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JOURNAL OF ANIMAL SCIENCES

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The "University of Sindh Journal of Animal Sciences (USJAS)" is dedicated to addressing critical issues within the fields of animal sciences and zoology. Focused on the promotion of research and the dissemination of knowledge, this journal covers a wide spectrum of subjects, including Biodiversity Conservation, highlighting the significance of preserving the planet's diverse animal species amid growing global challenges. Additionally, USJAS delves into the Ecological Impact of human activities, advocating responsible practices and ecosystem preservation. It also emphasizes Sustainable Food Production, recognizing the pivotal role of animals in the global food supply chain. Furthermore, USJAS explores Ecosystem Services provided by animals, contributes to understanding Human-Animal Interaction, encourages interdisciplinary research spanning Entomology, Endocrinology, Molecular Biology, Parasitology, Wildlife Management and Conservation, and Animal Diversity and Systematics. Serving as a platform for researchers, educators, and students, the journal facilitates the exchange of ideas and research findings while promoting access to advanced education programs. Lastly, USJAS underscores the urgency of Global Ecosystem Preservation, aiming to equip stakeholders with knowledge and strategies to safeguard our planet's ecosystems. Through these endeavors, the journal seeks to advance animal sciences, promote responsible practices, and address the intricate challenges faced by both animal species and human societies.

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The mission of the University of Sindh Journal of Animal Sciences (USJAS) is as follows:

Research Excellence: USJAS strives to establish a robust model for research collaboration, facilitating scholarly knowledge exchange, and the dissemination of innovative findings.

Advancing Zoological Sciences: The journal aims to promote the latest trends and developments in zoological sciences.

Ethical Awareness: USJAS seeks to raise awareness about the ethical treatment of animals.

Sustainability: USJAS recognizes its fundamental role in maintaining sustainable agriculture, protecting, and conserving animals within the country.

Environmental Preservation: It also endeavors to educate the public about the crucial need to protect and preserve the environment as a long-term goal for the welfare of future generations.

Research Propagation: The core focus of USJAS is to encourage and disseminate novel and innovative research among its readerships.

AIM OF THE JOURNAL

The primary aim of the University of Sindh Journal of Animal Sciences (USJAS) is to provide researchers with hands-on experience, enabling them to acquire the necessary knowledge for critical analysis of results and the formulation of appropriate recommendations in all fields of animal sciences. The journal aims to:

Encourage Publication: USJAS encourages researchers, investigators, and scientists to publish their findings, facilitating the wider dissemination of intellectual knowledge for the benefit of society.

Comprehensive Coverage: The journal covers a wide spectrum of specialties within the field of animal sciences, including original research articles, review articles, case reports, short commendations, and scientific findings within specified domains of zoology.

Adherence to Guidelines: USJAS strictly adheres to the guidelines proposed by the Higher Education Commission (HEC) Pakistan.

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SIGNIFICANT AND PURPOSE OF ESTABLISHING THE JOURNAL

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Knowledge Dissemination: The journal plays a pivotal role in disseminating valuable research findings, ensuring that the latest insights and discoveries reach a broader audience, including scientists, researchers, educators, and students.

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PUBLICATION
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CONTACT INFORMATION

Prof. Dr. Riffat Sultana
Editor,
Journal office, Department of Zoology, University of Sindh, Jamshoro Sindh, Pakistan.
Journal Website: *http://sujo.usindh.edu.pk/index.php/USJAS*
E-mail: *editor.usjas@usindh.edu.pk, riffat.sultana@usindh.edu.pk*
Contact: +92-333-2776771

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NEWLY DISCOVERED MALE OF *HETEROPODA LANGUIDA* SIMON, 1887 (ARANEAE: SPARASSIDAE) IN YUNNAN, CHINA

SHAKAL KHAN KORAI[*], KAI WANG

The State Key Laboratory of Biocatalysis and Enzyme Engineering of China, College of Life Sciences, Hubei University, Wuhan 430062, Hubei, China

ARTICLE INFORMATION

ABSTRACT

Heteropoda spiders are an essential predator in tropical and subtropical areas because they trap and eat insects, cockroaches, and other domestic soft-bodied pests in crops. Pantropical huntsman spiders do not use webs to catch prey, like other stray spiders do. They catch the insects they eat using their powerful chelicerae (jaws) and quick movements. More than 140 individuals belonging to *Heteropoda languida* Simon, 1887 species were collected from Xishuangbanna Dai Autonomous Prefecture and brought back to lab, and then stored in 75% ethanol. Among which 36 individuals were males and 104 individuals were females. Our research findings provide previously unknown male of *Heteropoda languida* Simon, 1887 for the first time, and the female is re-described from Xishuangbanna Dai autonomous prefecture, Yunnan, China. Our results also adding a little knowledge to *Heteropoda* species distribution, and may help to study the biogeography and dispersal route of *Heteropoda* spiders. In this paper, we provide the diagnosis, description, illustration, and field photos with distribution map for both males and females.

1. INTRODUCTION

According to Singh and Borkotoki (2014), spiders are a successful and old group of invertebrate animals, and they are also known as poisonous arthropods (Perveen & Jamal, 2012). Some scientists thought that spiders evolved into two groups' later one without extensor leg muscles and the other with them in the water where they first appeared. It is estimated that spiders first appeared 400 million years ago. Spiders come in a variety of sizes, shapes, and behaviors and are members of the class Arachnida, order Araneae, and phylum Arthropoda. They construct nests and egg sacs out of the silk as well as use it to hang from and wrap their prey (Turnbull, 1973; Nyffeler & Benz, 1987). According to Sharma et al., (2010), a single spider can manufacture more than half a dozen different types of silk. They make use of a wide range of niches in almost every biome on earth and among the diverse Arthropoda. Some spider species weave webs to catch their prey, whereas others don't. Spiders exhibit a diversity of ecological niches, are markers of environmental change and community level diversity, and are taxonomically varied (James, 2004).

About 40 spider species are thought to be capable of killing people, although *Heteropoda* species do not pose a threat.

Spiders are ferocious hunters, carnivores, and biological agents. Insect pests have been successfully managed by using natural predators like spiders and beetles, which is the most efficient method of management (Cave *et al.*, 2004). In agricultural settings, spiders are naturally occurring predators (Marc *et al.*, 1999; Nyffeler & Sunderland, 2003; Pearce & Zalucki, 2006). According to Tahir *et al.*, (2011) spiders also prey on eggs, larvae, and different stages of organisms in addition to adult insects. Insects that affect humans, such as mosquitoes and cockroaches, are also eaten by spiders. Spiders can consume hundreds of mosquitoes in their lifetime, which lowers the mosquito population (James, 2004). Given their significant function as predators in ecosystems, spiders are unquestionably an essential component of the world's biodiversity. One of the most prevalent groups of predators in the ecosystem, they hunt insects and other small invertebrates and control nuisance animals both inside and outside of our homes. Because of their capacity to create silk, most spiders spend their whole lives in a

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*Corresponding Author**: khanshakal7@gmail.com

single habitat and are prey for a variety of hunting animals, including insectivorous birds and reptiles (James, 2004).

The genus *Heteropoda* Latreille, 1804 is the largest genus of huntsman spiders belonging to subfamily Heteropodinae. It is almost exclusively distributed throughout the world, such as a worldwide invasive (*H. ventatoria*). Currently, 189 species are recorded globally, among which only 15 species are reported from China (World Spider Catalog, 2022). The *Heteropoda* species can be diagnosed by the sheath-like conductor, filiform embolus, and typical spiral windings of female copulatory ducts (Jäger, 2002b). They are widely distributed from tropical to sub-tropical areas, living on tree bark, leaf litter, under rocks, in caves and old houses (Jäger & Yin, 2001).

Xishuangbanna Dai autonomous prefecture (latitude 21°10′N–22°40′N, longitude 99°55′E–101°50′E) located in the southwestern part of China (Fig. 5), bordering Myanmar to the southwest and Laos to the southeast, with mild, warm and humid climate all year round with an annual average temperature from 18.6 to 22.9 °C, and an annual precipitation from 1347.4 to 1916.8 mm (Gao et al., 2020). Therefore, it is rich in animals and plants, belonging to Indo-Burma biodiversity hotspot (Liu *et al.*, 2010; Myers, 1988). Especially, Xishuangbanna Tropical Botanical Garden (XTBG) is a comprehensive research and education center engaged in scientific research, species preservation, popular science and scenic spot as well.

However, few *Heteropoda* spiders were reported and taken photos in this region with only *H. tetrica* Thorell, 1897 recorded by Liu *et al.*, (2010). During surveys on huntsman spiders, lots of individuals of *Heteropoda languida* were collected by the colleagues of Hubei University in Dai Autonomous Prefecture of Xishuangbanna. The male of *H. languida* is reported for the first time, and female is described as new record from China.

2. MATERIALS AND METHODS

Taxonomic description
The specimens were stored in 75% ethanol and examined with an Olympus SZX16 stereomicroscope. The details were further studied using the Olympus BX51 compound microscope. After dissection from spider body, male palp and the female reproductive organs were examined and illustrated. Epigynes were exposed with Proteinase K, habitus photos were taken with Leica 205C stereomicroscope and Olympus BX51 equipped with a

Micropublisher 3.3 RTV camera (QImaging, Surrey, BC, Canada).

