Sheep breeds raised under a subtropical environment revealed differential disease patterns and DNA damage

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Abstract- Environmental conditions such as heat or cold waves, as well as poor pasture and food quality, have a negative impact on sheep health and productivity. Adaptability to various climatic variables is an important quality for species progress. Twelve indigenous sheep breeds (seven fat-tailed and five thin-tailed) from Khyber Pakhtunkhwa, Pakistan, were maintained and studied in the Peshawar valley's subtropical environment. Over a two-year period, the breeds were observed for the presence of several disease symptoms (pulmonary discomfort, gastrointestinal parasite infestation, polioencephomalacia, digestive disorders, and maggot infestation). In addition, at the end of the study, a comet assay was performed to quantify DNA damage in blood cells from different sheep breeds. Overall, parasite infestation (44%) and pulmonary discomfort (35.9%) had the largest percentage in the flock. Variations in illness occurrence have been documented as a result of breed and age. The prevalence of parasite infestation and respiratory discomfort decreased over time in eight of the twelve breeds studied. During the summer months (May till August), there was a rise in the occurrence of illnesses. DNA damage has a distinct effect on different sheep breeds. Michni, Ghalji, Kari, and Madakhlasht sheep breeds have the least DNA damage. These findings provide a foundation for further research into the genetic underpinnings of illness occurrence and adaptability to environmental changes in sheep.

Index Terms- DNA damage, Gastrointestinal parasites, Indigenous sheep breeds, Polio encephomalacia, Pulmonary distress

I. INTRODUCTION

Sheep which were domesticated circa 8000 B.C., have been widely disseminated around the world in many forms and shapes adapted to local settings ranging from deserts to highlands and mountains [1]. Sheep are directly affected by changes in external temperature, as well as pasture and fodder quality, via thermoregulation [2]. Several studies have revealed that different sheep breeds adapt to changing environmental conditions at varying rates, implying genetic adaptation owing to polymorphism at the whole genome level [2]. In the current climate change situation, it is critical to identify sheep breeds with genetically greater acclimatizing efficiency. Several physiological and morphological markers in sheep have been investigated as indicators of climatic tolerance. Brazilian sheep breeds, for example, have been shown to respond to thermal changes in the climate by changing blood cell counts, body surface temperature, coat thickness, sweat gland size, and hair density in one research [3]. Whole-genome investigations of sheep selective adaptation have found a variety of related genomic areas, processes, and genes [4]. An integrated investigation of genome variation and environmental data revealed significant single nucleotide polymorphisms in genes involved in energy metabolism, the endocrine system, and the immune system [1]. Another study discovered a selection pressor of dryland adaptation on the genomic areas linked with immune response control, reproduction, body morphology and size, DNA damage and repair, and skin and hair pigmentation in Egyptian fat-tailed sheep [5]. Because of the current phenotypic variety in Pakistani sheep, identifying selected features for tolerance to harsh environmental changes is required. The majority of indigenous Pakistani sheep breeds have evolved an inherent adaptability to temperature changes throughout the year. Self-shedding of wool by some Pakistani sheep breeds, such as Balkhi, during the summer is one such adaptation [6]. In this study, we assess the adaptability of 12 sheep breeds native to Khyber Pakhtunkhwa, Pakistan, to the subtropical environment of the Peshawar valley over a two-year period by examining the occurrence of various health conditions. The comet assay is also used to assess the level of DNA damage in these by sampling the breeds' peripheral blood mononuclear cells (PBMCs). The existing phenotypic diversity in Pakistani sheep necessitates characterizing selectable traits for adaptability to the extreme environmental changes. Most of the indigenous Pakistani sheep breeds have been evolved an intrinsic adaptation to temperature variation through different seasons of the year. One such adaptation is self-shedding of wool by some Pakistani sheep breeds, such as Balkhi, during summer [6]. In this study, we evaluate the adaptability of 12 sheep breeds, native to Khyber Pakhtunkhwa, Pakistan, to the subtropical environment of Peshawar valley by analyzing the occurrence of different health problems over a period of two years. We also analyze the level of DNA damage in peripheral blood mononuclear cells (PBMCs) of these sheep breeds using the comet assay. This study demonstrates how environmental changes can affect sheep

populations, as well as the significance of genetic makeup in the adaptability of various sheep breeds.

II. MATERIALS AND METHODS

All tests were carried out in accordance with the ethical guidelines of the University of Agriculture, Peshawar's Animal Welfare Committee. The proposal was examined band approved by the Animal Care and Use Committee of the Institute of Biotechnology and Genetic Engineering, The University of Agriculture, Peshawar.

