Preliminary analysis of peripheral white blood cells in wild rodent species from Greece: comparison of morphology and proportion

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Introduction

Studies on the characteristics of White Blood Cells (WBCs) in rodent species, such as morphology and differential count, appear to be rather restricted to lab mice and rats, whereas much less knowledge exists for their wild populations. Far more profound is the lack of such data for wild populations of other rodent species, since for most of them even the slightest relevant information is missing. For example, no such research has been implemented so far on wild rodent populations from Greece. Therefore, the aim of this work is to examine the percentages of the different types of leukocytes in sampled wild rodents of Greece and to point out the possible differences among the studied species, regarding the count and the morphology of WBCs. Specimens are derived from wild populations, belonging to the mice of the genus *Apodemus* (Murinae), as well as the fossorial voles of the genus *Microtus* (Arvicolinae). This survey is part of a wider study (PhD Thesis of the first author) in Greece on wild rodent ectoparasites and pathogens, which can cause serious zoonotic diseases.

Methodology

A total of 69 animals (31 *Apodemus flavicollis,* 4 *A. sylvaticus,* 12 *A. epimelas* and 22 *Microtus thomasi*) from 15 localities of Peloponnese and Sterea Ellada (Fig. 1), were live-trapped, using either Sherman traps for *Apodemus* specimens or non-commercial, self-made traps, customized for *M. thomasi.* In addition, eight laboratory and twelve wild-caught mice (*Mus musculus domesticus*) were included in the study for comparison purposes. Animals were sedated with the administration of ketamine (Ketamidor®) and medetomidine hydrochloride (Domitor®) and recovery was facilitated with the administration of atipemazole (Antisedan®). While under anesthesia, body measurements, weight and sex were recorded for each specimen after a small quantity of blood was drawn from the saphenous veins of both lower limbs, using a micro-hematocrit capillary tube with heparin, mounted on a customized tool that enhances fast and continuous blood supply to the tube. Blood smears were immediately prepared after blood draw. Giemsa-stained and DPX-mounted preparations were studied, with regard to morphology and proportion of WBCs, under a Zeiss Axiophot microscope, equipped with a Leica 8MP digital camera. It should be pointed out that animals were finally released in the site of their collection.

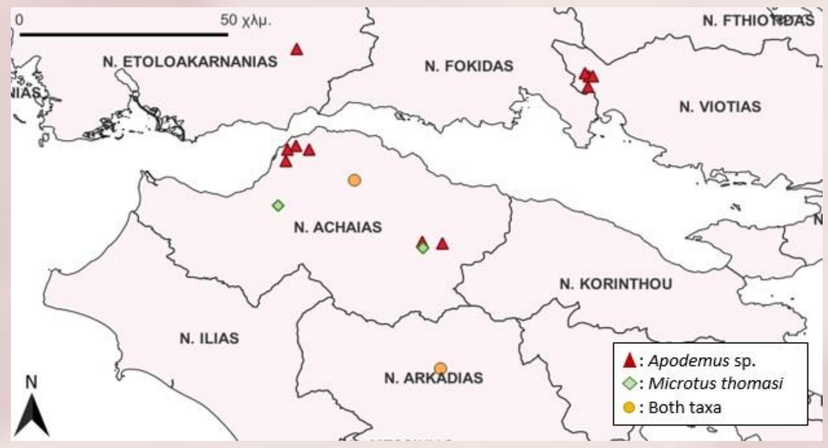

Fig. 1: Sampling localities of the studied specimens in Peloponnese and Sterea Ellada