Leg formula is given as follows total length (femur, patella, tibia, metatarsus, tarsus). The number of spines each segment is listed in the following order: prolateral, dorsal, retrolateral, ventral (in femora and patellae, ventral spines are absent and the fourth digit is omitted in the spination formula). The used terms and figure legends followed Li *et al.*, (2013). All measurements are in millimetres. The following abbreviation are used in the text and figures are given below:

Abbreviations

Depository institution
CBEE = Centre for Behavioural Ecology and Evolution, College of Life Sciences, Hubei University, Wuhan, China

Somatic morphology
ALE = anterior lateral eyes
AME = anterior median eyes
AW = anterior width of prosoma
BL = body length
CH = clypeus height
OL = opisthosoma length
OW = opisthosoma width
PLE = posterior lateral eyes
PME = posterior median eyes
PL = prosoma length
PW = prosoma width
Leg formula = I, II, III, IV–legs I to IV

3. RESULTS AND DISCUSSION

Family Sparassidae Bertkau, 1872
Subfamily Heteropodinae Thorell, 1873

Genus *Heteropoda* Latreille, 1804
***Heteropoda languida* Simon, 1887**
Figures (1–3, 4, 5)

See World Spider Catalog Version 23.0 (2022) for detailed taxonomic history of this species.

Material Examined: CHINA: Yunnan Province: 31 males & 82 females (CBEE), Xishuangbanna Dai Autonomous Prefecture, Mengla County, Menglun Town, Tropical Botanical Garden (21°55´41"N, 101°15´21"E, 550 m), 22–24 November 2019, J. Chen, J. Liu, Z.C. Li & B. Liang leg. (CBEE); 1 female (CBEE), Xishuangbanna Dai Autonomous Prefecture, Menghai County, Menghai Town, Xiding Village, Xianrenjiaoji (21°58´55"N, 100°28´38"E, 1340 m), 26 July 2020, R. Zhong, Z.C. Li, Z.W. Deng, W. Zhang & Y.T. Zhang leg. (CBEE); 4

males & 19 females (CBEE), Xishuangbanna Dai Autonomous Prefecture, Jinghong City, Mengyang Town, Primitive Forest Park (22°1´53"N, 100°53´27"E, 810 m), 6 August 2020, R. Zhong, Z.C. Li, Z.W. Deng, W. Zhang & Y.T. Zhang leg. (CBEE); 1 female (CBEE), Xishuangbanna Dai Autonomous Prefecture, Menghai County, Mengsong Township, Central Primary School (22°41´4"N, 100°33´55"E, 1340 m), 1 August 2020, R. Zhong, Z.C. Li, Z.W. Deng, W. Zhang & Y.T. Zhang leg. (CBEE); 1 female (CBEE), Xishuangbanna Dai Autonomous Prefecture, Menghai County, Bulang Township, Bulang Mountain (21°35´1"N, 100°24´60"E, 1250 m), 28 July 2020, R. Zhong, Z.C. Li, Z.W. Deng, W. Zhang & Y.T. Zhang leg. (CBEE); 1 male & 1 female (CBEE), Xishuangbanna Dai Autonomous Prefecture, Mengla County, Xiangming Yi Township, Anle Village, Kongming Mountain (22°5´30"N, 101°11´31"E, 1410 m), 9 August 2020, R. Zhong, Z.C. Li, Z.W. Deng, W. Zhang & Y.T. Zhang leg. (CBEE).

Diagnosis: This species is similar to *Heteropoda helge* Jäger, 2008 in having same arising point of embolus and conductor, slightly double S-shaped sperm duct, internal duct system with only one winding, glandular pores situated in the center of first winding, and anterior part of the internal duct system partly covering the posterior part (Jäger, 2008d: Figs 22–53), but can be distinguished from the latter by the following characters: both sexes: Two obvious dark-spots located in the anterior part of abdomen (Fig. 4A–D)**.** Male: 1. Embolus moderately long and slightly extending beyond the cymbial margin in this species, but relatively small and not extending in *H. helge*; 2. dRTA thick and slightly hook-shaped in this species, but thin and sharp in *H. helge*; 3. vRTA with distinct hump in this species, but almost reduced in *H. helge*. Female: 1. Median septum long and narrow in this species, but significantly small and wide in *H. helge***;** 2. Median margins of septum almost running straight in this species, but running laterally in *H. helge* (Figs 1–2A–C, 3A–B).

Description: Male. Measurements: BL 15.4, PL 7.4, PW 7.1, AW 0.2, OL 7.8, OW 5.0. Eyes: AME 0.30, ALE 0.54, PME 0.35, PLE 0.49, AME–AME 0.39, AME–ALE 0.18, PME–PME 0.49, PME–PLE 0.56, AME–PME 0.62, ALE–PLE 0.52, CH AME 0.72, CH ALE 0.66. Spination: Palp: 210, 000, 1210; Fe I-II 231, III 211, IV 121; Pa I-IV 201; Ti I-II 2401, III-IV 2312; Mt I-IV 0120. Measurements of palp and legs: Palp 11.5 (3.7, 1.7, 2.4,-, 3.5), I 32.7 (7.1, 4.1, 9.4, 9.4, 2.5), II 36.3 (7.9, 4.7, 10.2, 10.5, 2.8), III 24.8 (7.0, 3.3, 7.6, 4.8, 1.8), IV 28.9 (6.2, 2.8, 6.8, 10.0, 2.9). Leg formula: II-I-IV-III. Cheliceral furrow with 3 anterior, 4 posterior teeth and ca. 35 denticles.

Palp as in diagnosis: The length of cymbium is about one and half times as long as tibia. RTA arising distally on tibia, dRTA thick and hook-shaped, vRTA short. Embolus moderately long, arising from tegulum at a 5:30-o'clock-position, running a semi-circle and slightly extending beyond cymbial margin. Sperm duct slightly S-shaped in ventral view. Conductor short, arising in a 10:30-o'clock-position on tegulum, slightly extending beyond cymbial margin (Fig. 1–2A–C).

Colouration in ethanol: Dorsal shield of prosoma yellowish-brown to reddish-brown, with bright transversal crescent submarginally located at posterior part. Labium, sternum and gnathocoxae are yellowish-brown to light reddish-brown. Opisthosoma ventrally yellowish-brown, with two obvious markings located at the anterior part of abdomen and a dark marking located at posterior part of abdomen. Opisthosoma dorsally yellowish-brown, with V-shaped light marking located near to the spinnerets (Fig. 1D–E).

Description: Female. Measurement: BL 13.12, PL 6.7, PW 6.5, AW 3.6, OL 7.5, OW 5.3. Eyes: AME 0.32, ALE 0.44, PME 0.33, PLE 0.45, AME–AME 0.26, AME–ALE 0.12, PME–PME 0.43, PME–PLE 0.59, AME–PME 0.49, ALE–PLE 0.51, CH AME 0.39, CH ALE 0.52. Spination: Palp 131, 101, 2121, 1014; Fe I-III 323, IV 321; Pa I-IV 101; Ti I-II 1018, III -IV 2026; Mt I-II 0004, III 1014, IV 3036. Measurements of palp and legs: Palp 11.6 (3.4, 1.8, 2.2,-, 4.0), I 38.8 (9.9, 3.8, 10.6, 11.1, 3.3), II 43.8 (11.7, 4.2, 11.8, 12.8, 3.1), III 31.4 (8.8, 3.5, 8.1, 8.4, 2.5), IV 35.2 (9.4, 3.1, 9.2, 10.6, 2.8). Leg formula: II-I-IV-III. Cheliceral furrow with 3 anterior, 4 sposterior teeth and ca. 33 denticles.

Copulatory organ as in diagnosis: Epigynal field with short anterior bands. Median septum with almost rounded but not triangular subseptal pocket. Lateral lobes almost touching each other at posterior margin. Anterior part of internal duct system partly covering the posterior part with just one winding. Glandular pores are situated in the center of first winding, and posteriad. Fertilization ducts are separated by less than one width and visible in dorsal view (Fig. 3A–B).

Colouration in ethanol: Dorsal shield of prosoma reddish-brown, with dark-bright transversal crescent submarginally located at posterior part. Labium, sternum and gnathocoxae are yellowish-brown to reddish-brown. Opisthosoma ventrally yellowish-brown, with two obvious markings located at anterior part of abdomen and a light-dark marking located at posterior-abdomen. Opisthosoma dorsally yellowish-brown, with V-shaped light marking located near to the spinnerets (Fig. 3C–D).

Colouration in field: Dorsal shield of prosoma, palps and legs are reddish-brown. Dorsal shield of prosoma with

significantly sub-marginal and horizontal dark-band located posteriorly. Opisthosoma yellowish brown to dark brown, with two distinct anterior-dorsal black markings, with one obvious white marking located posteriorly in some individuals (Fig. 4A–D).

Variation: There are some individuals with obvious white marking (Fig. 4C) located in the posterior-dorsal abdomen for both male and female. While the marking is absent in most individuals (Fig. 4A–B, D).

Distribution: China (Yunnan Province, Fig. 5), Myanmar.

