



Estimation of Wild Boar Population and Assessment of Their Reproductive Hormone Level from Faecal Samples

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Abstract: Wild boar (*Sus scrofa*) population has increased constantly over the last three decades resulting into conflicts with the agro ecosystem. Damage to agricultural crops by wild boar is enormous and widespread. Therefore, understanding reproduction dynamics is necessary to control the local population successfully, which is of significant interest. One tool for evaluating reproductive functions in a wild population is to assess endocrine activity through hormone measurements in either blood, faeces, saliva, or other biological material. Endocrine analyses of steroid hormones enable us to evaluate reproductive status of animals; however, there is not much information available about sex hormone concentrations for the wild boar. The study involved a preliminary survey across different habitats during 2022-23 to estimate population of wild boar and variability in their reproductive hormone levels. Presence of wild boar was observed at village Ladhawal, Baranhara, Majarakalan, Rajjapur, Bhollewal, Dhanasu, Noorpur bet and Kharak of Ludhiana district of Punjab. Average herd size ranges from 5-9 individuals along the river Sutlej catchment areas where wild boars have ample habitat to roost in the day time and venture out during the night for feeding activities causing damage to agricultural crops. With the aim to study wild boar population with respect to their gender, faecal samples were collected from different habitats to analyse the reproductive hormones of wild boar. Results revealed distinct hormonal patterns corresponding to occurrence of male and female wild boars showing higher level of testosterone and progesterone during the months from July to December indicating more breeding activities as compared to from January to June. Non-invasive hormonal estimation of faecal samples may prove to be a valuable tool for monitoring the reproductive status of wild boar populations, offering a cost-effective and ethically sound alternative to traditional invasive methods. This approach, if thoroughly studied, could help in future wildlife management by guiding conservation efforts and population control tailored to wild boar reproductive dynamics across environments.

Key words: Wild boar (*Sus scrofa*), reproductive dynamics, hormonal estimation, management strategies.

Indian wild boar (*Sus scrofa* Linnaeus) commonly known as wild pig is widely distributed over geographical regions with an exception to Antarctica region (Barrios-Garcia and Ballari, 2012). It belongs to the following taxonomic classification: Phylum - Chordata; Class - Mammalia; Order - Artiodactyla; Family - Suidae; Genus - *Sus*; and species - *Sus scrofa*. The Indian wild boar is highly adaptable mammal with a wide distribution across diverse habitats, ranging from dense forests to open grasslands (Miettinen *et al.*, 2023). Various ecological and climatic factors have been identified to affect the population abundance and distribution of wild boar (Cuevas *et al.*, 2016) but still it is distributed globally because it can eat diverse food types encompassing leaves, roots, seeds, earthworms, insects, and mammals (Irizar *et al.*, 2004, Kim *et al.*, 2019). Furthermore, the high fertility rate, pronounced resilience to different environments and worldwide dearth of predators of wild boar have favoured increase in its populations as well as expansion of its distribution ranges (Johann *et al.*, 2020). Due to fragmentation of habitat, the wild boar population has become overabundant and creates all sort of menace to public. Indian wild boars are social animals that live in groups called sounders which typically consist of adult females known as sows and their off-springs also called as piglets. Adult males, known as boars, are generally solitary and form their own territories. They are considered one of the most courageous wild animals and often engage in territorial fights, even with predators like tigers. The adult Indian wild boar can reach a shoulder height of 83.8 to 91.4 cm and weigh between 90.7 to 136.1 kg.

