DOCTORAL (Ph.D.) THESIS

FARKAS CSABA LÁSZLÓ

MOSONMAGYARÓVÁR

2025

SZÉCHENYI ISTVÁN UNIVERSITY

Albert Kázmér Faculty of Mosonmagyaróvár

Wittmann Antal Multidisciplinary Doctoral School of Plant, Animal and Food Sciences

Ujhelyi Imre Doctoral Program of Animal Sciences

HEAD OF THE DOCTORAL SCHOOL:

PROF. DR. LÁSZLÓ VARGA, DSc

University Professor, Doctor of the Hungarian Academy of Science

PROGRAM ADVISOR:

PROF. EMERITUS DR. FERENC SZABÓ, DSc

Doctor of the Hungarian Academy of Science

SUPERVISOR:

PROF. DR. BORISZ EGRI, DSc, MRANH

University Professor

Comparative study of *Ascaris suum* and *Macracanthorhynchus hirudinaceus* infection in free ranging and captive wild boar populations in the Marcal Basin

Farkas Csaba László

Mosonmagyaróvár

2025

1. AIMS

The two most common and economically important parasites of wild boar that reside in the small intestine are *Ascaris suum*, which causes roundworm disease in pigs, and *Macracanthorhynchus hirudinaceus*, which causes thorny-head disease in pigs. Knowledge of different infection levels between captive and free-ranged populations is essential for planned game management. *A. suum* is one of the parasites causing economic losses and health issues**.** Although *M. hirudinaceus* is of lesser economic importance, its continuous global expansion are clear today. Both species pose a high risk of zoonosis. There has been no comprehensive endoparasitological research in Hungarian wild boar populations in the last twenty years. Prior to our studies, no similar survey had been conducted in wild boar populations in the Marcal Basin. Our studies were conducted in the area of the Marcal-Bitvaközi Hunting Company between 2015 and 2023.

During our examinations, we collected and analyzed data on:

- the geographic characteristics of the study area, including soil types, temperature, precipitation, relief, and water cover, based on the 2007 and 2018 Wildlife Management Plans of the Marcal Basin and the Marcal-Bitvaközi Wildlife Management Area;
- the age distribution, size, and health status of the wild boar population in the region, comparing the data with national statistics;
- the wild game meat sales system and the economic operation of the hunting association;
- the methods and results of investigating *A. suum* and *M. hirudinaceus* infections in the wild boar populations of the study area;
- the prevalence and intensity of *A. suum* and *M. hirudinaceus* infections in both captive and free-ranging populations**;**
- the potential relationship between body weight, health condition**,** and parasite infection rates;
- the correlation between *A. suum* and *M. hirudinaceus* infection concerning gender and age groups.

2. MATERIALS AND METHODS

2.1. Study locations

I conducted my research in the 11,893-ha hunting area of the Marcal-Bitvaközi Hunting Association between 2015 and 2023, located in the Marcal Basin game management region. Of the total area, 74.1% consists of arable land and grassland, while 19.4% is forested. More than 75% of the forests lie between Dabrony and Nemesszalók, connected by several narrow strips of forest. The wild boar enclosure is located in the Dabrony forest section, primarily composed of pedunculate and sessile oak, with pine and acacia at the edges. The Nemesszalók forest block is predominantly covered with acacia, which serves as a regenerating chewing forest and a hiding place for big game. Pine and oak occur sporadically. On the northwestern side of this forest, a 200-meter-wide swampy area extends from the forest to Road No. 834, predominantly covered with alder. The Marcal floodplain is a key wildlife habitat, featuring grasslands, reed beds, and poplar trees, providing ideal conditions for wild boars.

The 248.1-ha wild boar enclosure in the study area is classified as a small-scale facility. Originally designed as a separate hunting farm with dedicated areas for sows and boars, this management approach was abandoned due to the stress caused by the confined space, leading to low reproductive success. Consequently, the entire enclosure shifted towards breeding and hunting operations, resulting in increased reproduction rates and stabilized management.

Due to the epidemiological regulations introduced in 2019 in response to African swine fever, the hunting association suspended the operation of the wild boar enclosure indefinitely. To prevent parasitic infections, soil disinfection or replacement was not performed due to the nature of the forest cover. During the operational period of the wild boar enclosure, 19.8% of wild boar hunts took place within the captive area.

2.2. Sample Collection and Methods for Taxonomic Identification

Of the total population, 173 individuals (80%) lived in the free living area, the sex ratio: 82 (47.40%) females and 91 (52.60%) males. The examined free living area population consisted of 20 piglets (12♀ and 8♂), 92 juveniles (40♀ and 52♂) and 61 adults (30♀ and 31♂).

We killed 43 wild boars (20%) in a captive area, of which 22 wild boars (51.16%) were female and 21 individuals (48.84%) were male. There was no significant difference in the gender distribution of the entire population examined. In the population living in a captive area, we examined 10 piglets (5♀ and 5♂), 15 juveniles (8♀ and 7♂), and 18 adults (9♀ and 9♂).

