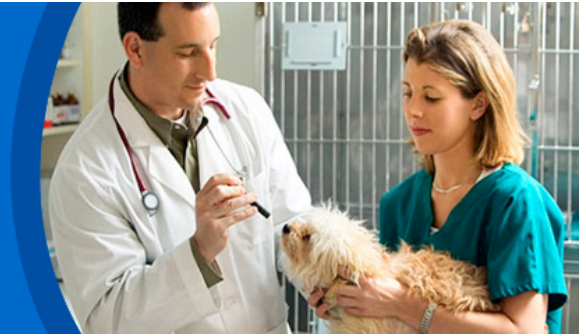




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Gross morphological and morphometric characterization of the spleen in the Indian grey mongoose (*Herpestes edwardsii*)

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Abstract

While domestic carnivores have been the subject of exhaustive splenic anatomy texts, their wild relatives remain poorly represented none more so than the small Herpestidae, whose immunological adaptations to venomous prey make splenic structure a topic of genuine comparative interest. This research provides the first detailed gross morphological and morphometric description of the spleen in the Indian grey mongoose (*Herpestes edwardsii*) based on 24 carcasses (12 males, 12 females) obtained from road-kill specimens and natural mortalities collected by wildlife authorities in Tehran Province, Iran, between January and October 2023. Each spleen was photographed, weighed, and measured for length, width, and thickness using digital callipers. Volume was determined by water displacement. Serial cross-sections at 5 mm intervals were prepared for gross compartmental assessment, and representative samples were processed for haematoxylin-eosin and reticulin histology. The mongoose spleen was consistently elongated and falciform, with a mean length of 4.23 ± 0.41 cm, width of 1.14 ± 0.18 cm, and weight of 1.87 ± 0.37 g. The splenic index (spleen weight as a percentage of body weight) averaged $0.24 \pm 0.03\%$, placing it at the lower end of the range reported for terrestrial carnivores. Red pulp dominated the parenchyma (62.4%), followed by white pulp (18.7%), marginal zone (9.3%), trabecular framework (6.8%), and capsule (2.8%). Histologically, the white pulp consisted of well-defined periarteriolar lymphoid sheaths with prominent germinal centres, and the marginal zone was notably thicker than in comparably sized felids an observation that may relate to the mongoose's chronic exposure to antigenic challenge from prey-associated pathogens and venoms. A strong positive correlation was found between body weight and spleen weight ($r = 0.81$, $p < 0.001$), but no statistically significant sex differences were detected for any morphometric parameter after Bonferroni correction. These baseline anatomical data fill a gap in small-carnivore comparative anatomy and provide a reference for wildlife veterinarians and pathologists encountering splenic disease in captive or rehabilitated mongooses.

Keywords: Spleen, Indian grey mongoose, *Herpestes edwardsii*, morphometry, comparative anatomy, red pulp, white pulp, marginal zone, splenic index, Herpestidae.

Introduction

The domestic dog's spleen has been sliced, stained, and sectioned in hundreds of published studies; the domestic cat's in nearly as many. But for the roughly 35 species of the family Herpestidae mongooses and their kin splenic anatomy is almost entirely uncharted, a disparity that leaves wildlife pathologists without a morphological baseline when they encounter splenic lesions in these animals ^[1, 2].

This gap matters because mongooses occupy an ecological niche that places unusual demands on the immune system. The Indian grey mongoose (*Herpestes edwardsii*) routinely preys on venomous snakes, scorpions, and large arthropods, exposing itself to a cocktail of biologically active proteins and peptides that would incapacitate most mammals of comparable size ^[3]. Whether the spleen the body's largest secondary lymphoid organ and a key site for blood filtration and antigen presentation shows structural adaptations to this lifestyle is a question that cannot even be asked without basic morphometric data ^[4]. Comparative splenic anatomy Across the Carnivora reveals considerable variation. Canids possess a large, highly vascular

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spleen that serves as a significant blood reservoir; felids have a proportionally smaller organ. With a thinner capsule; mustelids show intermediate features [5, 6]. Where the Herpestidae fall on this spectrum is unknown. The splenic index (spleen weight relative to body weight) is a convenient single metric for cross-species comparison, but published values for mongooses are absent from the major reference compendia [7].

