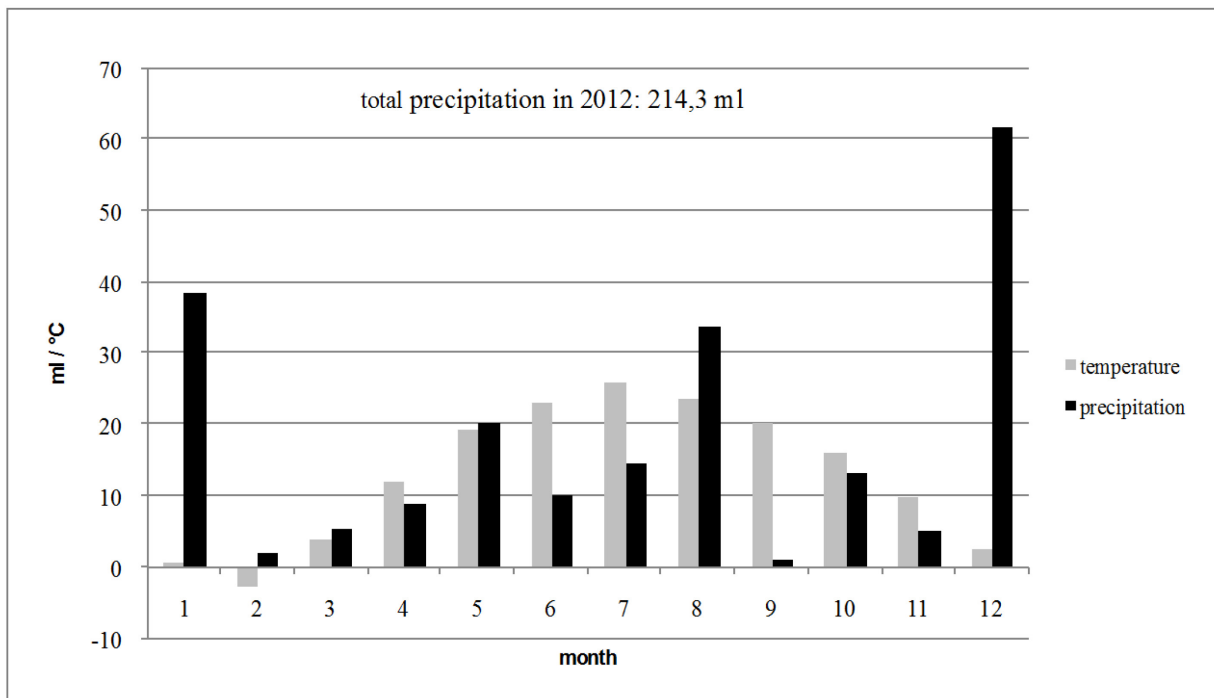


Fig. 4: Monthly mean temperature and precipitation in the Danube Delta in 2012 (ECA&D; ml/m²). The total precipitation in 2012 was 130,7 ml below the mean total precipitation (345ml; Vespremeanu-Storet et al., 2007).



Discussion

This study estimated the prevalence of gastrointestinal helminthes egg excretion in naturally infested feral horses in the Danube Delta, Romania. Strongyles eggs were predominant with a prevalence of 100% excreting animals, which is comparable with previous global findings (Hinney et al., 2011). The individual horses showed differences in fecal egg output (Fig. 1), which is similar to other findings (e.g. Crofton, 1971; Sréter et al., 1994; Galvani, 2003; Wood et al., 2013). Around 47% of the horses were significant egg shedders (epg >200; Startford et al., 2011). The environment probably has a considerably influence to the FEC frequency. The horse and cattle population occurred in a high density, which increases the risk of cross infections. Also overgrazing leads to an increased pressure of infection as horses are forced to graze closer to areas of fecal contamination and more likely ingest nematode larvae (Wood et al., 2013). It might therefore be questionable, if a universal cut off level should be used for every region in general.