Discussion: The long and narrow median septum with roughly parallel lateral margins, the narrow first winding with sub-parallel margins and the posteriad glandular pores situated in the center of the first winding indicate our individuals should belong to *H. languida* though we did not check the holotype. The *H. languida* female was first recorded and reported from Tavoy or presently called Dawei as the type locality, which is located in the Southern part of Myanmar (Simon, 1887; Jäger, 2014b). Dawei is situated about 928km far away from Xishuangbanna Tropical Botanical Garden, and it may fit into the distribution range of this species. We, here reported the male of *H. languida* for the first time. The *H. languida* male and female individuals were collected on the ground, mountains and old houses in Xishuangbanna Dai Autonomous Prefecture.

4. CONCLUSION

Heteropoda Latreille, 1804 is the most diverse genus in the spider family Sparassidae, and widely distributed in tropical and subtropical regions of south and Southeast Asia. The most effective form of management for insect pests has been the use of natural predators like spiders and beetles. Spiders are naturally occurring predators in agricultural environments. In this study, we reported a previously undiscovered male of *Heteropoda languida* for the first time, and redescribed the female from Xishuangbanna Dai autonomous prefecture, Yunnan, China. Additionally, we noticed that some *Heteropoda languida* individuals had clear white markings located in the posterior-dorsal abdomen (Fig. 4C). While the marking is absent in most individuals (Fig. 4A-B, D).