A. Experimental animals

During the year 2017, a survey of the Khyber Pakhtunkhwa province of Pakistan identified and located twelve sheep breeds (Figure 1). The animals pure to the breed were chosen based on the flocks' breeding history (information collected from farmers during probing) and specific phenotypic identification markers (e.g. colour) of the breed from the literature (Table 1). If a breed was not described in the literature, its distinct phenotypic in comparison to other populations in the area, as well as personal information from the shepherds, were taken into account. All purebred animals were carefully chosen and purchased after receiving various opinions from animal breeding and genetics experts, as well as local shepherds. A total of 12 mature sheep (10 ewes and 2 rams) per breed were purchased and moved to an open shed at the Sheep Resource Center, Agriculture University Dairy Farm, The University of Agriculture Peshawar. The flock is reared there with 5-6 hours of grazing per day and nighttime stall feeding of green fodder supplemented with wheat straw and wheat or maize grains. The animals have enough of clean water to drink. The flock is frequently vaccinated and dewormed. Any disease signs and symptoms that arise in the flock as a whole or in individual sheep are treated appropriately.

B. Collection of morbidity data

For a period of two years, the flock's morbidity data were acquired from the Sheep Resource Center's records (from Jan 2018 till Dec 2019). The current experimental data were collected for a total of 288 individuals from 12 breeds (Table 1). The occurrence of various health problems in the flock was reported based on the following disease signs and symptoms.
Pulmonary distress: nasal secretions, coughing, pneumonia.
Gastrointestinal parasitic infestation: submandibular edema, anemia fecal egg counts.
Polio encephomalacia (PEM): formation of white layer on the cornea.
Digestive disorders: diarrhea, tympany, anorexia, blot
Injury: injury of the head, hooves, limbs, neck or back
Maggots fistulation: invasion of maggots in wounds after injury

C. Blood sampling for PBMCs isolation

Seventy-two mature healthy sheep were chosen from the 12 sheep breeds (six per breed). The selected sheep were thoroughly evaluated for numerous health concerns based on the general indicators and symptoms of the disorders. Individuals displaying indications of any disease were not exposed to a blood test. With sterile syringes, blood was extracted from the sheep's jugular vein and immediately deposited in the designated EDTA tube. Before being delivered to the laboratory, the tubes were put in an icebox. Fresh blood samples were immediately processed in the lab to isolate PBMCs.

D. Isolation of PBMCs from blood

The RBC lysis technique was utilized to isolate PBMCs from whole blood. In a 15 mL falcon tube, 3 mL blood was collected and 7 mL RBC lysis solution (155 mM sodium citrate, 12 mM ammonium chloride, 0.1 mM EDTA) was added. The solution was gently mixed before being centrifuged at 5000 rpm for 25 minutes at room temperature. After centrifugation, the supernatant was carefully removed with a P-1000 micropipette. The PBMC-containing pellet was mixed thoroughly with 1 mL RBC lysis buffer. The PBMCs were transferred to a 1.5 mL Eppendorf tube after being suspended in 1 mL of RBC lysis buffer. The Eppendorf tubes containing PBMCs in RBC lysis buffer were spun at 2500 rpm for 5 minutes. After that, the supernatant was eliminated to separate the PBMC-containing pellet. The pelleted PBMCs were washed once with phosphate-buffered saline (PBS; Life Technologies; Catalog: 003002; Frederick, MD, USA). After that, the PBMCs were resuspended in 500 L PBS. The comet assay was carried out on cell isolates with vitality greater than 80%. The tubes containing prepared single-cell suspensions (after concentration adjustment) were placed on ice to inhibit cell assembly and impede DNA repair.

E. Analysis of DNA damage (Comet assay)

The isolated PBMCs were tested for single-strand DNA breakage and alkali-labile sites using the previously reported methodology of Olive and Banáth, [7] with minor modifications. To summarize, the cells were embedded in 1% low melting agarose on a microscope slide, lysed in alkaline lysis solution (A1), and washed in A2 solution (Table 2). The slides were then electrophoresed in A2 solution for 30 minutes at 30V. After electrophoresis, the slides were stained for 20 minutes in a 2.5 g/ml solution of propidium iodide (P1304MP, Themo Fisher Scientific) and carefully rinsed with distilled water. The slides were kept in a light-tight box at 4 °C until they were imaged with a fluorescent microscope. The samples were excited with light at 535 nm, and images of the emitted light (orange) from the propidium iodide linked to the DNA were saved.