Because of the dry summer (Fig.4), hatched larvae are expected to be reduced or killed by light and drying out and thus lower FECs were expected in autumn. Moreover, in autumn, it is likely that a proportion of L3 consumed would encyst in the large intestinal wall resulting in a downstream reduction in FECs, especially once resident adult worms reach the end of their lifespan (Wood et al., 2013). However, a significant higher mean FEC in November was demonstrated. It was not possible to differ between individuals and resampling of individuals might have influenced the results falsely. Also, strongyles may have found a refugium in a susceptible group of the population for the dry period. Furthermore, a kind of paradox hypobiotic stage of the small strongyles may have occurred, a phenomenon normally occurring during winter or spring in horses. It was not possible to incubated for larvae culture and determinate stongyle species. Hence, it is not possible to report which strongyle species had infested the horses. However, seasonal dynamics between parasites and hosts are well described (Wood et al., 2013; Polley, 1986; Ogbourne, 1975; Crofton, 1954), but the climatic impact on pasture contamination from one grazing season to the next is still not completely understand (Nielsen et al., 2007). Hayward et al. (2009) found significant higher FECs in

sheep due to markedly warmer and wetter weather in winter. The opposite correlation (cold and dry winters causing lower FEC) in following spring is therefore likely and could explain the present finding. The winter in 2012 (January to March) was considerably colder than usual. The average temperature in February 2012 was 4,32° below the average February temperature recorded between 2002-2011 (ECAD). While the infective larval stage and the eggs of strongyles can resist freezing temperatures, the alteration between frost and thaw, present in the winter 2012, can have deleterious effects (Nielsen et al., 2007). This holds especially true with little snow cover, also present in the winter 2012 (Fig. 3). A study in the central Ukraine came to the same conclusion in this context. Kuzmina et al. (2006) found a complete elimination of infective larval stages and eggs from soil under disintegrated fecal pats during a cold winter. To their opinion, this was caused by sporadic snow covering and the alternating freezing and melting of feces on pasture. Infectious larvae might therefore survived the winter 2012 in an energetically depleted state and subsequently were less infective in the following spring as demonstrated by the low FEC in May 2012 (Nielsen et al., 2007).

The prevalence of *Parascaris equorum* doesn't differ from other studies. Results with the sedimentation/ flotation technique have shown an overall ascarid prevalence from 1% to 26% in eastern Europe (e.g. Poland: Gawor et al., 1995; Hungary: Széll et al., 1999; Turkey: Uslu et al., 2007; Greece: Papazahariadou et al., 2009; Slovakia Republic: Königova et al., 2001). This is mirrored in other regions of the world (e.g. Germany: Epe et al., 2004; Spain: Francisco et al., 2009; Brazil: Pereira et al., 2006; India: Maria et al., 2012; Iran: Tavassoli et al., 2010; Ethiopia: Ayele, 2010; Australia: Brucknell et al., 1995; Kentucky: Lyons et al., 2000; Netherlands: Borgsteede & van Dyke, 1998; Mongolia: Painer et al., 2011), as ascarid eggs are predominately shed from young horses following a 75-80 days prepatent period while developing immunity (Clayton, 1986). The markedly increased prevalence in November in this study can be explained by a higher proportion of foals in the population and accordingly more of their feces being sampled.

Tapeworms are known as common intestinal parasites of equids (Höglund et al., 1998; Gasser et al., 2005) and high prevalence have been reported worldwide. However, in this study no evidence of *Anoplocephala* sp. was found. The lack of this species in the present study may be due to the possible absence of the orbitidae mite, its intermediate host in the biotope. The

occurrence of the tapeworm and accordingly the intermediate host has previously been reported from neighboring countries, but the specific local microclimates favorable for the mites likely changes quickly. The low sensitivity of the fecal flotation technique could also explain that eggs of this species were not identified (Skotarek et al., 2010; Traversa et al., 2008). Although the intermediate host *Galba truncatula* (Syn. *Lymnaea truncatula*) occurs in the Danube Delta (Cioboiu, 2006) and predisposing factors such as common pasturing with cattle and infections with strongyles were present (Nelis et al., 2010), trematodes were not found. Coproscopic examinations are described as inadequate for diagnosis, as horses rarely develop patent infections and therefore most flukes do not reach a generative stage (Nelis et al., 2010; Acici et al., 2013; Alves et al., 1988). Furthermore, the summer 2012 in the Danube Delta was very dry (average precipitation from May to November was 11,1 ml) and humidity is the essential factor for the development of this parasite and its intermediate host (Nelis et al., 2010; Campe et al., 2011). Hence the absence of liver flukes in this study should be considered carefully.