Wild boars are known for their destructive nature to crops, making them a threat to agriculture (Augustsson *et al.*, 2024). The boars are most active during the morning and evening hours, and they also feed at night. Wild boars are omnivorous and their feeding on different tree seedlings, saplings, and agricultural crops has resulted into pervasive human-animal negative interactions that caused alarming concern to the farming community (Khan and Ilyas, 2018). The coexistence of the forests with the cultivated fields i.e. agroforestry, induces an increase in crop damage (Serrani *et al.*, 2012). Agricultural fields are recognized as preferred

habitats for wild boars (Muthoka *et al.*, 2022) and are utilized more extensively during summer compared to other times of the year (Keuling *et al.*, 2009). The crop damage by wild animals is a very big problem in many parts of India as they damage crops by consuming them or by their rooting, trampling (Cappa *et al.*, 2021), and wallowing behaviours. During the vegetative stage of the crop, wild boars from the nearby forested area dig up the soil with their teeth in search of food, causing damage. Once the crop reaches maturity, these animals damage it by consuming the grains and wandering through the fields. The wild boars cause large damage to the maize (*Zea mays*), peanuts (*Arachis hypogea*), jowar (*Sorghum vulgare*), rice (*Oryza sativa*) some pulses and vegetables crops ranging between 10-75%, 5-56%, 5-30%, 10-35%, 5-20%, 10-30%, respectively in southern Telangana areas (Rao *et al.*, 2015). Singh and Kumar (2018) reported an average crop damage ranging from 15% to 20% across various crops, including maize, jowar, rice, Brassica spp., and vegetables. Throughout the world, substantial economic losses have been reported from wild boars, mainly through directly reducing crop yields and livestock disease transmission (Wang *et al.*, 2023).

Recent conflicts between humans and wild boars (i.e., crop damage, presence in downtown areas, and spread of disease) have become a serious concern, increasing the demand for an effective management plan. Population monitoring is a necessary step in wildlife and habitat management and specifically an accurate and precise monitoring is pivotal to factual decision-making. In order to formulate diverse management strategies, it's essential to recognize the habitat preferences of wild boars in their native habitat (Kovács *et al.*, 2017), their behaviour and interactions within the environment, and their reproductive biology. Comprehending the reproductive status of wild animals is crucial for wildlife management, conservation efforts, and gaining insights into population dynamics. An approach for examining the reproductive functions in a wild population is to determine the endocrine activity by measuring the hormone level either in blood, faeces, saliva, or other biological material (Schwarzenberger and Brown, 2013). Analysing steroid hormone levels through endocrine

assessments allows us to assess the reproductive status of animals; yet, there is limited data available on sex hormone concentrations for wild boars. Conventional techniques used to evaluate reproductive status in wild boars, like invasive blood sampling or direct observation, can be labor-intensive, stressful for the animals, and frequently impractical in field conditions. In recent times, non-invasive approaches employing faecal hormone analysis have emerged as a promising alternative for evaluating reproductive status in wildlife. The analysis of faecal hormones presents numerous benefits, such as minimizing stress on animals, avoiding significant disruptions to their natural behaviour, and enabling the non-invasive collection of samples over extended periods, facilitating longitudinal studies. Through the examination of faecal hormone metabolites, especially those linked to reproductive phases like estrous cycle and pregnancy, we can acquire valuable understandings into the reproductive physiology and status of wild boar populations. There is a lack of information on the faecal profile of sex hormones in this species, as previous studies have been mostly focussed on serum hormone levels. Knowledge on the level of sex hormones in different reproductive stages would contribute to the understanding of its reproductive physiology. As wild boars are of serious concern from agricultural perspective, so the present study was conducted to estimate the wild boar population and assessing their reproductive hormone level from faecal samples to generate baseline data for further studies.

Materials and Methods

Study area and survey methods: The preliminary surveys were conducted during 2022-23 for population assessment of wild boar in seven villages i.e. Ladhowal, Baranhara, Majarakalan, Rajjapur, Bhollewal, Dhanasu, Noorpur bet and Kharak of Ludhiana district (30.9010° N, 75.8573° E) of Punjab at a distance of 500 to 1000 m from each other. During data collection, for direct observations line-transect and point count methods (Glennie *et al.*, 2020) were used. For indirect observations, pug-marks method was followed (Alpizar-Jara, 2006). The observations were taken fortnightly at dusk and dawn. At the time of data collection, the presence of Indian Wild boar was recorded in agricultural crop fields at different period of

time and seasons. To ascertain the presence of wild boar, indirect observations like wallowing in crop field areas, presence of pug marks and presence of fecal matter was recorded and percentage was calculated.