F. Image analysis

Every slide's cells were examined for the creation of comet-like formations. The comets along the border were not studied. Depending on the population of cells present, numerous photos (up to 10) were captured from each slide. CASP Lab [8] image analysis software was used to calculate the percentage of DNA in the comet tail for individual comet photos.

G. Statistical analysis

Data from flock records were evaluated to determine the number of cases exhibiting symptoms of various health problems. SPSS version 23 was used to calculate the percentage distribution of various health conditions by breed, age, gender, and year. Using SPSS software, the comet assay results were evaluated for means and standard errors for % DNA in comet tail among different breeds. P-values for different breeds were calculated using one-way ANOVA in SPSS. To determine the differences across breeds, a pairwise comparison was performed using the Post-Hoc LSD test.

III. RESULTS

A. Health disorders observed in the flock

1. Breed-wise percentage of cases

The indigenous sheep breeds had the highest percentage of patients (44.0%) with gastrointestinal parasite infection and respiratory distress signs (35.9%). Other prominent indicators of health problems in the flock included PEM (7.4%), digestive issues (5.8%), injuries (5.4%), and maggots fistulation (1.6%) (Figure 2 and Table S1). Breed-wise variation was discovered based on the percentage of incidences of various health issues. Four of the twelve sheep breeds (Balkhi, Ghalji, Kutta, and Kaghani) tested positive for gastrointestinal parasite infestation (Figure 2A and Table S1). Michni sheep showed a higher percentage of instances of respiratory distress (64.3%) and a lower percentage of cases of gastrointestinal parasite infestation than other sheep breeds (14.3%). Similarly, as compared to other breeds, Madakhlasht sheep exhibited a higher percentage of pulmonary distress (40.9%) and PEM (36.4%) instances.

2. Age and gender wise percentage of cases

In both adult and lamb sheep, gastrointestinal parasite infestation and pulmonary discomfort were identified at a higher prevalence than the other health conditions (Figure 3A). In seven of the 12 breeds (Hashtnagri, Ghalji, Waziri, Kari, Kutta, Madakhlasht, and Kaghani), lambs had a higher percentage of gastrointestinal parasite infections than adults (Table S2). Adult sheep were shown to have a higher rate of respiratory distress than lambs (Figure 3A). Other than injuries, which were often detected in adult sheep, other health concerns were observed in both adult and lamb sheep.

Variations in the prevalence of several health issues in rams and ewes were also noted (Figure 3B). Rams were found to have a higher frequency of gastrointestinal parasites than ewes. A similar proportion of both sexes experienced pulmonary discomfort. Digestive problems and PEM were seen in a greater proportion of ewes than rams.

3. Year-wise changes in the occurrence of health issues

The 12 sheep breeds were evaluated for the occurrence of various health concerns after being transferred from their original habitat in Khyber Pakhtunkhwa over a two-year period. The findings revealed a larger percentage of incidences of various health concerns detected in the first year (2018) compared to the second year (2019) following transfer (Figure 4). In 2019, four sheep breeds (Ghalji, Mazai, Damani, and Kutta) had fewer incidences of gastrointestinal parasite infestation as well as pulmonary discomfort (Table S3). Balkhi and Kaghani had a lower percentage of parasite infestation in their gastrointestinal tracts, while Michni and Tirahi had fewer incidences of pulmonary distress. Injuries in Balkhi, Waziri, Kari, and Kaghani sheep breeds have decreased over time (Figure 4 and Table S3).

4. Month-wise occurrence of health issues

Figure S1 depicts the monthly distribution of the total number of cases of various health concerns in local sheep breeds. The findings revealed a specific season of development of health issues in several sheep breeds. The summer season saw the highest number of incidents in the majority of sheep breeds (May till Aug). Another rise of cases was seen in various sheep breeds in December 2018, including the Michni, Waziri, and Kutta breeds. However, the incidents did not reoccur in December 2019. PEM also appears to be a seasonal health concern, affecting the majority of the breeds during the months of March and April (spring). However, in Madakhlasht sheep, PEM was present all year. Injuries and digestive disorders were also common in spring (Mar & Apr).

B. DNA damage in local sheep breeds

The percentage of DNA in the comet tail (a measure of DNA damage) was higher in eight of the twelve sheep breeds studied (Figure 5). The remaining four breeds (Michni, Ghalji, Kari, and Madakhlsht) had a lower percentage of DNA in their comet tails. When compared to the other two central KP breeds, Michni sheep had a much lower percentage of DNA in the comet tail (2.1 ± 1.1 percent) (Balkhi and Hashtnagri). Ghalji sheep has the lowest percentage of DNA in comet tails among the southern KP breeds (1.4 ± 0.5 percent). When compared to Kutta and Kaghani from the same location, two breeds from northern KP (Kari and Madakhlasht) revealed lower values for DNA damage (comet tail DNA 0.8 ± 0.7 and 0.9 ± 0.1%, respectively).