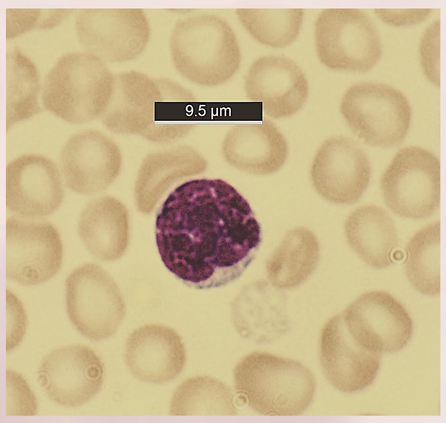

Fig. 2: Normal Lymphocyte

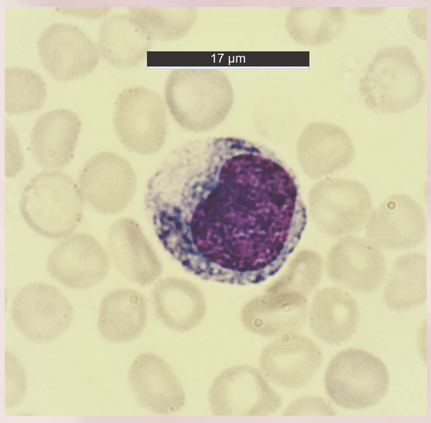

Fig. 3: Reactive Lymphocyte

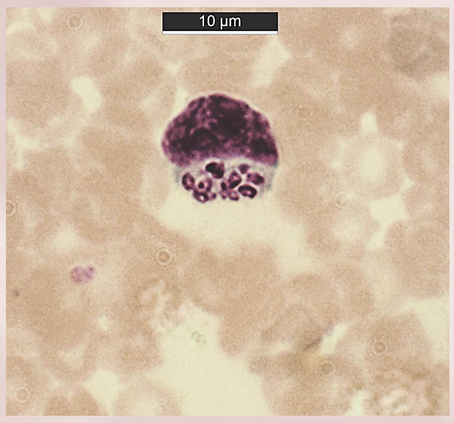

Fig. 4: Azurocyte, unique in *M. thomasi*

TABLE 1: Typical percentages of WBCs in the studied specimens

Percentage of types of WBCs \ Species	*Mus musculus domesticus* (Lab) (n=8)	*Mus musculus domesticus* (Wild) (n=12)	*Apodemus flavicollis* (n=24)	*Apodemus sylvaticus* (n=4)	*Apodemus epimelas* (n=11)	*Microtus thomasi* (n=16)
% Lymphocytes	85.71 ± 5.38	87.50 ± 6.24	80.83 ± 7.65	91.67 ± 4.04	86.73 ± 5.76	**59.93 ± 6.38**
% Neutrophils	11.14 ± 4.91	9.42 ± 5.12	15.36 ± 7.74	5.33 ± 3.21	9.55 ± 5.35	**36.53 ± 6.78**
% Monocytes	2.57 ± 1.40	1.92 ± 1.38	3.00 ± 1.82	1.67 ± 0.58	2.91 ± 1.64	2.38 ± 1.66
% Eosinophils	0.29 ± 0.49	1.08 ± 2.02	0.71 ± 0.86	1.33 ± 1.15	0.64 ± 0.67	0.14 ± 0.36
% Basophils	0.57 ± 0.79	0.08 ± 0.29	0.13 ± 0.34	0.0	0.18 ± 0.40	0.52 ± 1.21

Preliminary results

Our survey revealed certain differences among the studied species, regarding morphology and proportion of WBCs, as demonstrated in Table 1. As expected, lymphocytes and neutrophils were the most populous WBC types in all studied species, however with very different percentages in *M. thomasi* (Diagram 1). *M. thomasi* also had comparatively significantly lower WBC counts (data not shown). With regard to the morphology of WBCs, laboratory mice only had normal lymphocytes (Fig. 2) but wild mice (*M. m. domesticus*), possessed both normal and reactive lymphocytes (RL) (Fig. 3). RLs were also detected in some *Apodemus* individuals at a lower percentage, while they were rare in *M. thomasi*. Moreover, a very unique type of leucocyte, termed Azurocyte (AZ), was detected for the first time in eight adult *M. thomasi* individuals at varying frequencies (Fig. 4). Finally, some captured individuals (7 *A. flavicollis*, 1 *A. epimelas* and 6 *M. thomasi*) with an unhealthy appearance indeed presented some extreme high or low lymphocyte and neutrophil counts and thus the results of their study are presented separately from above (Table 2 - Diagrams 2, 3).