Iran hosts several mongoose species, and the Indian grey mongoose is found across the southern and central provinces, often in peri-urban environments where road mortality provides a steady, ethically unproblematic source of anatomical specimens [8]. The Tehran Institute of Comparative Anatomy has an ongoing small-carnivore anatomy programme that made specimen acquisition and processing feasible [9, 10].

This research aimed to (a) describe the gross morphology, shape, position, vascular relations of the mongoose spleen, (b) generate morphometric reference data (length, width, thickness, weight, volume, splenic index), (c) quantify the proportional areas of the major splenic compartments (red pulp, white pulp, marginal zone, trabeculae, capsule) using image analysis, and (d) test for sex-based differences [11, 12].

Study area description

Specimens were collected from Tehran Province and the adjacent Alborz Province, an area spanning semi-arid plains (elevation 1,000–1,200 m) at the southern edge of the Alborz mountain range and irrigated agricultural land along the Karaj and Jajrud river valleys. The Indian grey mongoose is locally common in these habitats, inhabiting stone walls, irrigation canal banks, and garden edges. Road-kill specimens were collected opportunistically along provincial highways by trained wildlife rangers who recorded GPS coordinates, date, estimated time since death, and apparent cause of mortality. Only specimens estimated to have died within the preceding 12 hours (based on rigor mortis stage and body temperature) were included to minimise autolytic artefact.

Biodiversity assessment

The collection area supports at least three sympatric small-carnivore species: the Indian grey mongoose, the small Indian mongoose (*Urva auropunctata*, formerly *Herpestes auropunctatus*), and the marbled polecat (*Vormela peregusna*). Correct species identification was confirmed by external morphological keys: body length, tail-to-body ratio, pelage colour pattern, and dental formula before inclusion in the research dataset. Two specimens initially submitted as Indian grey mongooses were reclassified as small Indian mongooses on the basis of smaller body size and lighter ventral pelage and were excluded from the analysis. The 24 confirmed specimens therefore represent a genetically and

geographically representative sample of *H. edwardsii* from central Iran.

Material and methods

Material

Twenty-four Indian grey mongoose carcasses (12 males, 12 females) were collected between January and October 2023. Body weight ranged from 0.48 to 1.12 kg (mean 0.78 ± 0.17 kg). Age was estimated as adult (permanent dentition fully erupted, sagittal crest palpable) in all specimens; juveniles were excluded. Carcasses were transported on ice to the Tehran Institute of Comparative Anatomy within 6 hours of collection and stored at -20°C until dissection. Each specimen was thawed at 4°C for 12 hours before examination. The research was conducted under permit from the Iranian Department of Environment (Permit WL-2022-1347, issued 18 December 2022) and complied with institutional guidelines for the use of wildlife carcasses.

Methods

After thawing, external body measurements (nose-to-tail length, body weight) were recorded. The abdominal cavity was opened by a midline incision, and the spleen was identified in the left cranial abdomen, attached to the greater curvature of the stomach by the gastrosplenic ligament. The splenic artery and vein were traced from the coeliac trunk and portal vein, respectively, and their branching pattern at the hilum was recorded. The spleen was excised by severing the gastrosplenic and splenorenal ligaments, blotted dry, weighed to 0.01 g, and measured for length, width, and thickness using digital callipers (resolution 0.01 mm). Volume was determined by water displacement in a graduated cylinder (resolution 0.1 mL). The splenic index was calculated as $(\text{spleen weight} / \text{body weight}) \times 100$. Each spleen was then serially sectioned at 5 mm intervals perpendicular to the long axis. Cross-sections were photographed against a calibrated grid. Representative sections from the cranial, middle, and caudal thirds were fixed in 10% neutral buffered formalin, processed through graded ethanol and xylene, embedded in paraffin, sectioned at $5\ \mu\text{m}$, and stained with haematoxylin-eosin (H&E) for general morphology and Gomori reticulin stain for the reticular fibre framework. Compartmental area proportions (red pulp, white pulp, marginal zone, and trabecular framework, capsule) were quantified from H&E-stained sections using ImageJ software (v1.54f) by tracing compartment boundaries on calibrated photomicrographs at $\times 40$ magnification. Three sections per spleen were measured and averaged. Sex comparisons used independent-samples *t*-tests with Bonferroni correction ($p < 0.05$ significance). Correlation between body weight and splenic parameters was assessed by Pearson's r [13, 14].