IV. DISCUSSION

According to studies, sheep's response to climate change is determined by their genetic make-up or breed type [5]. We investigated the occurrence of several health conditions and DNA damage in 12 phenotypically varied sheep groups after acclimation to the Peshawar valley's subtropical environment. Some sheep breeds may be less sensitive to climate differences in the hunt for selected features. Identifying such traits and genetic resources containing those features would aid in improving local populations' ability to perform better in the face of future climate shifts.

In this study, the sheep flock had the highest percentage of cases showing indications of gastrointestinal parasite infestation. Among the several gastrointestinal parasites detected in the flock's faecal egg counts in this investigation, Heamonchus controtus and Trichostrongylus colubriformis were the most common (unpublished results). Gastrointestinal parasites had previously been identified as one of the leading causes of productivity and weight loss in sheep herds around the world [9]. In the current study, the vulnerability of different sheep breeds to gastrointestinal parasite infestation ranged from 14.3 percent in Michni sheep to 66.7 percent in Balkhi sheep. Previous research has found a greater prevalence of gastrointestinal parasite infestation in sheep populations in western Ethiopia (75.3%) [10], India (68.75%) [11], and Pakistan (78.0%) [12]. If the environmental and management conditions in the current study were the same, difference in the frequency of gastrointestinal parasites among different sheep breeds could be attributed to genetic heterogeneity among these breeds, as previously stated [13].

The second most common health problem among the sheep breeds in this investigation was pulmonary distress. Respiratory difficulties have been identified as the principal causes of morbidity and production loss in 38.2 % of Ethiopian Horro and Menz sheep [14], which is consistent with the results of most breeds in this study. In the current study, Balkhi and Kaghani sheep were the least impacted by respiratory infections, implying that breed variety has a role in respiratory infection resistance. Other sheep breeds have had less morbidity as a result of respiratory ailments in the past [15, 16, 17].

Peaks in several health concerns were noted during the summer season in the current investigation. In contrast, recent research on Wolaita Soddo Zuria district sheep in southern

Ethiopia found higher morbidity rates (52.3 %) during the winter than the summer [17]. Higher morbidity rates during the summer season in most of the sheep breeds studied in this study could be attributed to higher summer temperatures. In comparison to the other local breeds, Michni had higher cases of health problems in the winter than in the summer. These findings show that Michni sheep have adapted to the summer temperatures of the Peshawar valley, as Michni were initially bred in this region [6].

Other health difficulties, including as PEM, digestive disorders, and injuries, usually occurred in different breeds during the spring season. Thiamin insufficiency may be the cause of sheep PEM in the current investigation. This condition has previously been documented to occur throughout the spring and autumn seasons due to rapid changes in feed composition [18] (17 Hepworth, 2018), as was the case in this study. In the current study, the sheep having PEM recovered with repeated thiamin injections along with antibiotics (field observations). The same phenomenon (feed change) that occurs in the spring may also contribute to the digestive issues (Diarrhoea, tympany, and blot) seen in the current study. The majority of injuries occurred as a result of rams fighting for breeding during the spring season.

The assay employed to detect single-strand DNA breaks in the current work is extremely sensitive for assessing DNA damage [19]. In this experiment, the percentage of DNA in the comet tail and the length of the comet tail are thought to be directly proportional to DNA damage. In the current study, differences in the level of DNA damage were found between sheep breeds from different areas. When compared to the northern KP breeds, sheep breeds from central and southern KP have more DNA damage. The DNA damage in sheep blood cells in the current study could be due to the emergence of the diseases, particularly gastrointestinal parasite infection and pulmonary discomfort. Previous studies have indicated that viral diseases such as FMD can cause DNA damage in sheep blood cells [20].

V. CONCLUSION

The sheep in the current study were predominantly impacted by parasite infestations in their gastrointestinal tracts and respiratory discomfort. The incidence of many health issues varied amongst sheep breeds. Adults were more likely to be infected with gastrointestinal parasites, whereas lambs showed signs of respiratory distress in the majority of cases. During acclimatization to the new environment, the occurrence of various health issues decreased over time. The rate of acclimatization varied by sheep breed, with a significant reduction in illness cases in eight of the twelve breeds. The majority of the health issues happened during the summer season. A genetic investigation, focusing on specific genes involved for DNA repair, could provide more information on the DNA damage effects in different sheep breeds.