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

6. ACKNOWLEDGEMENTS

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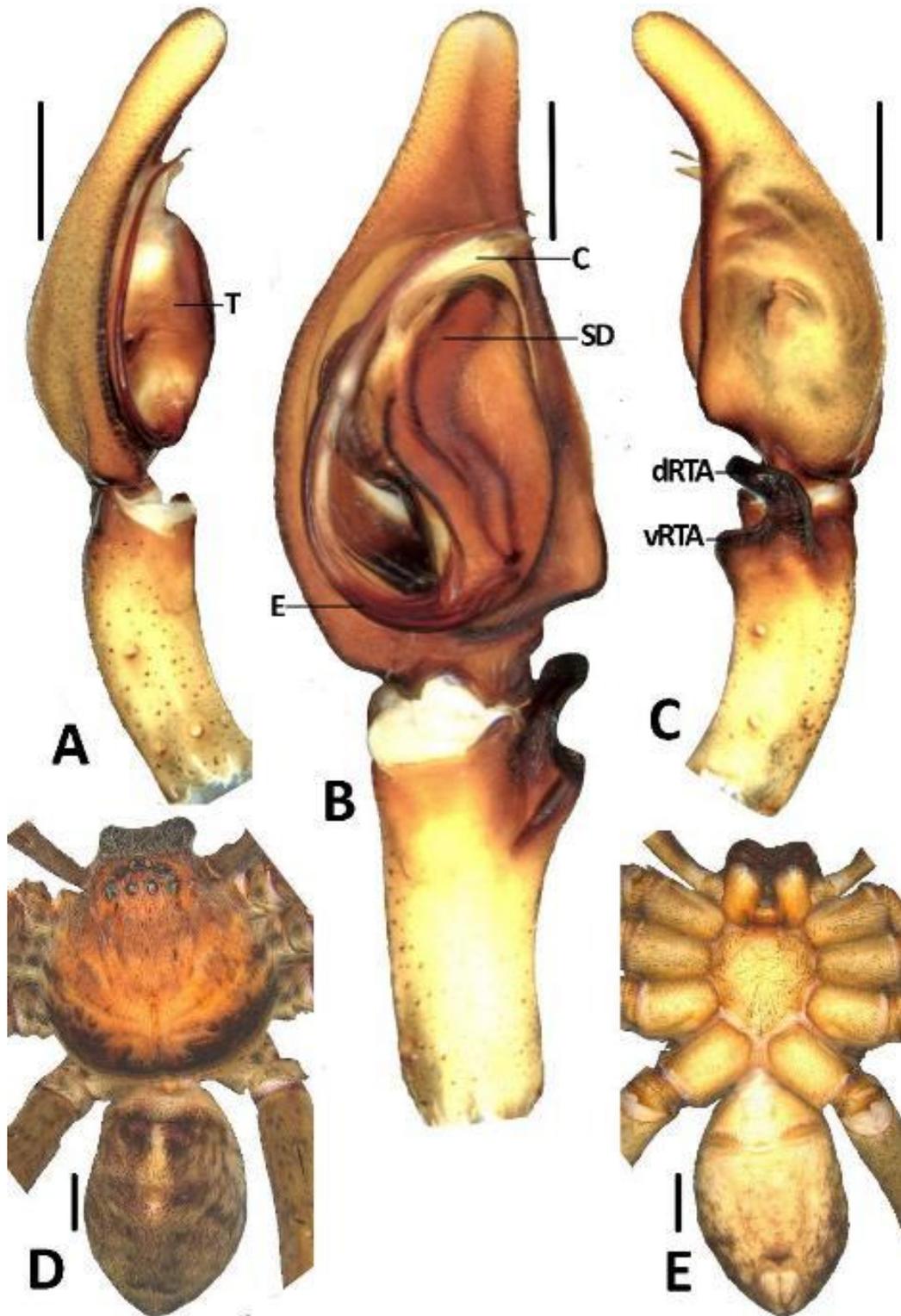
Figure. 1. *Heteropoda languida* Simon, 1887, male from Xishuangbanna Dai Autonomous Prefecture. A–C left male palp (A prolateral, B ventral, C retrolateral); D–E male habitus (D dorsal, E ventral). Abbreviations: C, conductor; dRTA, dorsal part of retrolateral tibial apophysis; E, embolus; SD, sperm duct; vRTA, ventral part of retrolateral tibial apophysis; T, tegulum. Scale bars: A–C 0.5 mm, D–E 2 mm.
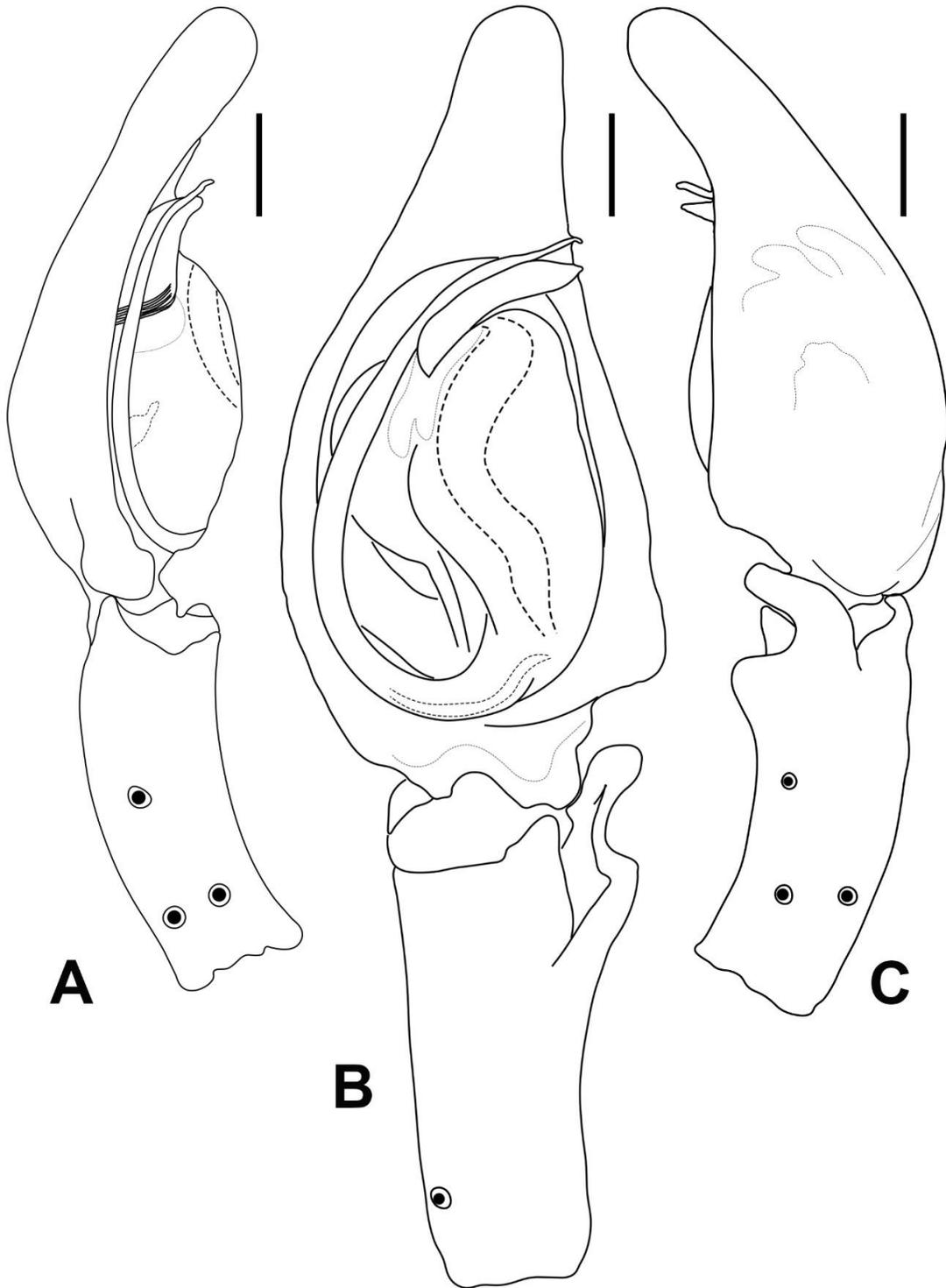
Figure.2. *Heteropoda languida* Simon, 1887, male from Xishuangbanna Dai Autonomous Prefecture. A–C left male palp (A prolateral, B ventral, C retrolateral).
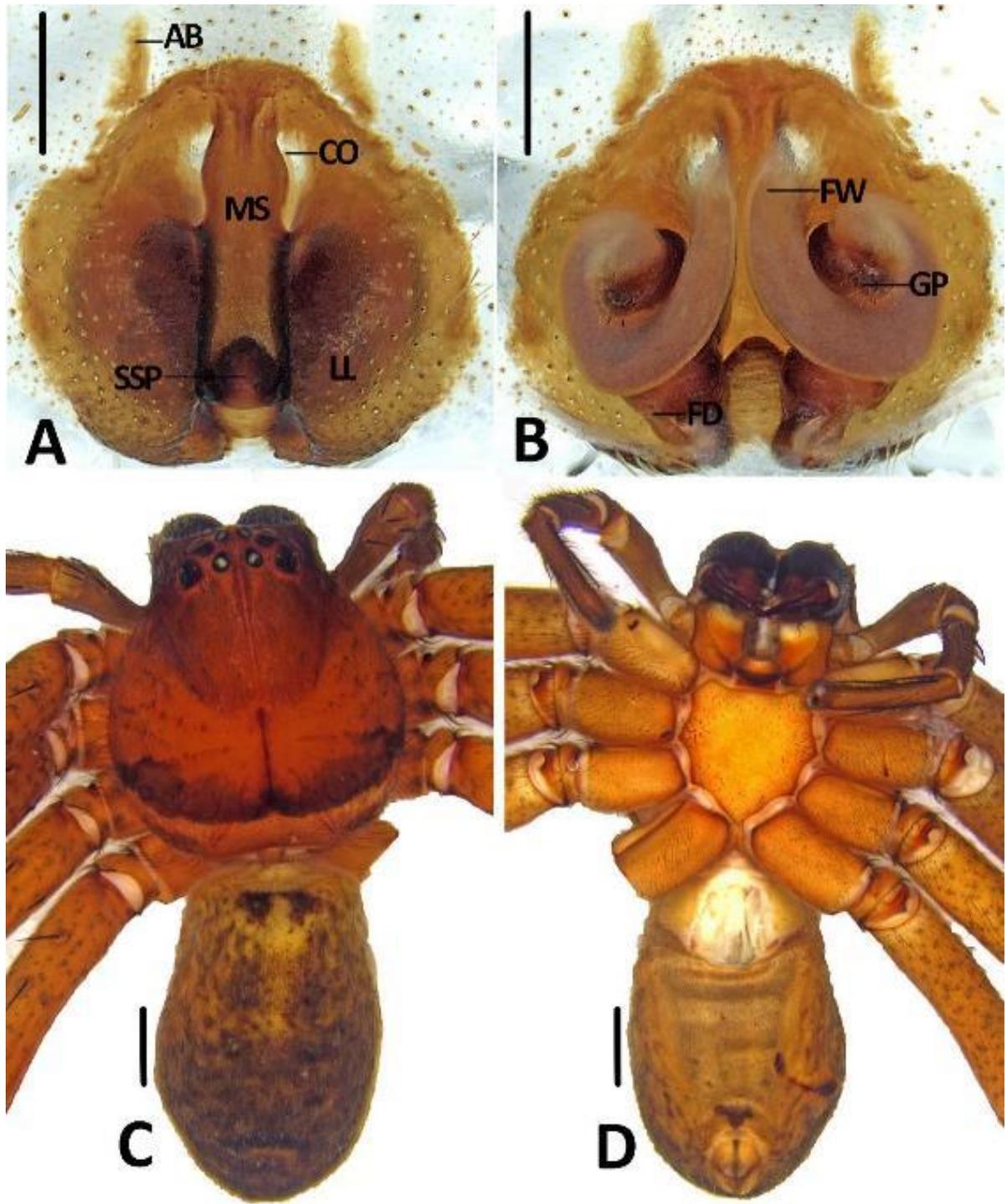
Figure. 3. *Heteropoda languida* Simon, 1887, female from Xishuangbanna Dai Autonomous Prefecture. A–B (A epigyne, ventral; B vulva, dorsal); C–D female habitus (C dorsal, D ventral). Abbreviations: AB, anterior bands; CO, copulatory openings; MS, median septum; LL, lateral lobes; SSP, subseptal pocket; FW, first winding; GP, glandular pores; FD, fertilization duct. Scale bars: A–B 0.5 mm, C–D 2 mm.
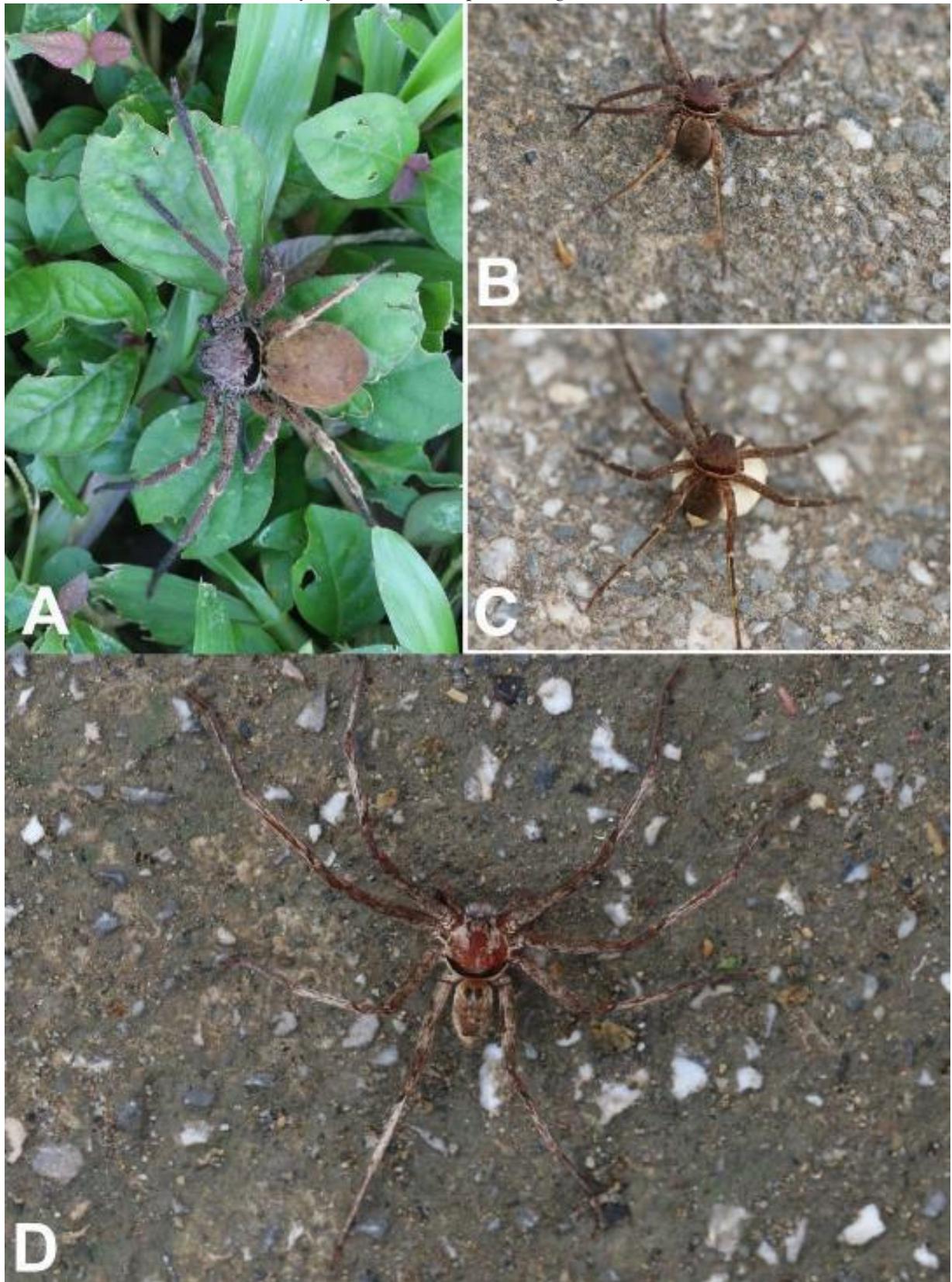
Figure. 4. *H. languida* Simon, 1887 from Xishuangbanna Dai Autonomous Prefecture, Tropical Botanical Garden, Yunnan, China, habitus: female (A–C); male (D), photos by Liang Bing.
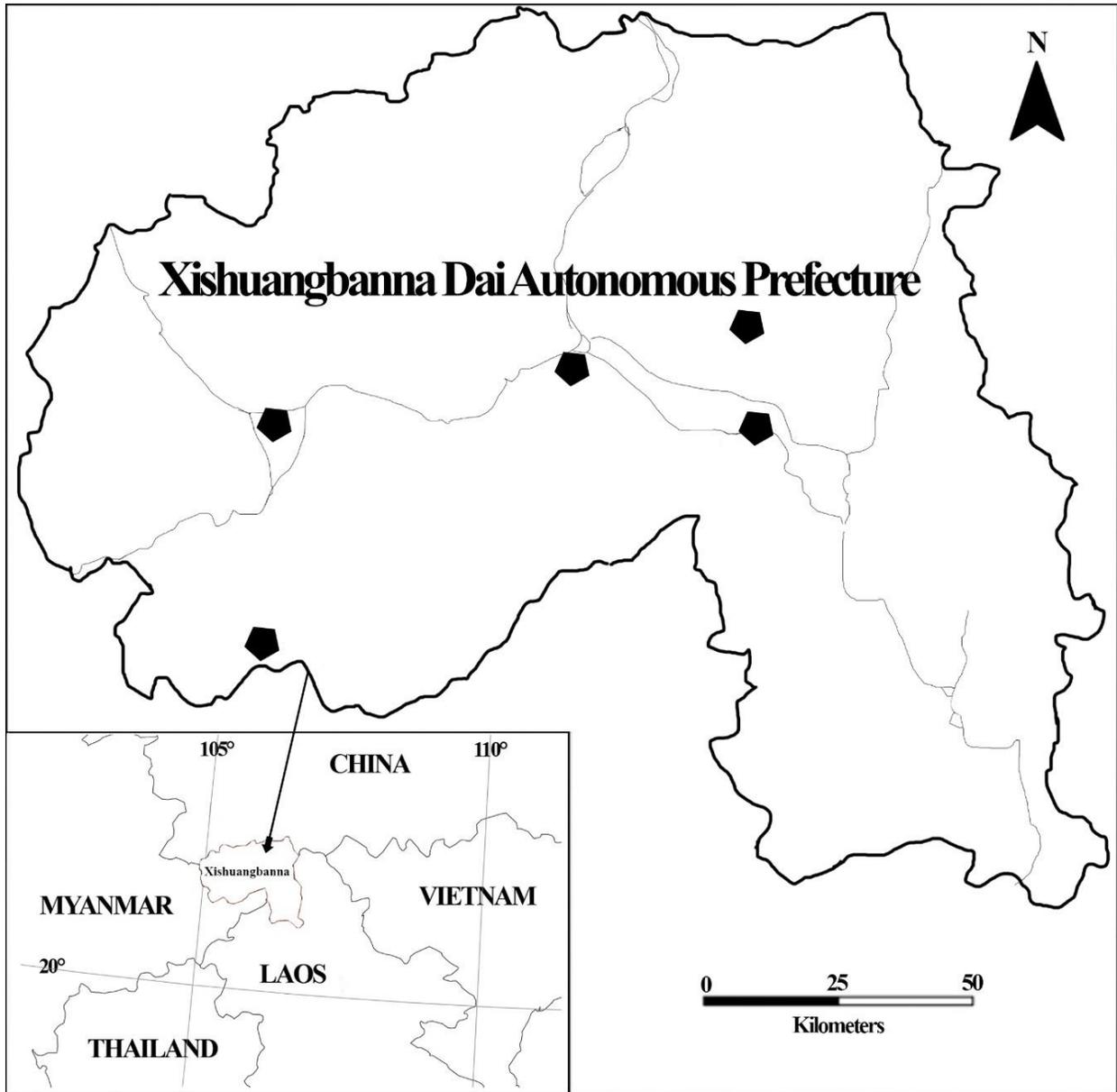
Figure. 5. Locality records for *H. languida* Simon, 1887 from Xishuangbanna Dai Autonomous Prefecture.