During our examination, the sampling, evisceration and documentation of the 216 shot game examined in total were carried out in the same way. During this, we recorded the age, body weight, sex, health status, possible injuries of the game, as well as the unique game identification number issued by the Hunting Authority of the sampled shot game, which was also used to identify the sample until laboratory determination. We recorded the GPS coordinates of the exact location of the shooting. In each case, the sampling began with the removal and separation of the viscera. The internal organs were either purposefully or completely exposed. During the dissection of the examined animals, in order to accurately determine endoparasite infection, the stomach and small intestine were exposed along their entire length and washed separately along their entire length according to the guidelines of NAGY et al. (2014), then, in order to find the parasites, they were covered on an examination table illuminated from below and equipped with a glass plate, and spread out thinly on it.

Afterwards, the parasites found were stored in glass jars with an identification number and labeled, containing a pre-prepared solution of 90% alcohol and 5% glycerol, at a temperature of 4°C, in a refrigerator. The species of the parasites were determined using PZ0 MST131, a Zeiss Ergaval and a Zeiss Discovery V8 stereomicroscope. We usually worked with 3.2x5 and 6.3x5 magnification. The photos were taken with a Panasonic DMC-G6 camera attached to a Zeiss Discovery V8 stereomicroscope, at an eight-fold magnification, which provided a three-dimensional image of the examined parasites.

3. RESULTS

3.1. *A. s*uum and *M. hirudinaceus* infection rates in free living and captive areas

To assess the impact of living conditions on infection level, we used a chi-square test. Results indicated that infection rates were significantly higher in captive populations than in free-ranging ones. The chi^2 test value was: $x^2(1) = 19.409$, the empirical significance: $p<0.001$, the Cramer's V index, which indicates the strength of the relationship, was 0.300, $p<0.001$. The prevalence of shot game under investigation in the captive management area was 69.8%, which is 36.9 percentage points higher than the prevalence of shot game kept and shot in the free-range area, which was only 32.9%. Determining the average number of examined nematodes per infected animal, it is clear that this indicator (5.5 helminths/individual) was higher in the case of the captive individuals than in the case of the free-range population (4.11 helminths/individual). The number of infections did not follow a normal distribution based on our tests (Shapiro-Wilk test and Q-Q graph). Based on this result, we also used the Mann-Whitney U-test and the Mood median test. Based on the results of both tests, it can be stated that the keeping technology of the populations had a great influence on the intensity of the infection. The intensity value was higher in the examined individuals of the captive population.

The level of nematode infections examined showed a significant difference in both parasitosis cases, regarding whether the animal was shot in a captive or free treated area. In the case of those shot in a captive area, the average number of infections per animal was higher for both infections than in the case of those shot in a free living area. The difference is significant ($U=2491.000$, $p<0.001$, $U=2660.500$, $p<0.001$, $U=2165.000$, $p<0.001$). The result of the Kruskal-Wallis test also showed a significant difference ($x^2(1)=14.299$, $p<0.001$; $x^2(1)=22.432$, $p<0.001$; $x^2(1)=2.858$, $p=0.001$).

The average number of infections per infected animal by area type was as follows: The number of *A. suum*, *M. hirudinaceus* and total infections showed a significant difference in *M. hirudinaceus* infection in the comparison by area, according to the Kruskal-Wallis test statistic. ($x^2(1)=1.646$, $p=0.200$; $x^2(1)=9.350$, $p=0.002$; $x^2(1)=2.777$, $p=0.096$). Using the Mann-Whitney U test, we also obtained a similar result: in the case of *M. hirudinaceus* infection, the difference was significant: $U=714.000$, $p=0.200$; $U= 562.000$, $p=0.002$; $U=670.000$, $p=0.096$.

The number of *A. suum, M. hirudinaceus* and total infections per animal, when compared by area infected with both parasite species, showed a significant difference for *A. suum* and *M. hirudinaceus* infections, however, the difference was not significant for all examined nematode infections, according to the results of the Kruskal-Wallis test: ($x^2(1)=9.502$, $p=0.002$; $x^2(1)=17.841$, $p<0.001$; $x^2(1)=1.488$, $p=0.222$). The result also showed a significant difference for *A. suum* and *M. hirudinaceus* infections according to the Mann-Whitney U test. $U=43.500$, $p=0.002$; $U=14.500$, $p=0.001$; $U=89.500$, $p=0.232$.

The number of nematode infections in the infected animals was also examined. Wild boars shot and examined in the free-ranged area were less infected than those shot in the captive area, the difference was significant for the total number of infections: $x^2(1) = 5.395$, $p=0.020$. In the case of *A. suum* infection, this difference was not significant: $x^2(1) = 0.635$, $p=0.425$, while in the case of *M. hirudinaceus* infection it was significant: $x^2(1)=4.121$, $p=0.042$.