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REFERENCES

[1] F. H. Lv, S. Agha, J. Kantanen, L. Colli, S. Stucki, J. W. Kijas, S. Joost, M.H. Li and P. Ajmone Marsan, Adaptations to climate-mediated selective pressures in sheep. Mol Biol Evol, 2014, 31: 3324-3343

[2] A. Nielsen, G. Steinheim, A. Mysterud, Do different sheep breeds show equal responses to climate fluctuations? Basic App Ecol, 2013, 14: 137-145

[3] M. H. A. Pantoja, S. N. Esteves, M. A. C. Jacinto, J. R. M. Pezzopane, C. C. P. Paz, J. Silva, J. B. Lourenco Junior, F. Z. Brandao, A. B .B. Moura, N. Romanello, D. Botta, A. R. Garcia, Thermoregulation of male sheep of indigenous or exotic breeds in a tropical environment. J Therm Biol, 2017, 69: 302-310

[4] J. Yang, W.-R. Li, F.-H. Lv, S.-G. He, S.-L. Tian, W.-F. Peng, Y.-W. Sun, Y.-X. Zhao, X.-L. Tu, M. Zhang, X.-L. Xie, Y.-T. Wang, J.-Q. Li, Y.-G. Liu, Z.-Q. Shen, F. Wang, G.-J. Liu, H.-F. Lu, J. Kantanen, J.-L. Han, M.-H. Li, M.-J. Liu, Whole-Genome Sequencing of Native Sheep Provides Insights into Rapid Adaptations to Extreme Environments. Mol Biol Evol, 2016, 33: 2576-2592.

[5] J. M. Mwacharo, E.S. Kim, A.R. Elbeltagy, A.M. Aboul-Naga, B.A. Rischkowsky and M.F. Rothschil, Genomic footprints of dryland stress adaptation in Egyptian fat-tail sheep and their divergence from East African and western Asia cohorts. Sci Rep, 2017, 7: 17647.

[6] M. Ibrahim, S. Ahmad, Z.A. Swati, G. Ullah, Fat-tailed sheep production systems in the Khyber Pakhtunkhwa Province of Pakistan. Trop Anim Health Prod, 2011, 43: 1395-1403

[7] P. L. Olive J. P. Banáth, The comet assay: a method to measure DNA damage in individual cells. Nature protocols, 2006, 1(1), 23-29.

[8] A. L. Adeel, S. Jahan, F. Subhan, W. Alam, R. Bibi, Total anti-oxidant status: a biochemical predictor of human male fertility. Andrologia, 2012, 44, 20-25.

[9] F. Mavrot, H. Hertzberg, P. Torgerson, Effect of gastro-intestinal nematode infection on sheep performance: a systematic review and meta-analysis. Parasit Vect, 2015, 8: 557.

[10] F. Regassa, T. Sori, R. Dhuguma, Y. Kiros, Epidemiology of gastrointestinal parasites of ruminants in Western Oromia, Ethiopia. Int J App Res Vet Med, 2006, 4: 51-57.

[11] V. Singh, P. Varshney, S. Dash, H. Lal.. Prevalence of gastrointestinal parasites in sheep and goats in and around Mathura, India. Vet World, 2013, 6: 260-262.

[12] M. Raza, M. Younas, E. Schlecht, Prevalence of gastrointestinal helminths in pastoral sheep and goat flocks in the Cholistan desert of Pakistan. J Anim Pl Sci, 2014, 24: 127-134.

[13] M. K. Dappawar, , B.S. Khillare, B.W. Narladkar, G.N. Bhangale, Prevalence of gastrointestinal parasites in small ruminants in Udgir area of Marathwada. J Entomol Zool St, 2018, 6: 672-676.

[14] T. Bekele, T. Woldeab, A. Lahlou-Kassi, J. Sherington, Factors affecting morbidity and mortality on-farm and on-station in the Ethiopian highland sheep. Acta Tropica, 1992, 52: 99-1098.

[15] E. Mukasa-Mugerwa, A. Lahlou-Kassi, D. Anindo, J. Rege, S. Tembely, M. Tibbo, R. Baker, Between and within breed variation in lamb survival and the risk factors associated with major causes of mortality in indigenous Horro and Menz sheep in Ethiopia. Sm Rumin Res, 2000, 37: 1-12

[16] V. B.Sharma, M.R. Verma, Incidence and effects of diseases in sheep in Uttar Pradesh. Economic Affairs, 2014, 59: 579-584.