COENURUS CEREBRALIS ASSOCIATED PATHOLOGICAL FINDINGS IN GOATS: RESEARCH COMMUNICATION

MOBUSHRA IRSHAD[1], QAMAR UN NISA[1*], GULBEENA SALEEM[1], MUHAMMAD AVAIS[2], SYED KHURRAM FAREED[3]

[1]Department of Pathology, University of Veterinary and Animal Sciences, Lahore-Pakistan.
[2]Department of Veterinary Medicine, University of Veterinary and Animal Sciences, Lahore-Pakistan.
[3]Baqai College of Veterinary Sciences, Baqai Medical University, Karachi, Pakistan

ARTICLE INFORMATION

ABSTRACT

Coenurosis, which is also known as gid is a stage, which causes infection especially in goats, sheeps and sometimes also in human. This is the intermediate stage of the tapeworm of carnivores, known as Taenia multiceps. Blood, CSF, brain and cyst samples of total 30 (N=30) were obtained from the affected goats.CBC, serum biochemistry, PCR, cytology and histopathology was performed. The data about owner, management and conditions were recorded. Chi-square test and Independent t-test was used for analysis.Statistical analysis was performed on Statistical Package for the Social Sciences (IBM SPSS version 25) software. This study done yield data about the status of acute and chronic form of *Coenurosis cerebralis* in goats population of district Lahore and its cytological effects and histopathological lesion,as well as, its hematological effects and molecular characterization.The study findings helped in designing some strict measures for minimizing economic losses in livestock industry of Pakistan.

1. INTRODUCTION

Livestock is considered as one of the fastest growing subsectors of agriculture in Pakistan and its share in agriculture is 56% and 11% in GDP. Livestock promote socio-economic development and approximately 35 million people are involved in raising animals for meat and milk purposes. Due to increase in population and food demand, it becomes a vigorous source of income for the rural and plays an important role in alleviation of poverty as they earn 10-25% of their income from livestock. According to economic survey of Pakistan goat population is 53.5 million numbers and meat, milk and skin obtained from goats (Rehman *et al.,* 2017).

Within rural society goats are an important animal in livestock productions systems. Because of fewer resources and maintenance cost, low input requirements goat comprises the total wealth of poor families of rural areas. Quick reconstitution of flocks according to demand and after disaster needs only short period.

Diseases due to poor management and poor breeding policies are the major constraints in small ruminants' production systems. Goat productivity is mostly affected by helminthes parasites in Pakistan (Shiferaw & Abdela, 2016). In small ruminant *Ceonurus cerbralis* is considered as the major cause of stockbreeding losses (Sun *et al.,* 2017).

Coenurus cerebralis causes neurological parasitic disease, which is known as staggers, gid and sturdy, is a stage, which causes infection especially in goats, sheeps and in humans. This is the larval stage of the tapeworm of canid known as *Taenia multiceps* and normally present in intermediate host (Oryan *et al.,* 2015a). Tapeworms of Cyclophyllidea are among the crown groups of the subclass eucestoda. Among this Taeniidae family which consist of tapeworms of livestock in the genus Taenia. The general characteristics of different species exibit as specific arrangement of rosteller hooks on scolex in cyst (Hoberg, 2002).

During the 17th century reliable records of the disease appeared in literature although the details of nervous symptoms of the disease have been discovered in literature from the time of Hippocrates. The adult tape worm inhabits the small intestine of canids which are definitive hosts and eggs are excreted within the feces and ingested by herbivores that act as intermediate host for parasite.

Other species which affect canines or feline and rodents are *Taenia pisiformis* and *Taenia taeniaeformis* and those which are of zoonotic importance include *Taenia solium* which is found in pigs and *Taenia saginata* present in cattle (Hoberg 2002). The metacestodal form was first time recognized by (Schuster *et al.,* 2015). The neurological form of *Coenurus cerebralis* in goat was reported first from Lahore, Pakistan by Greig (1977) which is caused by intermediate stage of *Taenia multiceps* (Rahman *et al.,* 2017). The prominent feature associated with gid developed sporadically and progressively became more visible prior to the death of the animal postmortem findings revealed simultaneous occurrence of cerebral and hepatic form of coenurosis. While considering pathological findings in the affected organs, a different strain of *Taenia multiceps* was involved in the caprine coenurosis. Normally Coenurusis affects the brain, but its stray locations may also be recorded sometimes (Sharma *et al.,* 1995). There is also another form of this infection, which is caused by *Coenurus gaigeri* in: intramuscular, sub-cutaneous and connective tissues, as well as, liver, and lung also affected in goats (Oryan *et al.,* 2015b).

2. MATERIALS AND METHODS

Study Area
The second largest city as well as the capital of Punjab is Lahore. The total area is 1,772 square kilometers. The total population of Lahore is 11,126,285. The study was carried out on the infected goats which were randomly selected after physical examination at different veterinary hospitals and local slaughterhouses from the surroundings of Lahore.

Experimental Design
A total of 30 goats (n=30) were included in this study. Inclusion criteria was the suspected goats. The samples including blood, cerebrospinal fluid, brain, and cyst were collected. All the samples were collected by following standard protocol to minimize pain and discomfort to the animals while conducting these experiments.

The study was conducted to determine cytological examination, molecular characterization, haematological analysis, and histopathological lesions of *Coenurus Cerebralis* in goats.