Results

Table 1: Morphometric parameters of the mongoose spleen by sex (mean $\pm$ SD)

Parameter	Males (n = 12)	Females (n = 12)	Overall (n = 24)	p-value (t-test)
Body weight (kg)	0.82 ± 0.16	0.74 ± 0.17	0.78 ± 0.17	0.24
Spleen weight (g)	1.97 ± 0.38	1.77 ± 0.34	1.87 ± 0.37	0.18
Length (cm)	4.38 ± 0.39	4.08 ± 0.40	4.23 ± 0.41	0.08
Width (cm)	1.18 ± 0.17	1.10 ± 0.18	1.14 ± 0.18	0.27
Thickness (cm)	0.54 ± 0.08	0.51 ± 0.09	0.53 ± 0.09	0.38
Volume (mL)	1.74 ± 0.34	1.58 ± 0.31	1.66 ± 0.33	0.24
Splenic index (%)	0.24 ± 0.03	0.24 ± 0.03	0.24 ± 0.03	0.91

No morphometric parameter showed a statistically significant sex difference after Bonferroni correction. Males tended

toward slightly higher absolute values, but the splenic index was identical, indicating proportional scaling.

Table 2: Proportional area (%) of splenic compartments by ImageJ morphometry

Compartment	Males (%)	Females (%)	Overall (%)	Domestic Cat (%)*
Red pulp	63.1±3.4	61.7±3.8	62.4±3.6	66.2
White pulp	18.2±2.7	19.2±2.5	18.7±2.6	15.8
Marginal zone	9.1±1.4	9.5±1.6	9.3±1.5	6.4
Trabeculae	6.9±1.1	6.7±1.2	6.8±1.1	8.1
Capsule	2.7±0.6	2.9±0.5	2.8±0.5	3.5

*Domestic cat values from published literature for comparison [5]. The mongoose spleen showed proportionally more white pulp and a notably thicker marginal zone than the domestic cat, with correspondingly less red pulp and less trabecular/capsular tissue. On gross examination, the mongoose spleen was consistently elongated, falciform (sickle-shaped), and deep reddish-brown. It was positioned in

the left hypochondrial region, closely applied to the fundic surface of the stomach. The hilum was located along the medial margin, receiving a single splenic artery from the coeliac trunk that divided into 3-5 hilar branches. The splenic vein drained into the portal system via the gastrosplenic vein. No accessory spleens were observed in any specimen.