[17] M. Tifashe, A. Hassan, T. Herago, G. Tesfamariam, Analysis of Morbidity and Mortality of Sheep and Goat in Wolaita Soddo Zuria District, Southern Ethiopia. Global Vet, 2017, 18: 168-177

[18] K. Hepworth, Thiamine deficiency induced polioencephomalacia (PEM) of sheep and cattle. Agricultrue and Food Division, Department of Primary Industries and Regional Development, Government of Western Africa, 2018: https://www.agric.wa.gov.au/livestock-biosecurity/thiamine-deficiency-induced-polioencephomalacia-pem-sheep-and-cattle

[19] A. Azqueta, J. Slyskova, S.A. Langie, I. O'Neill Gaivão, A. Collins, Comet assay to measure DNA repair: approach and applications. Front Genet, 2014, 5: 288.

[20] H. A.Abou-Zeina, , S.M. Nasr, S.A. Nassar, T.K. Farag, M.K. El-Bayoumy, E.B. Ata, N.M. Hassan, S.H. Abdel-Aziem, Beneficial effects of antioxidants in improving health conditions of sheep infected with foot-and-mouth disease. Trop Anim Health Prod, 2019, 51: 2379-2386.

Table 1: Sample size and other characteristics of the indigenous sheep breeds.

Provincial Origin	Purchased from	Breed	Bodyweight (Kg)	Tail type	Coat color
Central Khyber	Random places	Balkhi	45-60	Fat	White or brown
Pakhtunkhwa	Random places	Hashtnagri	30-45	Fat	White, black spots on head
	Peshawar valley	Michni	28-43	Fat	Brown or black
Southern Khyber	Hangu	Ghalji	40-60	Fat	Brown
Pakhtunkhwa	South Waziristan	Mazai	22-37	Fat	Brown or light brown
	Tirah	Tirahi	40-55	Fat	Mostly Brown
	Bannu	Waziri	35-50	Fat	White
	D. I. Khan	Damani	30-45	Thin	White
Northern Khyber	Chitral	Kari	12-27	Thin	Mostly White
Pakhtunkhwa	Swat	Kutta	20-35	Thin	Dark brown
	Chitral	Madakhlasht	18-33	Thin	White
	Hazara	Kaghani	25-40	Thin	brown or black

Table 2: Composition of Alkaline lysis solution and Alkaline rinse solution for the comet assay.

Solution	Purpose	Ingredients	Concentration
A1	Cell lysis	NaCl	1.2 M
		EDTA	100 mM
		Sarcosyl	0.1%
		NaOH	0.26 M
A2	Rinsing and electrophoresis	NaOH	0.03 M
		EDTA	2 mM

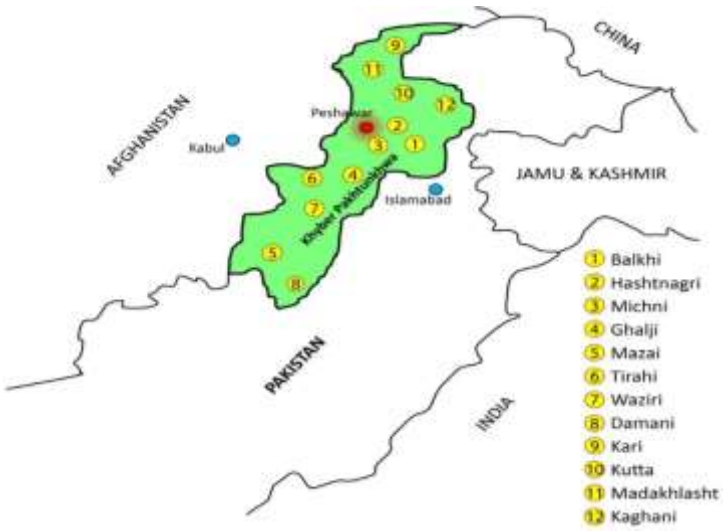

Figure 1: Map indicating the location of the 12 sheep breeds in the Khyber Pakhtunkhwa province of Pakistan. Red dot indicated the capital city Peshawar where the experiment was conducted. Yellow dots with numbers shows different sheep breeds.