Collection of Cerebrospinal fluid
Collection of CSF samples was performed by cerebellomedullary cisternal puncture.

Collection of Blood and Serum Samples
The blood samples were collected from the jugular vein of goats located in the neck region.

Sample collection for histopathology
The heads from 30 goats (n=30) were collected at the Abattoir after slaughtering. The head separated from the rest of the carcasses and brought to the UVAS postmortem laboratory where brain and cyst samples were collected from it.

Cytology of CSF
Cerebrospinal fluid samples were evaluated after simple centrifugation and stained with Giemsa.

Molecular Study
DNA was extracted from 200µl cerebrospinal fluid samples with genomic purification kit catalogue# 51304, using the QIAamp DNA Mini Kit (50) (Qiagen, Hilden, Germany) according to the manufacturer's protocol for PCR and for the confirmation of *Coenurus Cerbralis* in the collected CSF samples. DNA was stored at –20 °C. PCR was performed in a thermo cycler by following Initial denaturation at 95 °C for 5 minutes, denaturation at 94 °C for 1 minute, annealing at 55 °C for 1 minute, elongation at 72 °C for 1 minute and final elongation at 72 °C for 5 minutes was performed. A total of 35 cycles of denaturation, annealing and elongation were performed. The PCR products obtained from the mitochondrial region were electrophoresed on a 1.5 % agarose gel and stained with ethidium bromide (10 mg/ml). The amplification products were visualized under a UV trans illuminator and photographed.

Hematological analysis
From blood sample complete blood count was performed to check the changes in the level of white blood cells, lymphocyte, monocyte, granulocyte, hemoglobin, red blood cell count, PCV, MCV, MCH, MCHC, RDW, PDW, HCT, PCT and platelets by using the MS4Se fully automated hematology cell counter (Melet Schloesing Laboratories, France) according to the manufacturer's protocol.

Serum chemistry
Total protein estimation from collected blood sample was performed by using Edif Sphera Chemistry Analyzer (Edif Instruments S.r.I Via Ardeatina, 132 – 00179 Roma, Italy) according to the manufacturer's protocol.

Histopathology

Collected tissues samples were examined histopathologically by using standard techniques. To prevent any autolytic changes tissue samples were fixed in 10% neutral buffered formalin with the ratio of 1:10 and allowed it to be adequately fixed for 24-48 hours. The sample was further proceeded by following steps; dehydration, clearing, sectioning, embedding, and staining with H&E stain.

3. RESULTS AND DISCUSSION

The research study was performed with the objective of observing the changes in blood parameters of goats affected with fatal infection and for diagnosis of *Coenurus cerebralis* based on PCR. Cytology of samples was performed for the observation of effects of infection on cerebrospinal fluid. The alteration in brain tissues of goats including presence of cyst was observed. Histopathology of brain and cyst for the observation of changes in tissues was performed. The alterations in blood were checked by performing complete blood count including various parameters like packed cell volume, MCV, MCH, MCHC, red cell distribution width, red blood cell, white blood cell, lymphocyte, monocyte, granulocyte, hemoglobin, and platelets. For observation of serum chemistry total protein estimation was performed. Cytology of samples was performed for the observation of effects of infection on CSF.

For the fulfillment of these objectives, suspected goats showing nervous signs including froth from nostrils, frequent bleating, circling, staggering gait, blindness, bumps into objects and paralysis were used. A total of 30 goats (n=30) were used in this study. The samples were collected from the local Abattoir and clinics by following standard protocol. The sampling was performed by following three steps; firstly, the blood samples of 4-5 ml were collected for CBC and serum chemistry, secondly CSF samples of 4-5ml were collected for PCR and cytology, and thirdly different brain sections and cyst were collected for histopathology. All the samples were collected by using sterile and standard techniques and transported to the laboratories of UVAS Lahore. The history of animal including sex, age, breed, area, duration, purchase or marketed and signs was recorded. The data regarding the owner including name, contact number and residential address was collected.

In this study first the identification of showing certain nervous signs was performed. The visually suspected goats were mostly showing signs of high temperature, restlessness, high respiratory rate, unilateral blindness, paralysis, froth from nostril and mouth and downer condition. After slaughter the presence of cysts were confirmed as it is specific sign of this infection as shown in figure 1, 2 and 3.

CSF samples cytology show presence of different cells in cerebrospinal fluid. The presence of the following cells: eosinophils, red blood cells, white blood cells, lymphocytes, neutrophils, and monocytes were detected during microscopic examination of slides from the infected goat.

By the PCR, 21 goats were found positive, and 9 goats were found negative. The suspected goats, which were confirmed with PCR later, show that there were significant differences in values of blood parameters between affected and healthy goats as shown in table 1.

The result of serum biochemistry shows there was significant difference in the value of total protein concentration of effected goats as shown in table 2.
Total protein concentration was increased in the blood of the gid infected goats. The most prominent changes observed grossly were hemorrhages, thinning and atrophy of overlying cerebral tissues. During microscopic examination macrophages infiltration, Leukocytic infiltration along with red blood cells was also observed. Perivascular cuffing microgliosis, cestode cutis debris and fewer lymphocytes were also observed microscopically. Chronic inflammatory cells, degenerative mononuclear cells, histiocytes, langerhans giant cells, necrosis and congested blood vessels were also noticed as shown in figure 4 to 16.

Meat production is one of the major activities of Pakistani Livestock Sector. Currently owing to changing lifestyle and awareness regarding balanced diet, meat demand has drastically increased. Regardless of the nature of the economy, the agriculture sector plays pivotal roles in the economic development of the developing countries. Livestock is the single largest contributor to overall agriculture i.e., 53.2 percent. Livestock is considered as one of the fastest growing subsectors of agriculture in Pakistan and its share in agriculture is 56% and 11% in GDP. Approximately 35 million of people are involved in raising animals for meat and milk purposes. On the other hand, the population growth, increase in per capita income and export revenue is fueling the demand of livestock and livestock products and population growth, urbanization and income growth in developing countries are fuelling a massive global increase in demand for food of animal origin. That is why it becomes a vigorous source of income for the rural and playing an important role in alleviation of poverty as they earn 10-25% of their income from livestock. From goat meat ,milk and skin obtained (Rehman *et al*., 2017)

Coenurosis *is* a fatal zoonotic disease of small ruminants and known as gid, sturdy, staggers. It is neurological parasitic infection caused by intermediate stage of metacestode of *Taenia multiceps* which is a tape worm of canids (Oryan *et al*., 2015a). *Coenurus Cerebralis* causes economic losses in exports by condemnation of the effected organs of the animal like brain or by killing the animal or by reduction in productivity of the animals (Abera *et al*., 2016)**.**

In this study the suspected goats were diagnosed by physical and neurological examination of the animal as described by (Zobba *et al*., 2014a) as well as (Li *et al*., 2017). The same method of diagnosis from history and clinical manifestations was described. In current study the identification of infection showing certain nervous signs was performed. The visually suspected goats were mostly showing the signs of drowsiness, irregular gait, circling and failure to hold head straight as observed by (Hassanein & Elghaffar 2016). High temperature, restlessness, high respiration, appetite loss and unable to stand were also observed as described by (Shiferaw & Abdela, 2016). Other neurological symptoms which were observed include blindness of either left/ right or both eyes as observed by (Oryan *et al*., 2014) . In chronic condition paralysis also observed as by (Al-Riyami *et al*., 2016)

In the present study, CSF was collected from suspected goats for cytological examination. Collected CSF was transported to the laboratory using standard procedure, and immediately slides were prepared by centrifugation and staining with Giemsa. Cytological evaluation of CSF showed increased eosinophils as well as presence of lymphocytes. Other cells including red blood cells, lymphocytes and neutrophils were also present in s the smear of CSF. The presence of eosinophilia in CSF smear has been considered as effective pre-method for the diagnosis of parasitic infection like gid as observed by (Oruç & Uslu, 2006)

In this study polymerase chain reaction was performed on CSF samples for the diagnosis of *Coenurosis cerebralis* infection in goats. For this purpose, 4-5 ml of CSF samples were collected from the suspected goats and transported to the laboratory following standard protocol. For PCR the first mitochondrial DNA was extracted from CSF by using QIAamp DNA Mini Kit. PCR was performed by following certain conditions as described by (Oryan *et al*., 2015a) . The presence of parasite was confirmed by gel electrophoresis after seeing through trans illuminator. The PCR of 30 goats were performed out of which 21 were positive and 9 were found negative.

So PCR also used for identification of parasite in ruminants as described by (Oryan *et al*., 2015a) . There were some variations in results were recorded. Some goats results showed positive but they do not had cyst in brain. Similarly, some results were negative even for the goats having cyst in brain. Variation in results may be due to some external factors. But still examination of the brain containing cyst having thin wall and filled with transparent fluid remain as definitive diagnosis despite the availability of these tests(Afonso *et al*., 2011). Similar method was described for diagnosis as separation of head after slaughtering and by visible examination of brain (Miran, 2013) and by checking localization of cyst in brain (Scott, 2012).