Faecal sample collection and steroid estimation: The locations with high population density of Indian wild boar were selected for collection of faecal samples fortnightly. Wet samples (n=6 each for male and female) were collected from the area near foraging and roosting sites of wild boar throughout the year and stored at 4°C in zip lock bags along with date and month of collection. The faecal samples were segregated into two groups on the basis of months of collection i.e. group I from January to June and group II from July to December and steroids were extracted from faecal samples by using the method of Kusuda *et al.*, (2013) followed by diluting the samples in dilution buffer. The reproductive hormones i.e. progesterone (P₄) and testosterone (T) were assayed by using standard ELISA (Enzyme Linked Immuno Sorbent Assay) kits. The level of hormones was estimated from per cent sensitivity observed through ELISA Reader (Tecan, i20).

Results and Discussion

Surveys conducted across all selected villages in Ludhiana district, Punjab-namely Ladhowal, Baranhara, Majarakalan, Rajjapur, Bhollewal, Dhanasu, Noorpur Bet, and Kharak-indicated the presence of wild boars through indirect signs such as wallowing activity, pugmarks, and feces (Fig. 1, Table 1). The direct observations recorded as the number of herds observed and herd size are also given in table 1.

At location Ladhowal and Dhanasu, two herds each were observed, whereas at remaining locations single herd was observed during the study period. The average herd size ranged from 5 to 9 individuals with maximum 9 at village Dhanasu and minimum 5 at Majarakalan village. Maselli *et al.*, (2014) observed that the social structure of the wild boar included solitary adult males who live isolated from the herd, and groups of females with young boars, having four individuals representing the most frequent group size. During the 120 days of observations, they recorded the activities of 1431 wild boar individuals forming 345 social groups. The number of observations was highest during summer and was lowest in winter. The

Table 1. Observations on prevalence of wild boar in different villages

Village	Direct observations		Indirect observations (%)		
	Number of herds	Herd size (Average)	Wallowing	Pug marks	Faeces
Ladhowal	2	8	15.40	79.23	5.37
Baranhara	1	6	32.92	64.27	2.81
Majarakalan	1	5	30.47	69.53	0.00
Rajjapur	1	7	35.02	58.73	6.25
Bhollewal	1	5	28.20	67.83	3.97
Dhanasu	2	9	20.94	73.91	5.15
Noorpur bet	1	6	15.05	80.74	4.21
Kharak	1	6	24.35	75.65	0.00

basic social unit of wild boar population is the sow and her litter. Males are usually solitary and remain secluded except when participating in breeding groups (Sparklin 2009).

Out of total observations, the highest percentage was of pug-marks ranging from 58.73 to 80.74% followed by wallowing activity ranging from 15.05 to 35.02% and lowest of faecal matter ranging from 2.81 to 6.25%. At two locations -Majarakalan and Kharak only wallowing activity and pugmarks were observed, while no excreta was detected in these study areas. Erdtmann and Keuling, (2020) in their study on behavioural patterns of wild boar studied the comfort behaviour as a behavioural category which mostly serves for two functions *i.e.* personal hygiene behaviour and resting behaviour. Wild boar uses wallowing as a personal hygiene behaviour for thermoregulation because they are not able to sweat and a mud layer also keeps stinging insects away. Further the pug-mark of animals has its own unique individual and class characteristic which help in identifying and differentiating them from other groups of animals (Yadav *et al.*, 2020). Wildlife scientists more likely to see signs of a wild boar, such

as scat, than to see a boar therefore try to find excreta etc as was reported in present study also.