No eggs of pinworms could be found. *Oxyuris equi* is described as an endoparasite in mostly young horses (Durham and Coles et al., 1988; Reinemeyer et al., 2010) but infections with pinworms appear to be more rarely. On the other hand eggs are not shed regularly with the feces making periodic fecal examinations unsuitable for diagnosis (Reinemeyer et al., 2010). Similarly, *Eimeria Leuckarti* organisms were not found. It should be noted, that infections of *Coccidia* might be undetected due to the irregular presence of organisms in the feces, especially at lower infection loads (Studzinska. et al 2008). Furthermore, this species is a very uncommon cause of problems in horses, so even if small numbers were present the clinical relevance is absent.

Conclusion

According to their appearance, domestic horses in the Danube Delta seem to be healthy and therefore cope well with their parasite load. This is in contrast to the findings reported for seasonally free-ranging domestic horses in Mongolia (Painer et al., 2011). Possibly, the social structure (rather the grazing strategy) of the feral horse population and an intact co-evolved endoparasite population in these feral horses limits actual parasitic disease. The immune system of these animals may be better adapted to the parasitic burden when compared to horses in conservative management systems, as the individual immune response is related to the individual environment exposure (Cornell et al., 2008). More research into these host-parasite interactions, the climatic influences and the use of anthelmintics appears warranted. The equine parasitic fauna in the Danube Delta could constitute a valuable comparative resource in this regard.

Zusammenfassung

Zur Erhebung der Prävalenz von endoparasitären Infektionen einer verwilderten Pferdepopulation im rumänischen Donaudelta wurden im May und November 2012, 96 frische Kotproben gesammelt. Eier wurden mithilfe des Kombinierten Sedimentations-Flotations-Verfahren nachgewiesen, zusätzlich wurde an einer Sammelprobe die Methode nach Benedek durchgeführt. Strongilideneier wurden mit dem modifizierten McMaster-Verfahren gezählt. Strongiliden und *Parascaris equorum* waren die einzig vorhandenen Spezies. Alle Pferde waren mit Strongiliden infiziert, die allgemeine Prävalenz von *Parascaris equorum* betrug 22%. Die durchschnittliche Eizahl pro Gramm unterschied sich im May und November ($p=0,05$), was möglicherweise durch den vorangegangenen Winter beeinflusst wurde. Die vorliegende Studie unterstreicht das Phänomen unterschiedlich hoher Eisausscheidungsraten von Individuen und die hohen Prävalenz von Strongiliden in natürlich infizierten Pferden.

Acknowledgements

I wish to thank R. Hengel, O. Rosu and the family of A. Kiselev for their great support as well as the Research Institute of Wildlife and Ecology for its financial support. Special thanks to Dr. B. Hinney for her friendly instructions and advises, also K. I. Nicholson for reviewing this study. Thanks to B. Piédallu and everyone else, who was somehow involved.

