DOCTORAL (Ph.D.) THESIS

FARKAS CSABA LÁSZLÓ

MOSONMAGYARÓVÁR
2025

SZÉCHENYI ISTVÁN UNIVERSITY
ALBERT KÁZMÉR FACULTY OF MOSONMAGYARÓVÁR
DEPARTMENT OF ANIMAL SCIENCE

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. EMERITUS DR. BORISZ EGRI, DSC, MRANH
UNIVERSITY PROFESSOR

COMPARATIVE STUDY OF *ASCARIS SUUM* AND *MACRACANTHORHYNCHUS HIRUDINACEUS* INFECTION IN FREE LIVING AND FREE-RANGE WILD BOAR POPULATIONS IN THE MARCAL BASIN

MADE BY:
CSABA LÁSZLÓ FARKAS

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.

I conducted my studies in the area of the Marcal-Bitvaközi Hunting Company between 2015 and 2023. During my observations

- I present the location of the given study area, its soil types, temperature and precipitation conditions, as well as its relief and water cover characteristics, based on the 2007 and 2018 Wildlife Management Plans of the Marcal Basin and the Marcal-Bitvaközi Wildlife Management Area;
- I present the game population found in the region, within which I detail the age composition of the wild boar population, as well as its quantitative and qualitative aspects.

I compare the range and population estimation data with national data;

- I describe the sales system of game meat and shot game brought to the table by those entitled to hunt, and the economic operation of the hunting company;
- I present the methods and results of the investigation of *A. suum* and *M. hirudinaceus* infection in wild boar populations in the area were presented. In connection with the above, we primarily sought to answer the question of the significance of *A. suum* and *M. hirudinaceus* infection and their relationship to each other in the populations managed in captive and free living areas in the Marcal Basin.;
- I examined whether there is a relationship between the body weight and condition of the wild boar and the endoparasite infection?
- I compared the relationship between *A. suum* and *M. hirudinaceus* infection by sex and age group.

2. MATERIALS AND METHODS

2.1. Locations of the tests

I conducted my research on the 11,893 ha hunting area - which includes a 248.1 ha wild boar farm - of the Marcal-Bitvaközi Hunting Company (code number: 19-301250-508) between 2015 and 2023, which is located in the Marcal Basin game management region. 74.1% of the areas suitable for game management in the game management unit are arable land and grassland, and 19.4% are forests. More than 75% of the forests are located between Dabrony and Nemesszalók, which are connected by a narrow forest strip in several places like a bridge. The wild boar garden is located in the Dabrony forest section, the vast majority of which is made up of pedunculate and sessile oak, with pine and acacia on the edges. The Nemeszalók forest block is mostly covered with acacia, which functions as a young chewing forest sprouting from the stem in the partially cut forest area every year and as a hiding place for big game. Pine and oak can be found in spots. On the northwestern side of this forest, there is a swampy, slightly marshy area with a width of about 100-200 m, which stretches from the forest to road no. 834. The typical tree species is alder. The Marcal floodplain is of particular importance from a wildlife management perspective, where most of the grasslands and reed beds are also found. This area, which is swampy in many places, dotted with reed beds, bushes and summer cottages, is an ideal habitat for big game, including 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 - du e to the domestic appearance of African swine fever - (regardless of th e fact that the hunting company had all the necessary permits for the w ild boar park), the hunting association suspended the operation of the wild boar park for an indefinite period. In order to prevent parasitic in fections, the hunting association did not carry out soil disinfection or s oil replacement due to the structure of the forest cover. 19.8% of wild boar killings occurred in closed areas during the period when the wild boar park was in operation.

2.2. Sample collection and methods used 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 sows (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 sows (8♀ and 7♂), and 18 adults (9♀ and 9♂).

During our studies, 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 big game identification number issued by the Hunting Authority of the sampled shot game, which was also used to identify the given 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 autopsy 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. To compare *A. suum* and *M. hirudinaceus* infection in captive and free areas, and to determine their relationship to each other, the Shapiro-Wilk test, Q-Q graph, Cramer's V coefficient, Kruskal-Wallis test, Mann-Whitney test, Mood's median test, Youle's association coefficient, chi-square test and z-test statistical methods were used.