Of the 399 nematodes we identified, 287 (71.9%) were *A. suum*, while 112 (28%) were *M. hirudinaceus*.

3.2. Comparison between the sex of wild boars and their rate of nematode infection

53.85% of all females were infected with *A. suum* and 51.16% with *M. hirudinaceus*. The prevalence of *A. suum* and *M. hirudinaceus* infection in all examined boars was 27.68% and 27.67%, respectively. Of all examined wild boars, 21 females (9.72%) and 10 males (4.63%) were infected with both parasites simultaneously.

Statistical analysis showed that infection rates significantly differed between sexes**.**

3.3. Examining the relationship between age groups and nematode infection

When the examined wild boar population was divided into age groups, a clear correlation between nematode infection rates and age emerged. The value of the chi^2 test statistic is: $x^2(5)=19.973$, the empirical significance is: $p=0.001$, i.e. the relationship between the sex of the animal and the infection is significant.

The nematode infection by sex showed significant differences for the entire sample, *A. suum*, *M. hirudinaceus*, and all infections. The results of the Mann-Whitney U test were: $U=4277.0$, $p<0.001$; $U=5089.5$, $p=0.009$; $U=4234.0$, $p<0.001$.

However, if we examine the nematode infection by sex and age group for the entire sample, we find a significant difference in the case of *A. suum* infection and all infections, but the difference was not significant in the case of *M. hirudinaceus* infection.

When we narrowed the study to all nematode-infected individuals, we no longer obtained significant results due to the smaller difference in infection between sexes in the indoor population.

Examining the average infection rate of females by age group, *A. suum* infection showed a significant difference for the entire sample. The results of the Kruskal-Wallis test were: $x^2(2)=6.369$, $p=0.041$, $x^2(2)=0.277$, $p=0.871$, $x^2(2)=4.980$, $p=0.083$.

In the case of males, the average nematode infection by age group did not show significant differences for the entire sample. The results of the Kruskal-Wallis test were: $x^2(2)=1.221$, $p=0.543$, $x^2(2)=0.353$, $p=0.838$, $x^2(2)=1.262$, $p=0.532$.

In the piglets, the nematode infection by sex showed significant differences in the total sample for *A. suum* infection and total infections. Here, the results of the Mann-Whitney U test were: U=59.0, p=0.023; U=95.0, p=0.318; U=58.0, p=0.020.

Among the juveniles, the nematode infection by sex - for the entire sample - and also for all nematode infections showed significant differences. The results of the Mann-Whitney U test were: U=919.0, p<0.001; U=1197.0, p=0.023; U=919.5, p<0.001.

When examining adult wild boars, based on the nematode infection by sex (for the entire sample), we did not obtain any significant results for any nematode infection. The results of the Mann-Whitney U test were: U=731.5, p=0.585; U=716.0, p=0.297; U=710.0, p=0.431.

4. NEW SCIENTIFIC RESULTS

1. For the first time, a comparative analysis of *A. suum* and *M. hirudinaceus* infections in free-ranging and captive wild boar populations in the Marcal Basin has been conducted, revealing differences in prevalence and infection intensity.

2. A novel methodological refinement for small intestine dissection has been developed, enhancing the detection of nematode infections.

3. A significant correlation between wild boar sex and nematode infection rates was confirmed, with females exhibiting higher prevalence rates.

5. LIST OF PUBLICATIONS BY THE AUTHOR ON THE TOPIC

1. Farkas, Cs., Egri, B. (2017) Vaddisznó állományok endoparazitológiai fertőzöttségének vizsgálata az elmúlt évtizedekben. **Vadbiológia**, 19. pp. 13-26.
2. Farkas, Cs., Fekete, B., & Egri, B. (2021). Comparative Examination of the Roundworm (*Ascaris suum*, Goeze, 1782) and Giant Thorny-Headed Worm (*Macracanthorynchus hirudinaceus*, Pallas, 1781) Infestations of Free-Ranging (Living in Game-Preserve) and Free Living Wild Boar-Stocks in Midwest Hungary. **International Journal of Zoology and Animal Biology** *(IZAB)*, 4(3), doi: https://doi.org/10.23880/izab-16000308
3. Farkas, C., Juhász, A., Fekete, B., Egri, B. (2024a) Comparative Analysis of *Ascaris suum* and *Macracanthorhynchus hirudinaceus* Infections in Free-Ranging and Captive Wild Boars (Sus scrofa) in Hungary. **Animals**, *14*(6), 932.
4. Farkas, Cs., Juhász, A., Fekete, B., & Egri, B. (2024b). Parasitological Examination of the Digestive System of Wild Boar from a Practical Point of View - Endoparasitological Sampling under Field Conditions. **Methods and Protocols**, *7*(4), 65.