References

- ACICI, M., BOLUKBAS, C. S., GURLER, A. T., UMUR, S., BUYUKTANIR, O. (2013): Seroprevalence of fasciolosis in equines of the Black Sea region, Turkey. *J Equine Vet Sci* **33**, 62-66
- ALVES, R. M., VAN RENSBURG, L. J., VAN WYK, J.A. (1988): Fasciola in horses in the Republic of South Africa: a single natural case of Fasciola hepatica and the failure to infest ten horses either with F. hepatica or Fasciola gigantica. *Onderstepoort J Vet Res* **55** (3), 157-163
- AYELE, G. DINKA, A. (2010): Study on strongyles and parascaris parasites population in working donkeys of central Shoa, Ethiopia. *Livest Res Rural Devel* **22** (12)
- BELLO, T.R, ABELL, J.E. (1999): Are equine tapeworms an emerging disease? A retrospective study. *J Equine Vet Sci* **19** (11), 723-727
- BRUCKNELL, D. G., GASSER, R. B., BEVERIDGE, I. (1995): The prevalence of gastrointestinal parasites of horses in Victoria, Australia. *Intern J Parasitol* **25** (6), 711-724
- BORGSTEEDE, F. H. M., VAN BEEK, G. (1998): Parasites of stomach and small intestine of 70 horses slaughtered in the Netherlands. *Vet Quarterly*, **20**(1), 31-34
- CAMPE, J., VYT, PH., DUCHEYNE, K.(2011): Leverbot op een Belgische stoeterij | [Fasciolosis in horses on a Belgian stud farm]. *Vlaams Diergeneeskundig Tijdschrift* **80** (6), 403-406
- CHAPMAN, M. R., FRENCH, D. D., KLEI, T. R. (2002): Gastrointestinal helminths of ponies in Louisiana: A comparison of species currently prevalent with those present 20 years ago. *J Parasitol* **88** (6), 1130-1134
- CIOBOIU, C. (2006): Diversity of Gastropoda in the Romanian sector of the Danube lower hydrographic basin. *Proceedings 36th International Conference of IAD. Austrian Committee DanubeResearch / IAD, Vienna. ISBN 13: 978-3-9500723-2-7 pp.230-235*
- CLAYTON, H. M. (1986): Ascarids: Recent advances. *Vet Clinics North Am Equine Pr* **2** (2), 313- 328
- CROFTON, H. D. (1954): Nematode parasite populations in sheep on lowland farms. 1. Worm egg counts in ewes. *Parasitol* **44** (3-4), 465-477
- CROFTON, H. D. (1971): A model of host-parasite relationships. *Parasitol* **63** (3), 343-364

- CORNELL, S- J., BJORNSTAD, O. N., CATTADORI, I. M., BOAG, B., HUDSON, P. J. (2008): Seasonality, cohort-dependence and the development of immunity in a natural host-nematode system. *Proc R Soc B* **275** (1634), 511-518
- DURHAM, A. COLES, G. (1988): Possible resistance in equine pinworms. *Onderstepoort J Vet Res* **55** (3), 157-163
- ECA&D (European Climate Assessment & Dataset): Datasets: Daily mean temperature TG; Daily precipitation amount RR. Source 969
<http://eca.knmi.nl/dailydata/predefinedseries.php>
- EPE, C., COATI, N., SCHNIEDER, T. (2004): Ergebnisse parasitologischer kotuntersuchungen von pferden, wiederkäuern, schweinen, hunden, katzen, igeln und kaninchen in den jahren 1998-2002 | [Results of parasitological examinations of faecal samples from horses, ruminants, pigs, dogs, cats, hedgehogs and rabbits between 1998 and 2002] *Deutsche Tierärztl Wochenszts* **111** (6), 243-247
- Fox, J. (2005): The R Commander: A Basic Statistic Graphical User Interface of Statistical to R. *J Stat Softw*, **14** (9), 1-24
- FRANCISCO, I., ARIAS, M., CORTIN, F. J., FRANCISCO, R., MOCHALES, E., SÁNCHEZ, J. A., URIARTE, J. SUÁREZ, J. L. MORRONDO, P. SÁNCHEZ-ANDRADE, R. SA., DÍEZ-BANOS, P., PAZ-SILVA, A., (2009): Silvopastoralism and autochthonous equine livestock: Analysis of the infection by endoparasites. *Vet Parasitol* **164**, 357-362
- GALVANI, A. P. (2003): Immunity, antigenic heterogeneity, and aggregation of helminth parasites. *J Parasitol* **89** (2), 232-241
- GASTESCU, P. (1993): The Danube Delta: Geographical characteristics and ecological recovery. *GeoJ* **29** (1), 57-67
- GASSER, R. B., WILLIAMSON, R. M. C., BEVERIFGE, I. (2005): *Anoplocephala perfoliata* of horses - Significant scope for further research, improved diagnosis and control. *Parasitol* **131** (1), 1-13
- GAWOR, J. J. (1995): The prevalence and abundance of internal parasites in working horses autopsied in Poland. *Vet Parasitol* **58** (1-2), 99-108
- HAYWARD, A. D., WILSON, A. J., PILKINGTON, J. G., PEMBERTON, J. M., KRUK, L. E. B. (2009): Ageing in a variable habitat: Environmental stress affects senescence in parasite resistance in St Kilda Soay sheep. *Proc R Soc B* **276** (1672), 3477-3485