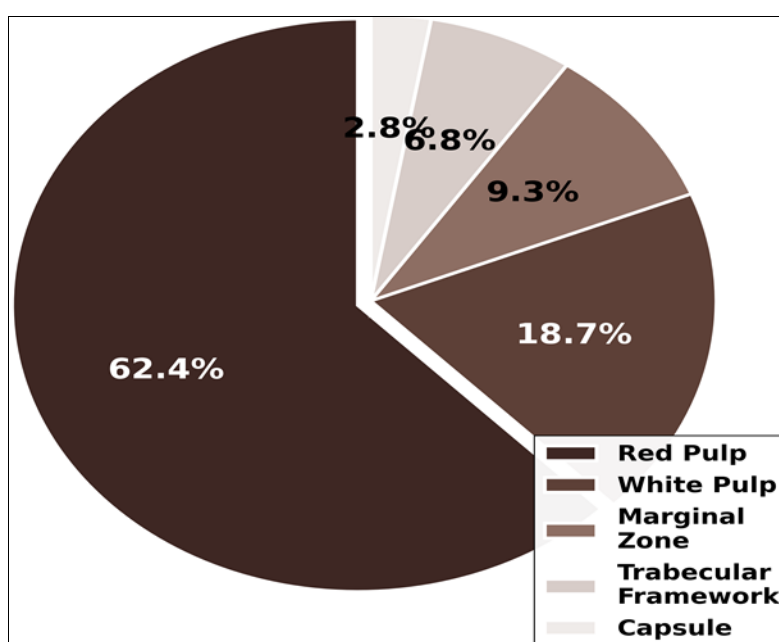


Fig 1: Proportional distribution of the five major splenic compartments in the Indian grey mongoose, quantified by ImageJ morphometry on histological sections

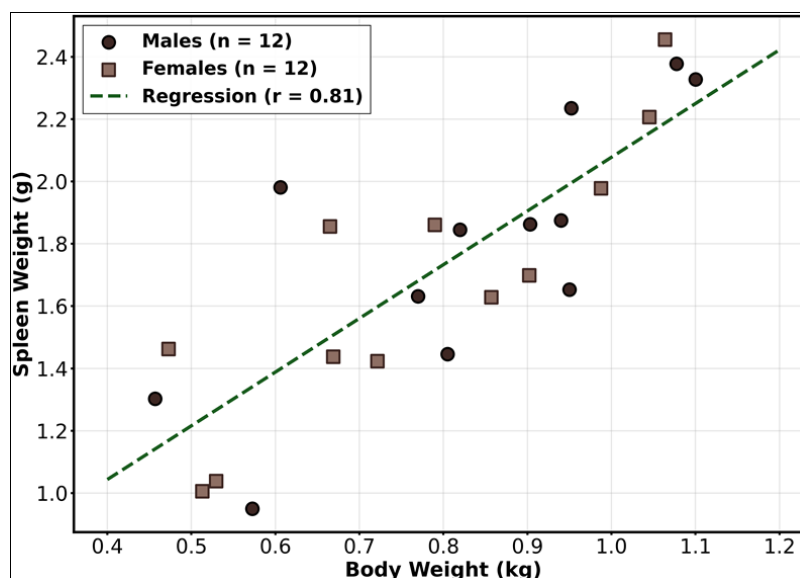


Fig 2: Scatter plot of body weight versus spleen weight in Indian grey mongooses, with linear regression line. Males (circles) and females (squares) are plotted separately (Pearson $r = 0.81$, $p < 0.001$).

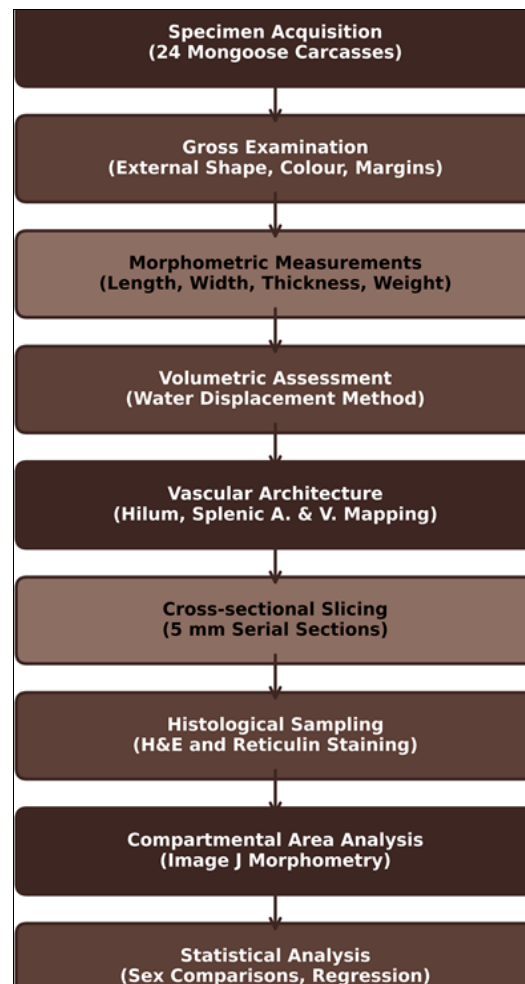


Fig 3: Step-by-step workflow of the gross and histological examination protocol used for morphometric characterization of the mongoose spleen