In the hormone assay, progesterone and testosterone estimations were done. Out of these, progesterone provides an important indication of ovulation and pregnancy whereas testosterone helps to identify the sex, reproductive state and behaviour in many mammals. In the present study six samples were studied and ELISA sensitivity for both the hormones was observed to be 80% (Fig. 2). EIA (Enzyme Immuno Assay) sensitivity was 80% with 0.27 and 1.02 pg well⁻¹ binding of Progesterone and testosterone, respectively. Intra-assay co-efficient of variation (CV) for P₄ and T were 5.3 and 5.09% while Inter-assay co-efficient of variation (CV) for P₄ and T were 13.2 and 13.11%.

During the months January to June, out of the samples tested for male wild boar, four were having testosterone level comparatively higher than the female hormone ranging from 1.6 to 2.3 ng g⁻¹ indicating the dominance of male wild boar (Fig. 3). Whereas during July to December months the testosterone level

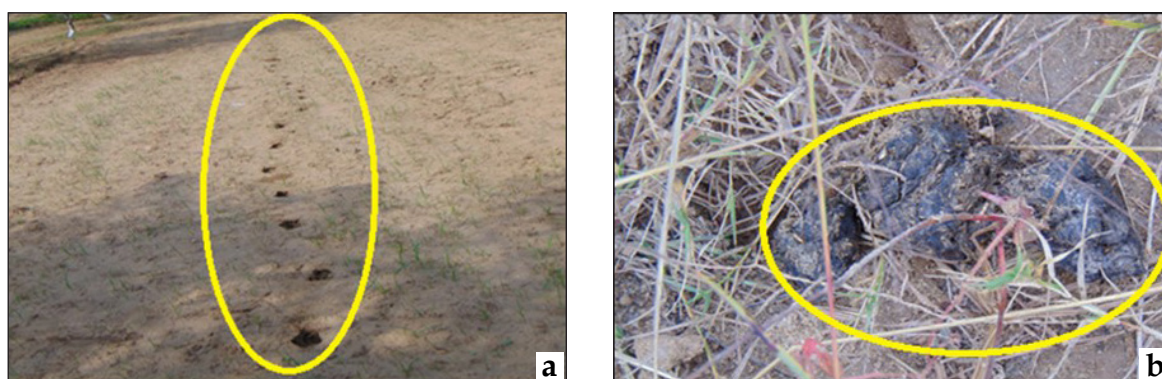


Fig. 1. a. Pug-marks of wild boar b. Excreta of wild boar

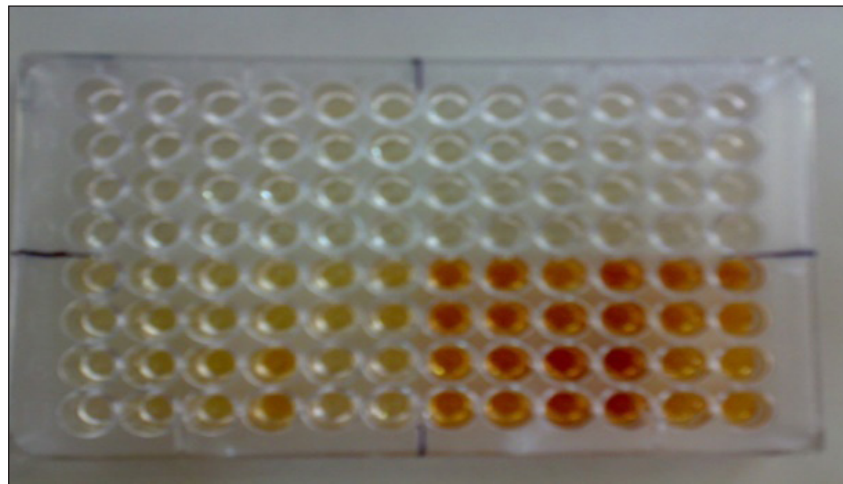


Fig 2. ELISA plate showing hormone binding R1-4 Blank, R 5-8, C1-6 Standard, C7-12 Sample.

showed an increase ranging from 2.1 to 4.1 ng g^{-1} (Fig. 4) attributing to sexual activities during the breeding season of wild boar. Muehlenbein and Watts, (2010) estimated faecal testosterone levels in wild chimpanzee which were directly associated with dominance rank, and both testosterone and cortisol were directly associated with intestinal parasite richness. So, the level of testosterone is also an indicator of social behaviour of wild animals. Testosterone may facilitate attainment of high rank therefore causing increased reproductive success, by modifying other behaviours like aggression, mate seeking, courtship, mate guarding and physical attributes *i.e.*, secondary sexual characteristics and muscle anabolism.