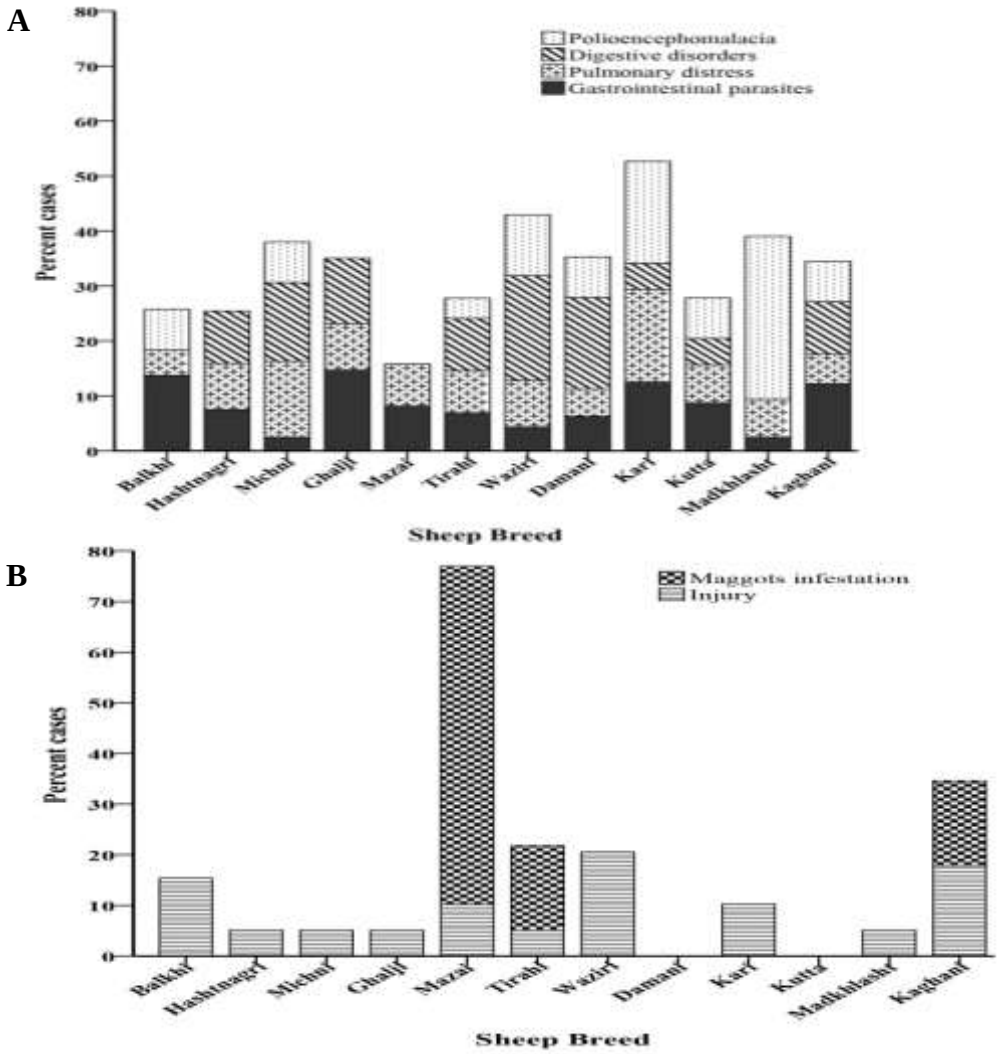

Figure 2: Breed-wise percentage of cases showing signs of different health disorders. (**A**) Percent cases of diseases observed in different breeds. (**B**) Percent cases of physical injuries and maggots fistulation in sheep breeds.