TABLE 2: Differences in WBCs proportions in healthy and presumably unhealthy specimens

Percentage of types of WBCs \ Species	*Apodemus* sp. (Normal) (n=37)	*Apodemus* sp. (Unhealthy) (n=8)	*Microtus thomasi* (Normal) (n=16)	*M. thomasi* (Unhealthy Variant 1) (n=4)	*M. thomasi* (Unhealthy Variant 2) (n=2)
% Lymphocytes	83.95 ± 7.01	**49.5 ± 11.02**	59.93 ± 6.38	**82.50 ± 7.68**	**25.50 ± 17.68**
% Neutrophils	12.30 ± 6.72	**46.5 ± 10.43**	36.53 ± 6.78	**16.25 ± 7.41**	**71.51 ± 20.50**

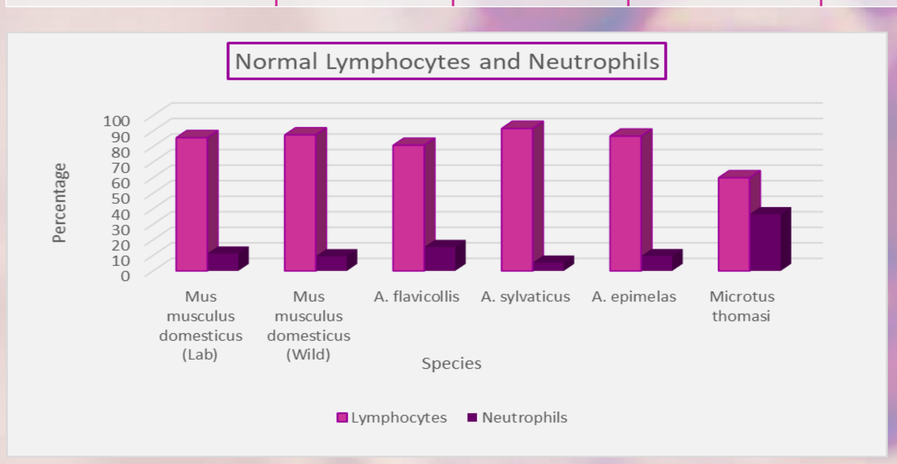

Diagram 1: % of Lymphocytes and Neutrophils in studied species

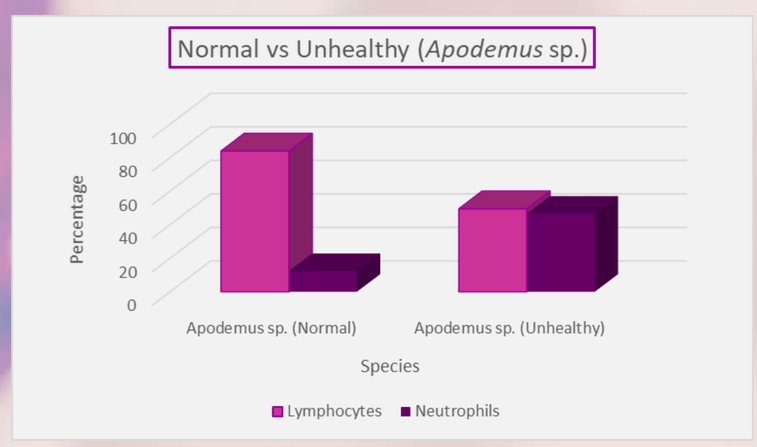

Diagram 2: % of Lymphocytes and Neutrophils of normal and presumably unhealthy individuals of *Apodemus* sp.