- HENNEKE, D.R., POTTER, G.D., KREIDER, J.L., YEATES, B.F. (1983): Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Vet J* **15** (4), 371-372
- HINNEY, B., WIRTHERLE, N. C., KYULE, M., MIETHE, N., ZESSIN, K. -H., CLAUSEN, P. -H. (2011): Prevalence of helminths in horses in the state of Brandenburg, Germany *Parasitol Res* **108** (5), 1083-1091
- HÖGLUND, J., NILSSON, O., LJUNGSTRÖM, B. -L., HELLANDER, J. OSTERMAN LIND, E. UGGLA, A. (1998): Epidemiology of *Anoplocephala perfoliata* infection in foals on a stud farm in south-western Sweden. *Vet Parasitol* **75** (1), 71-79
- IUCN (International Union for Conservation of Nature and National Resource) Summary of world heritage nomination (1991): 588: Danube Delta biosphere reserve (Romania) http://whc.unesco.org/archive/advisory_body_evaluation/588.pdf
- KAPLAN, R. M., NIELSEN, M. K. (2010): An evidence-based approach to equine parasite control: It ain't the 60s anymore. *Equine Vet Edu* **22** (6), 306-316
- KISS, B.J., MARINOV, M., VASILE, A., SÁNDOR, A.D. (2012): First records of the pine marten (*Martes martes*) in the Danube Delta. *North-Western J Zoo* **8** (1), 195-197
- KRECEK, R. C., LOUW, J. P., SNEDDON, J. C. (1995): Parasites of feral horses from the Namib Desert, Namibia. *J Helminthol Soci Washingt* **62** (1), 84-86
- KÖNIGOVÁ, A., VÁRADY, M., ČORBA, J. (2001): The prevalence of equine gastrointestinal parasites in the Slovak Republic. *Helminthol* **38** (4), 211-214
- KUZMINA, T. (2012): Analysis of regional peculiarities of strongylid (Nematoda, strongylidae) biodiversity in domestic horses in Ukraine. *Vestnik Zoologii* **46** (1), e7-e15
- KUZMINA, T. A., KUZMIN, Y. I., KHARCHENKO, V. A. (2006): Field study on the survival, migration and overwintering of infective larvae of horse strongyles on pasture in central Ukraine. *Vet Parasitol* **141** (3-4), 264-272
- KUZMINA, T. A., KHARCHENKO, V. A., STAROVIR, A. I., DVOJNOS, G. M. (2005): Analysis of the strongylid nematodes (Nematoda: Strongylidae) community after deworming of brood horses in Ukraine. *Vet Parasitol* **13** (3-4), 283-290
- LYONS, E. T., SWERCZEK, T. W., TOLLIVER, S. C., BAIR, H. D., DRUDGE, J. H., ENNIS, L. E. (2000): Prevalence of selected species of internal parasites in equids at necropsy in central Kentucky (1995-1999). *Vet Parasitol* **92** (1), 51-62
- LYONS, E. T., TOLLIVER, S. C., DRUDGE, J. H. (1999): Historical perspective of cyathostomes: Prevalence, treatment and control programs. *Vet Parasitol* **85** (2-3), 97-112