In the present study blood samples were collected from the suspected goats and there were significant differences recorded in values of hematological parameters between affected and healthy goats. There was an increase in white blood cells, lymphocytes, monocytes, granulocytes and red blood cells. The decrease in MCV, MCH and MCHC were also recorded. There was increase in RDW and platelets. In some cases decrease in hemoglobin recorded as observed by (Ghosh *et al*., 1998) but others parameters were contrast from the results . In some cases no effect on hematological parameters recorded as recorded by (Kumar *et al*., 2002)

In the present study the blood samples were collected from the suspected goats and the result shows there was significant difference in the value of total protein concentration. Total protein concentration was increased which were different from previous data as (Kumar *et al*., 2002) . In the similar serological studies the decrease in the level of total protein was recorded which is different from our current study (Saleh, 2004). There was no significant increase recorded in serum protein according to (Altintaş *et al*., 1997). Variations in the result of these studies can be due to geographical, species variations and due to some other factors influencing the change in natural infections.

In the present study head of the slaughtered goat was collected from slaughterhouse and clinics and transported to University of Veterinary and Animal Sciences laboratory where further steps of processing of the tissues were performed according to the standard protocol. On opening of the skull thinning and atrophy of the overlaying cerebral tissues was recorded. In addition, there was presence of visible cyst, which was ruptured on opening and fluid discharge abruptly as observed by (Haridy *et al*., 2013). The surrounding brain tissues revealed perivascular cuffing as observed by (Oryan *et al*., 2015a).The cellular debris were also recorded as by (Ioannidou *et al*., 2015)

4. CONCLUSION

From the results of the present study, it can be concluded that there are significant tissue changes in brain and formation of cyst as well as many blood alterations occurs. PCR can be used for the diagnosis of *Coenurus cerebralis.*

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

6. ETHICAL STATEMENT

Hereby, Mobushra Irshad consciously assures that this material is the author's 'own research and analysis in a truthful and complete manner. All sources used are properly disclosed. All authors have been personally and actively involved in substantial work leading to the paper and will take public responsibility for its content.

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Figure 1. Cyst from the brain of goat

Figure 2. Sample of brain showing presence of cyst

Figure 3. Presence of hemorrhages and cyst in brain
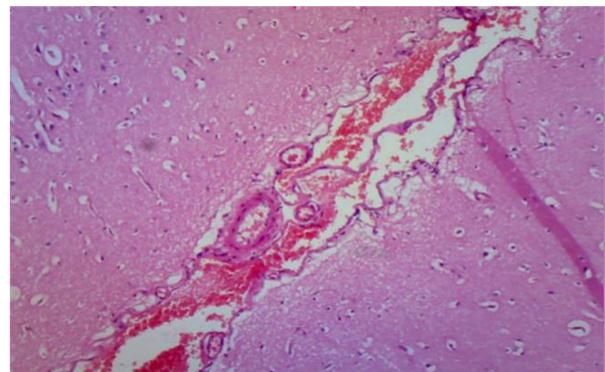
Figure 4. Micrograph of brain cross section showing congested blood vessels (H&E, 10X)
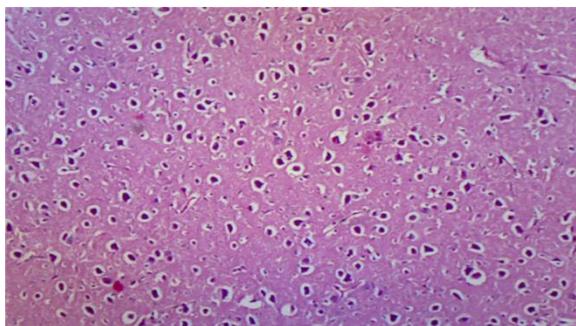
Figure 5. Microphotograph of brain cross section showing gliosis (Hematoxylin-Eosin, 10X)
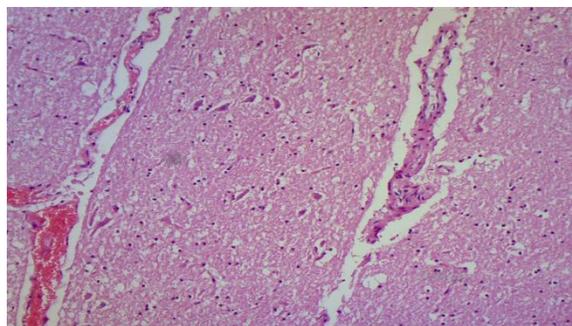
Figure 8. Micrograph of brain cross section showing congested blood vessels (H&E, 10X)
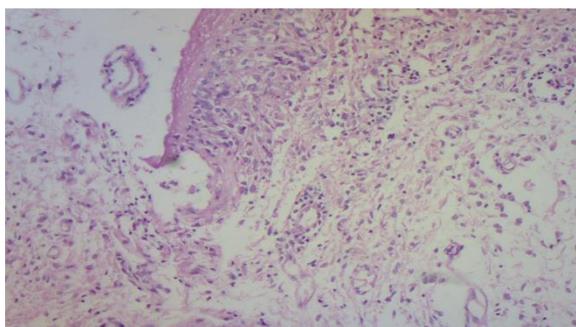
Figure 6. Photomicrograph of brain cross section showing leukocytic infiltration (Hematoxylin-Eosin, 10X)
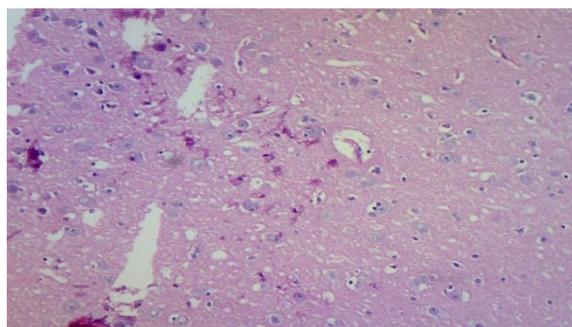
Figure 9. Micrograph of brain cross section showing gliosis (Hematoxylin-Eosin, 10X)
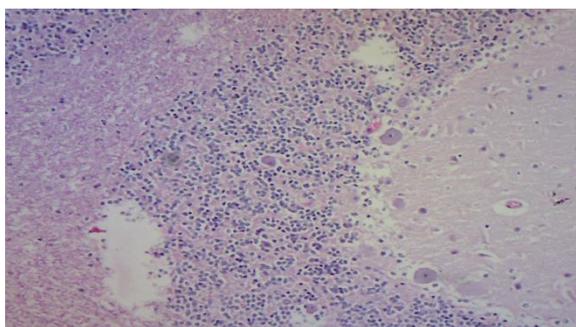
Figure 7. Photomicrograph of brain cross section showing leukocytic infiltration (Hematoxylin-Eosin, 10X)
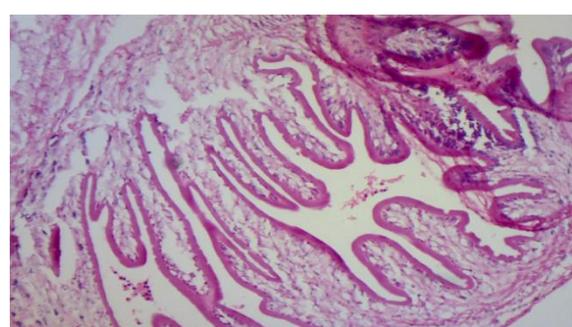
Figure 10. Photomicrograph of cyst cross section (Hematoxylin-Eosin, 10X)
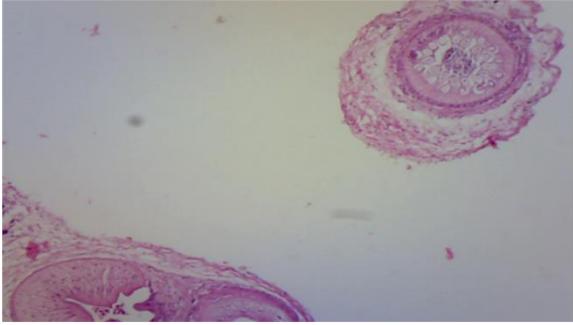
Figure 11. Photomicrograph of cyst cross section (Hematoxylin-Eosin, 10X)
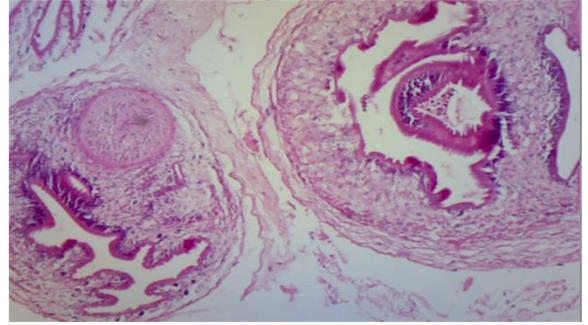
Figure 14. Photomicrograph of cyst cross section (Hematoxylin-Eosin, 10X)
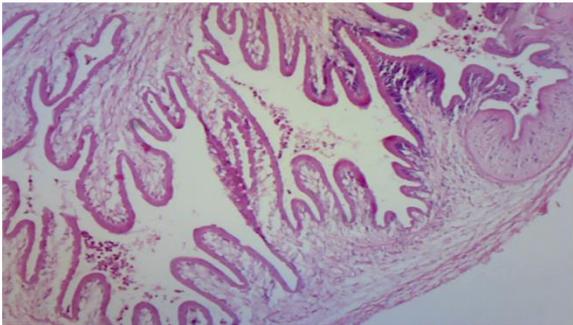
Figuure 12. Photomicrograph of cyst cross section (Hematoxylin-Eosin, 10X)
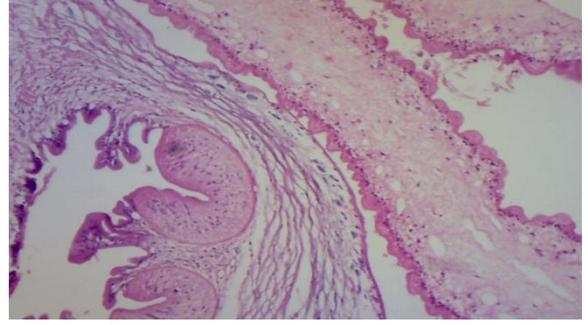
Figure 15. Photomicrograph of cyst cross section (Hematoxylin-Eosin, 10X)
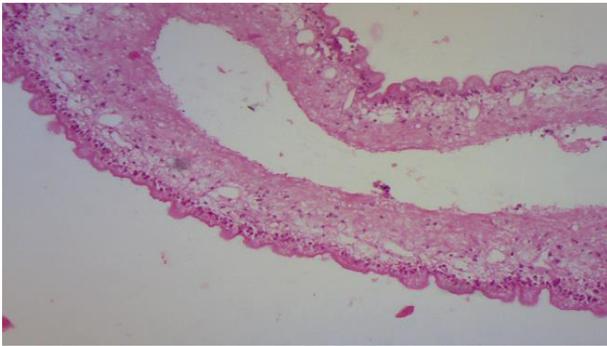
Figure 13. Microphotograph of cyst cross section showing cutis debris (Hematoxylin-Eosin, 10X)
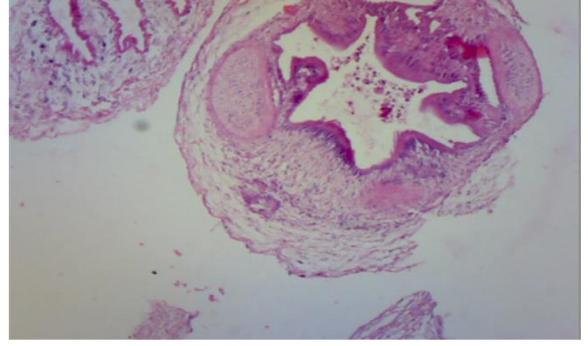
Figure 16. Photomicrograph of cyst cross section (Hematoxylin-Eosin, 10X)