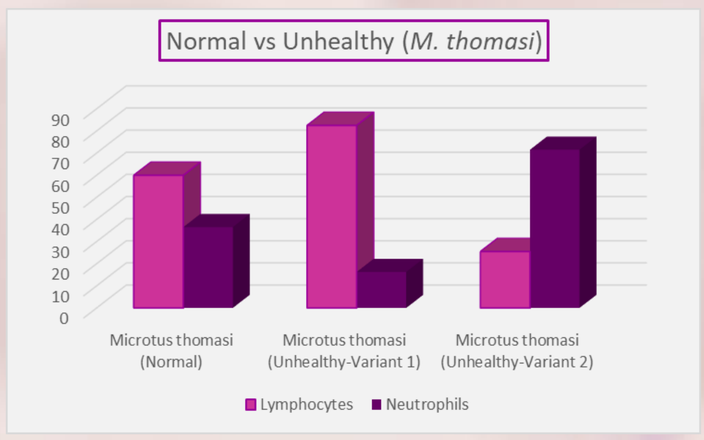

Diagram 3: % of Lymphocytes and Neutrophils of normal and presumably unhealthy individuals of *M. thomasi*

Discussion

Based on our results, *Mus* and *Apodemus* representatives of our sample, do not present significant differences in the WBCs characteristics, given above, whereas *M. thomasi* is the only species that somehow differs from the above species. Our results on the Lymphocyte and Neutrophil counts for the studied vole species correspond to equivalent records for *M. arvalis* and *M. levis* [3]. In addition, previous works have underlined that blood cells levels in voles show a seasonal variation [1,7], which, however, has not been confirmed so far in our study, since the trapped animals, during both spring and autumn, presented no differences. This will be further tested as sampling continues in different seasons. Furthermore, the remarkably lower number of WBCs recorded in *M. thomasi* agrees with the results of a previous work, that demonstrated the same count difference between *Mus musculus* on one hand and the voles *Microtus pennsylvanicus* and *M. oeconomus* on the other [8].

The appearance of RLs only in wild individuals, albeit with varying percentages in different species, may be related to the differences in the living conditions between wild and laboratory mice. RLs are usually $CD8^+$ cells and their numbers increase in response to infections (mainly viral or bacterial). The fact that wild rodents are expected to be subjected more in their natural environment to such infections than laboratory ones, might explain this difference in RL percentages. The rarity of RLs in *M. thomasi*, remains to be further scrutinized.

So far, the presence of AZs appears to be a trait within the genus *Microtus*, since apart from *M. thomasi,* it has previously been detected only in four other *Microtus* species, i.e. *M. pennsylvanicus* [4,5], *M. arvalis*, *M. levis* [3] and *M. agrestis* [1,2]. In fact, these five species belong to four different subgenera of *Microtus* (*M. thomasi: Terricola; M. arvalis & M. levis: Microtus, M. agrestis: Agricola; M. pennsylvanicus: Mynomes*), which may imply that AZs may be an old trait in the genus *Microtus*, occurring in several different subgenera! In this sense, it would interesting to check if this trait is indeed restricted to the genus *Microtus* or it appears in other Arvicolinae, as well.

In our sample, we observed AZs at high levels in two female M. *thomasi* individuals in advanced pregnancy and at low levels in six adult male voles. Indeed, even though their role has not been clarified completely, their levels raise with progestins [4,6] and AZ numbers increase during gestation, but also as a response to infection [2,5]. It has been further suggested that their role is linked to abortion at the first semester of reproductive activity during unfavorable circumstances. AZs have many morphological and functional similarities with mammal cells with Natural Killer (NK) activity (Kurloff cells at guinea pigs and Large Granular Lymphocytes at humans and rats), a fact that has led scientists to believe that these cells may be NK cells, unique to voles [2], perhaps playing a very important role in their immune system.

Acknowledgments

We thank Dr. Ioannis L. Oikonomidis, DVM, PhD, DipACVP (Clin Path), AFHEA, MRCVS – Clinical Pathologist at IDEXX Laboratories and Dr. Theodora Tsouloufi, DVM, PhD, MRCVS – Resident in Veterinary Clinical Pathology at IDEXX Laboratories for their comments and suggestions on the identification of the leukocytes of our specimens.

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