- MARIA, A., SHAHARDAR, R. A., BUSHRA, M. (2012): Prevalence of gastrointestinal helminth parasites of equines in central zone of Kashmir Valley. *Ind J Anim Sci* **82** (11), 1276-1280
- MARTIN, L. B., WEIL, Z. M., NELSON, R. J. (2008): Seasonal changes in vertebrate immune activity: Mediation by physiological trade-offs. *Phil Trans R. Soc B* **363** (1490), 321-339
- MATTHEWS, J. B. (2011): Facing the threat of equine parasitic disease. *Equine Vet J* **43** (2), 126-132
- MONAHAN, C. M., CHAPMAN, M. R., TAYLOR, H. W., FRENCH, D. D., KLEI, T. R. (1996): Comparison of moxidectin oral gel and ivermectin oral paste against a spectrum of internal parasites of ponies with special attention to encysted cyathostome larvae. *Vet Parasitol* **63** (3-4), 225-235
- OGBOURNE, C. P. (1975): Studies on the epidemiology *Strongylus vulgaris* infection of the horse. *Int J Parasitol* **5**, 423-426
- NELIS, H., GEURDEN, T., DEPREZ, P. (2010): *Fasciola hepatica* bij het paard | [*Fasciola hepatica* in the horse] *Vlaams Diergeneeskundig Tijdschrift* **79** (6), 436-444
- NIELSEN, M. K. (2012): Sustainable equine parasite control: Perspectives and research needs. *Vet Parasitol* **185** (1), 32-44
- NIELSEN, M. K., BAPTISTE, K. E., TOLLIVER, S. C., COLLINS, S. S., LYONS, E. T. (2010): Analysis of multiyear studies in horses in Kentucky to ascertain whether counts of eggs and larvae per gram of feces are reliable indicators of numbers of strongyles and ascarids present. *Vet Parasitol* **174** (1-2), 77-84
- NIELSEN, K. M., KAPLAN, R. M. THAMSBORG, S. M. MONRAD, J., OLSON, S. N. (2007): Climatic influences on development and survival of free-living stages of equine strongyles: Implications for worm control strategies and managing anthelmintic resistance. *Vet J* **174** (1), 23-32
- PAINER, J., KACZENSKY, P., GANBAATAR, O., HUBER, K., WALZER, C. (2011): Comparative parasitological examination on sympatric equids in the Great Gobi "B" Strictly Protected Area, Mongolia. *Eur J Wildl Res* **57** (2), 225-232
- PAPAZAHARIADOU, M., PAPDOPOULOS, E., DIAKOU, A. PTOCHOS, S. (2009): Gastrointestinal Parasites of Stabled and Grazing Horses in Central and Northern Greece. *J Equine Vet Sci* **29** (4), 233-236
- PEREIRA, J. R., VIANNA, S. S. S. (2006): Gastrointestinal parasitic worms in equines in the Paraíba Valley, State of Sao Paulo, Brazil. *Vet Parasitol* **140**, 289-295
- PETROV, A. M., GAGARIN, V. G. (1953): Veterinarno-gelmintologicheskie issledovaniya. In: *Laboratornye Metody Issledovaniya v veter- inarii* [Laboratory methods of investigations in veterinary], Gos. izd-vo selskokhoziajstvennoj literatury, Moscow, 588