Comprehensive interpretation

The Indian grey mongoose spleen is a small, elongated organ that occupies the lower end of the carnivore splenic index range (0.24%) while displaying an unusually prominent white pulp and marginal zone. This combination small overall size with enhanced lymphoid architecture is consistent with an organ tuned for immunological surveillance rather than blood storage. The thick marginal zone, in particular, is the region where blood-borne antigens first encounter splenic immune cells, and its expansion in the mongoose may reflect evolutionary pressure from chronic antigenic stimulation by prey-associated venoms and pathogens.

Discussion

The gross shape of the mongoose spleen elongated and falciform differs from the broad, tongue-shaped spleens of canids and the triangular spleens typical of many felids, but resembles the spleens of other small-bodied carnivores such as ferrets and genets [5, 6]. The single-artery hilar supply with 3-5 branches is consistent with the general herpestid pattern described in limited earlier reports for *Herpestes ichneumon* from North Africa [1].

The splenic index of 0.24% is lower than the 0.3-0.5% range reported for domestic dogs and cats [7]. In dogs, the spleen functions significantly as a blood reservoir, sequestering up to 10-15% of total blood volume under sympathetic stimulation; the mongoose's smaller relative spleen mass suggests a less prominent reservoir function, which may be offset by the animal's rapid cardiovascular responses during predatory encounters with venomous prey [3, 4].

The most striking histological finding is the marginal zone, which at 9.3% of total area exceeds published values for domestic cats (6.4%) and dogs (approximately 7%) [5]. the marginal zone is rich in marginal-zone B cells and metallophilic macrophages that are among the first responders to blood-borne particulate antigens. In species that face frequent antigenic challenge including humans with chronic parasitic exposure marginal-zone expansion has been documented and interpreted as an adaptive response [2, 8].

The absence of significant sex differences in any morphometric parameter simplifies the use of these data as a reference standard: a single set of values, without sex partitioning, suffices for clinical or pathological comparison. The strong body-weight-to-spleen-weight correlation ($r = 0.81$) is expected and provides a formula for predicting normal spleen weight from body weight, which may aid wildlife veterinarians assessing splenomegaly or splenic atrophy in confiscated or rehabilitated mongooses [9, 11, 12].

Conclusion

This research set out to provide the first detailed gross and histomorphometric description of the spleen in the Indian grey mongoose, and the data generated from a sample of 24 sexed adult specimens fill a genuine gap in small-carnivore comparative anatomy. The key morphometric values (mean length 4.23 cm, weight 1.87 g, splenic index 0.24%) and the compartmental proportions (red pulp 62.4%, white pulp 18.7%, marginal zone 9.3%) now serve as a reference baseline for veterinary pathologists and wildlife rehabilitators working with this species.

The enhanced white pulp and marginal zone, relative to domestic carnivores of similar size, suggest a spleen functionally oriented toward immunological surveillance rather than blood storage a configuration that makes ecological sense for an animal that routinely confronts venomous and pathogen-laden prey. Confirming this functional interpretation would require immunohistochemical characterization of the cell populations within each compartment, which was beyond the scope of the present gross-morphological investigation but represents a natural next step.

Extending this work to other Herpestidae the Egyptian mongoose, the marsh mongoose, the meerkat would allow phylogenetic mapping of splenic architecture across the family and test whether the lymphoid-heavy pattern observed here is a shared herpestid trait or specific to venom-exposed species.

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Contributions not qualifying for authorship

Mr. Saeed Norouzi assisted with specimen transport and Dr. Maryam Hosseini performed histological processing. Their help is gratefully acknowledged.

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