3. RESULTS

3.1. *A. suum* and *M. hirudinaceus* infection rates in open and closed areas

To assess the impact of the husbandry (and living) conditions on the level of infection, we used a chi-square test. Our results showed that the infection in closed areas was dominant, while the free-range populations were less infected. Of the 399 endoparasites we identified, 287 (71.9%) were *A. suum*, while 112 (28%) were *M. hirudinaceus*. When calculating the infection rate of the free living population, the value of the chi-square test is: $x^2(1) = 19.409$, the empirical significance was: $p<0.001$. The value of 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 living 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 living 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 herds had a great influence on the intensity of infection, but the intensity value was higher in the examined individuals of the captive herd. Thus, in the case of those brought to the table in the captive area, the average number of infections per animal was higher in both cases of infection than in the cases of those shot in the 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 level of endoparasite infections examined showed a significant difference in both parasitosis cases, regarding whether the animal was shot in a captive or free living area. In the case of animals brought to the table in a captive area, the average number of infections per animal on both infection days was higher than in the cases of animals shot in the free living area. The difference was 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, but not for all parasites infections examined. According to the results of the Kruskal-Wallis test: ($x^2(1)=9.502$, $p=0.002$; $x^2(1)=17.841$, $p<0.001$; $x^2(1)=1.488$, $p=0.222$). According to the Mann-Whitney U test, a significant difference was also shown for A. suum and M. hirudinaceus infections. (U=43.500, p=0.002; U=14.500, p=0.001; U=89.500, p=0.232.)

We also examined the infection of infected animals for both parasites separately and together. Wild boars shot and examined in the free living 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: $x^2(1)=4.121$, $p=0.042$.

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

Based on the above, it appears that the parasite infection of animals depends on their sex.

3.3. Examining the relationship between age groups and endoparasite infection

Based on the entire study sample and divided into age groups, the endoparasite infection of the studied wild boar population shows a correlation with the sex of the animals. The value of the chi-square 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 was 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, when nematode infection is examined by sex and age group for the entire sample, significant differences were found for *A.*

suum infection and total infections, while the difference was not significant for *M. hirudinaceus* infection. However, when we restricted the study to individuals infected with all nematodes and acanthocephlas, 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 parasite 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 parasite 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 endoparasite infection by sex - for the entire sample - and also for all endoparasite 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.

3.4. Examination of the relationship between condition and nematode infection

The comparison of the infection rate by condition pair in the case of wild boars shot in a captive area showed the following results. According to the calculations, there was no significant difference in the infection rate between individuals with different conditions in a captive area. This was also confirmed by the result of the independence test. In the case of wild boars shot in a captive area, the infection rate was independent of the condition, $x^2(2)=3.228$, $p=0.199$, $V=0.274$, $p=0.199$.

In the case of wild boars in good condition examined in the free living area, the level of infection was significantly lower than in the case of their counterparts in worse condition These indicators were determined from the infection rates, using a z-test testing the difference in rates. The latter result was also confirmed by the results of the independence test. In the case of animals shot in free-living areas, the degree of infection showed a correlation with the condition ($x^2(2)=67.690$, $p<0.001$, $V=0.626$, $p<0.001$).

4. NEW SCIENTIFIC RESULTS

1. For the first time, a comparison of *Ascaris suum* and *Macracanthorhynchus hirudinaceus* infection rates in 216 wild boars living in free and game reserve areas of the Marcal-Bitvaközi Hunting Company was conducted in relation to the husbandry technology and prevalence.
2. We were the first to determine that the examined endoparasite infection in the wild boar population living in the Marcal basin is related to the sex, age and condition of the animals, as the relationship between the sex, age and condition of the animals and the infection is significant.
3. Small intestine dissection, which facilitates the determination of targeted nematode and acanthocephala infection, has been improved with new, practical method refinements.

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.