- POLLEY, L. (1986): Strongylid parasites of horses: experimental ecology of the free-living stages on the Canadian prairie. *Am J Vet Res* **47**, (8), 1686-1693
- R CORE TEAM (2012): R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna
- REHBEIN, S., VISSER, M., WINTER, R. (2013): Prevalence, intensity and seasonality of gastrointestinal parasites in abattoir horses in Germany. *Parasitol Res* **112** (1), 407-413
- REINEMEYER, C. R., PRADO, J. C., NICHOLS, E. C., MARCHIONDO, A. A. (2010): Efficacy of pyrantel pamoate and ivermectin paste formulations against naturally acquired *Oxyuris equi* infections in horses. *Vet Parasitol* **171** (1-2), 106-110
- SAVE Foundation (Safeguard for Agricultural Varieties in Europe): <http://www.agrobiodiversity.net/regional/>
- SCMIDT, U. (1971): Parasitologische Kotuntersuchung durch ein neues Verdünnungsverfahren. *Tierärztl Umsch* **26**, 229-230
- SCHNIEDER, T. (2000): Veterinärmedizinische Parasitologie. Parey in MVS Medizinverlage Stuttgart GmbH&Co. KG, Stuttgart, p **89-92**
- SINGH, B. RAM, H., BANERJEE, P. S., GARG, R., YADAV, C. L. (2002): Parasites of equines in Uttaranchal and Uttar Pradesh. *Ind J Anim Sci* **72** (10), 861-862
- SLIVINSKA, K., DVOJNOS, G., KOPIJ, G. (2006): Helminth fauna of sympatric Przewalski's Equus przewalskii Poljakov, 1881 and domestic horses E. caballus in the Chernobyl exclusion zone, Ukraine. *Helminthol* **43** (1), 27 - 32
- SKOTAREK, S. L., COLWELL, D. D. GOATER, C. P. (2010): Evaluation of diagnostic techniques for *Anoplocephala perfoliata* in horses from Alberta, Canada. *Vet Parasitol* **172** (3-4), 249-255
- SOTIRAKI, S. T., BADOUVAS, A. G., HIMONSA, C. A. (1997): A survey on the prevalence of internal parasites of equines in Macedonia and Thessalia-Greece. *J Equine Vet Sci* **17** (10), 550-552
- SRÉTER, T., MOLNÁR, V., KASSAI, T. (1994): The distribution of nematode egg counts and larval counts in grazing sheep and their implications for parasite control. *Int J Parasitol* **24** (1), 103-108
- STRATFORD, C.H., MCGORUM, B.C., PICKLES, K.J., MATTHEWS, J.B. (2011): An update on cyathostomins: Anthelmintic resistance and diagnostic tools. *Equine Vet J* **43** (SUPPL.39), 133-139
- STUDZINKA, M. B., TOMCZUK, K., SADZIKOWSKI, A. B. (2008): Prevalence of *Eimeria leuckarti* in young horses and usefulness of some coproscopical methods for its detections. *Vet Inst Pulawy* **52**, 541-544

- SZÉLL, Z., TÓTH, J., VARGA, I. (1999): Adatok a magyarországi lovak parazitás fertőzöttségéhez bélsárvizsgálatok alapján | [Prevalence of internal parasites of horses in Hungary by fecal examination] *Magyar Allatorvosok Lapja* **121** (2), 70-74
- TAVASSOLI, M., DALIR-NAGHADEH, B. ESMAEILI-SANI, S. (2010): Prevalence of gastrointestinal parasites in working horses. *Pol J Vet Sci* **13** (2), 319-324
- THIENEPONT, D. (1990): Diagnose von Helminthosen durch Koproskopische Untersuchungen. Janssen Research Foundation, Belgien, 2. Aufl
- TRAVERSA, D., FICHI, G., CAMPIGLI, M., RONDOLOTTI, A., IORIO, R., PROUDMAN, C. J., PELLEGRINI, D., PERRUCCI, S. (2008): A comparison of coprological, serological and molecular methods for the diagnosis of horse infection with *Anoplocephala perfoliata* (Cestoda, Cyclophyllidea). *Vet Parasitol* **152** (3-4), 271-277
- TURNER, W.C., GETZL, W. M. (2010): Seasonal and demographic factors influencing gastrointestinal parasitism in ungulates of Estoshia National Park. *J Wildl Dis* **46**(4), 1108–1119
- UMUR, S., ACICI, M., (2009): A survey on helminth infections of equines in the Central Black Sea region, Turkey. *Turk J Vet Anim* **33**(5), 373-378
- USLU, U., FEYZULLAH, G. L. (2007): Prevalence of endoparasites in horses and donkeys in Turkey. *Bull Vet Inst Pulawy* **51**, 237-240
- VESPREMEANU-STROE, A., PREOTEASA, L. (2007): Beach-dune interactions on the dry-temperate Danube delta coast. *Geomorphology* **86** (3-4), 267-282
- WOOD, D., MATTHEW, J. B., STEPHENSON, S. SLOTE, M. NUSSEY, D. H. (2013): Variation in fecal egg counts in horses managed for conservation purposes: Individual egg shedding consistency, age effects and seasonal variation. *Parasitol* **140** (1), 115-128
- YOUNG, K. E., GARZA, V., SNOWDEN, K. DOBSON, R. J., POWELL, D., CRAIG, T. M. (1999): Parasite diversity and anthelmintic resistance in two herds of horses. *Vet Parasitol* **85** (2-3), 205-214