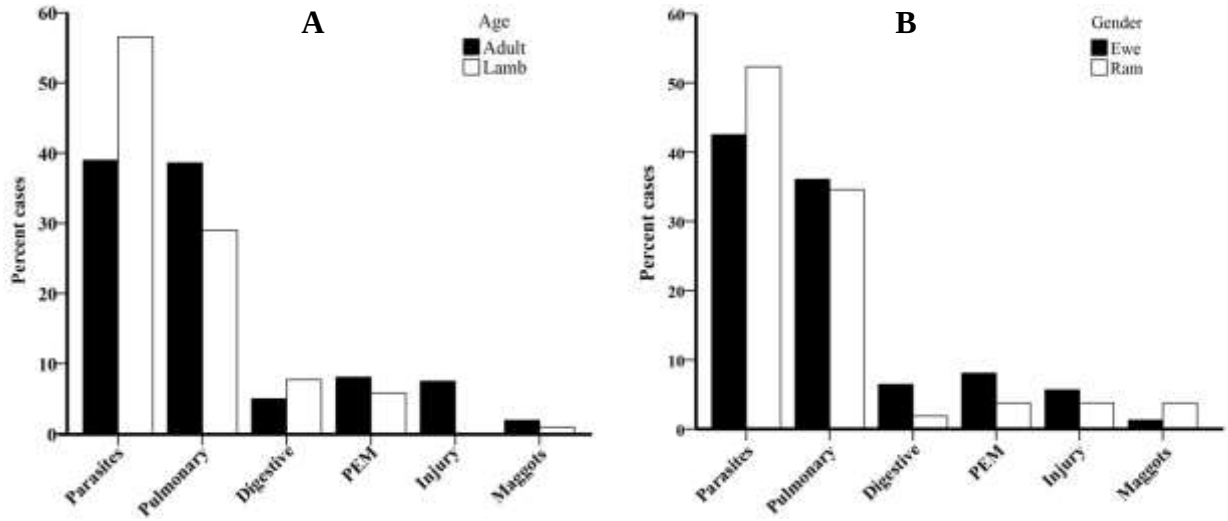

Figure 3: Age (A) and gender (B) wise distribution of the health issues in local sheep breeds. PEM = polioencepomalacia.

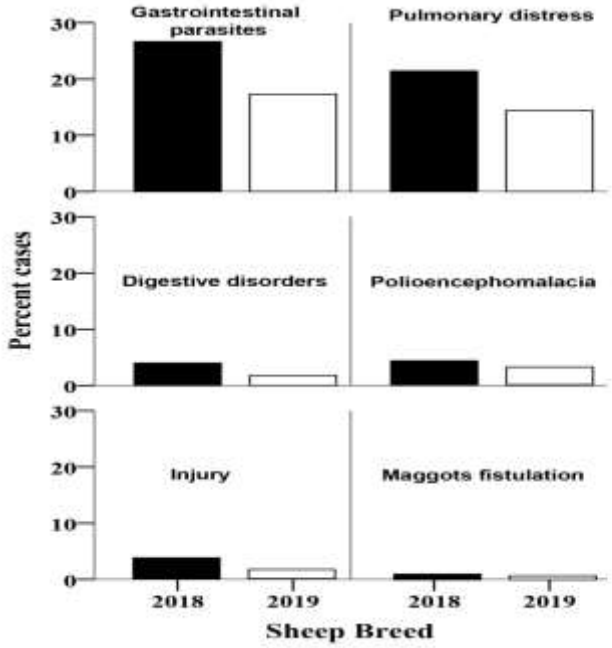

Figure 4: Changes in the occurrence of different health disorders in the flock after being transferred from their habitat

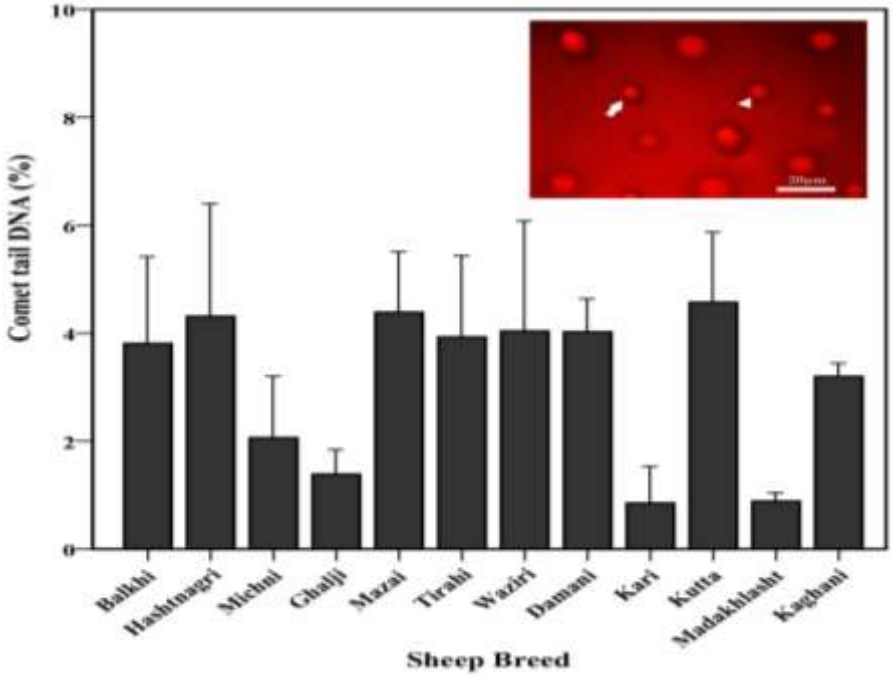

Figure 5: Mean percent DNA in the comet tail in samples from different sheep breeds. A representative microscopic image for the comet assay is shown in the upper left corner. The arrow represents cell with DNA damage forming a comet like structure, arrow head shows a round cell with no DNA damage.