In present study, the level of progesterone in female faecal samples during the months January to June was very low ranging from 0.69 to 1.02 ng g^{-1} (Fig. 3) which could be related to post-partum or non-reproductive behaviour. Ahuja-Aguirre *et al.* (2017) assessed progesterone and estradiol profiles in different reproductive stages of captive collared peccary

(*Pecari tajacu*) females from faecal metabolites and found low level of progesterone as an indicator of lactating or non-pregnant females. In contrast, the levels of progesterone in female faecal samples collected from July to December were notably higher, ranging from 4.7 to 7.1 ng g^{-1} (Fig. 4). This increase strongly indicates heightened sexual receptivity and mating activity during the wild boar's breeding season. Similar findings were reported by Ahuja-Aguirre *et al.* (2017) in their study. On similar lines, faecal P_4 profiles showed a marked increase or decrease seasonally in female golden takins which was considered to correspond to the oestrous cycle (Yoshida *et al.*, 2024). Progesterone is the major hormone in females and is involved in the menstrual cycle, pregnancy, and embryogenesis (Croxatto, 2017). Levels of P_4 were also reported higher in females exposed to high predation or hunting pressure as well as those roaming in a group as compared to females that were alone, with no distinguishable differences in cortisol levels (Davidson *et al.*, 2021) but no such indication was reported in present case. The high faecal

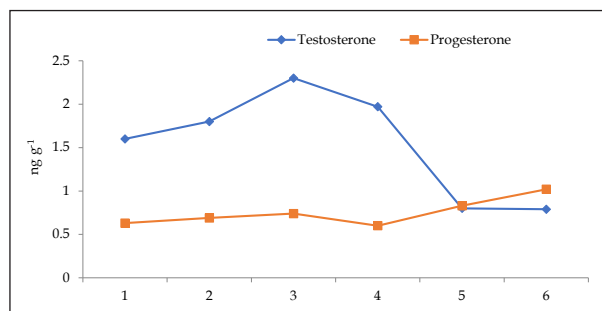


Fig. 3. Level of hormones in faecal samples of wild boar from January to June (group I).

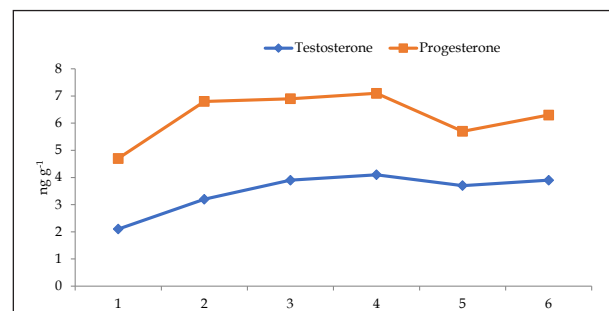


Fig. 4. Level of hormones in faecal samples of wild boar from July to December (group II).

P₄ level of female hormone also highlights the social group dominated and led by females.

Conclusion

Wild boar was reported at all the selected locations attributed to availability of food and water sources in the vicinity. Further distinct faecal hormonal profile corresponding to occurrence of male and female wild boars were reported showing higher level of testosterone and progesterone during the months from July to December indicating more breeding activities as compared to the months from January to June. The non-invasive hormonal estimation of faecal samples throughout the year may prove to be a valuable tool for monitoring the reproductive status of wild boar populations. It will prove a cost-effective and ethically sound alternative to traditional invasive methods. However, the present study was conducted on a small sample size so this approach if studied comprehensively, holds promise for future wildlife management strategies tailored to the specific reproductive dynamics of wild boar populations across different habitats.

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