Table 1: Comparative values of hematology infected and non-infected animals

Hematological parameters	Groups		P-Value (P<0.05)
	Infected animals	Non-infected animals	
WBC count (10^9/L)	37.76± 283.35	11.66±2.33	0.013
Lymphocytes (10^9/L)	22.72±346.58	5.82±0.24	0.132
Lymphocytes (%)	52.54±576.76	59.60±24.96	0.620
Monocytes (10^9/L)	1.32±0.67	2.33±0.72	0.63
Monocytes (%)	4.39±3.63	3.10±0.37	0.260
Granulocytes (10^9/L)	11.75±34.33	3.36±0.02	0.021
Granulocytes (%)	38.43±455.06	42.66±116.33	0.740
Hemoglobin (g/dL)	8.26±0.93	9.46±0.30	0.045
Red blood cells (10^{12}/L)	19.56±22.32	12.70±14.32	0.022
PCV (%)	30.11±17.43	30.47±44.40	0.894
MCV (fl)	17.68±30.73	19.27±9.02	0.633
MCH (pg)	4.87±2.20	6.60±1.44	0.053
MCHC (g/dl)	25.56±31.62	31.70±9.25	0.076
RDW	17.72±12.08	10.36±1.90	0.001
Platelets	471.48± 354.10	393.66± 50.90	0.710
MPV (fl)	5.74±0.41	5.46±0.90	0.508
PCT (%)	0.38±0.13	0.03±0.01	0.117

Table 2: Comparative values of serum biochemistry of infected and non-infected animals

Serum biochemistry	Groups		P-Value (P<0.05)
	Infected animals	Non-infected animals	
Total protein g/dl	7.3± 0.13	6.5±0.04	0.001

EXPLORING THE AVIAN DIVERSITY OF CHAKESAR VALLEY, SHANGLA, KHYBER PAKHTUNKHWA, PAKISTAN

WAQAS AHMAD[1*] JALAL HAYAT KHAN[2], QUDRAT ULLAH[1], MUHAMMAD QASIM[3], MUHAMMAD ATHER RAFI[4]

[1]Department of Zoology, University of Peshawar, Peshawar, Pakistan.
[2]Rangeland Research Institute, National Agricultural Research Center, Islamabad, Pakistan.
[3] Department of Zoology, Kohsar University Murree, Pakistan.
[4] National Insect Museum, National Agricultural Research Center, Islamabad, Pakistan.

ARTICLE INFORMATION

ABSTRACT

The avian fauna of Chakesar Valley, Shangla, was explored from March 2019 to March 2022. The result yielded a total of 38 bird species belonging to 20 families under the orders Passeriformes, Columbiformes, Coraciiformes, and Accipitriformes. Order Passeriformes, representing 34 species in 17 families, are *Acridotheres tristis* (L)*, A. fuscus* (Wagler)*, Sturnia pagodarum* (Gmelin)*, Passer domesticus, P. montanus* (L)*, Pycnonotus leucogenys* (L)*, P. cafer* (Grey), and *Hypsipetes leucocephalus* (Gmelin)*. Phylloscopus xanthoschist* (Grey & Grey), *Phylloscopus* sp.*, Terpsiphone paradisi* (L.)*, Motacilla cinerea* (Tunstall)*, M. alba* (L.)*, Oriolus kundoo* (Sykes)*, Emberiza lathami* (Grey),*Phoenicurus fuliginous* (Vigors)*, P. leucocephalus* (Vigors)*, Copsychus saularis* (L.)*, Eumyias thalassinus* (Swainson)*, Saxicola maurus* (Pallas)*, Saxicola caprata* (Linnaeus)*, Hirundo rustica* (L.)*, Cecropis daurica* (Laxmann)*, Hirundo smithii* (Leach)*, Dicrurus macrocercus* (Vieillot)*, Corvus splendens* (Vieillot)*, C. macrorhynchos* (Wagler)*, Dendrocitta vagabunda* (Latham)*, Parus cinereus* (Vieillot)*, Lanius schach* (L.)*, Chloris spinoides* (Vigors)*, Prinia crinigera* (Hodgson)*,* and *Lonchura punctulate* (L.) Two species, *Halcyon smyrnensis* (L.) and *Alcedo atthis* (L.), represented the order Coraciiformes**,** while *Spilopelia senegalensis* (L.) and *Gyps himalayensis* (Hume) represented the orders Columbiformes and Accipitriformes**,** respectively**.** *Emberiza lathami* was rarely sighted. All reported species are new for the area. These preliminary findings will be valuable for further research in the area.

1. INTRODUCTION

Avifaunal research is a vital ecological tool that serves as a key indication in evaluating different habitats both qualitatively and quantitatively (Bilgrami, 1995). Birds are excellent markers of the health of an environment because they perform several crucial ecological tasks such aspect control, pollination, seed propagation, and nutrient dynamics (Wenny *et al.,* 2011). Bird species richness and diversity act as a standard that determines the productivity and health of almost any ecosystem; this is the main reason they have a special place in science and culture that drive us towards deeper scientific inquiries into their varied ways of life (Paruk, 2018). Pakistan has every possible environment for a wide variety of bird species (Khan, 2023).

In Pakistan, there are approximately 660 to 680 bird species belonging to 74 families and 278 genera. (Khan, 2023; Roberts, 1991). However, because of climate changes and increased anthropogenic activity, global bird diversity is quickly declining (Roy *et al.,* 2012). Shangla is a district in Khyber Pakhtunkhwa Province of Pakistan and Located in Hindukash region (Khan *et al.,* 2013). However according to Dong *et al.* (2010), since the climate and vegetation of the eastern Hindu Kush are increasingly like those of the neighboring Himalayas, most bio-geographers refer to these mountain ranges as the Hindu Kush-Himalaya (HKH) group. Shangla is a high mountain landscape with moist temperate and coniferous vegetation that covers 11,528 acres. (Swati *et al.,* 2002). The Shangla district is made up of tiny valleys wedged between hills and encircled by tall, forested mountains. Thick and open gymnospermy forests can be

found in the region, which is located at a high elevation above sea level. High mountains and confined valleys make up most of the topography of the Shangla district.

No work has been done on avian diversity of Shangla so the current Preliminary study was conducted in Chakesar Valley, Shangla. The aim of the study was to assess the avian diversity in Chakesar Valley, Shangla, and provide valuable insights into the bird species present in the region. Additionally, the findings of this study can contribute to conservation efforts and help in understanding the ecological importance of Shangla for avian populations.

2. MATERIALS AND METHODS

Study area
The present study performed to explore the avian fauna of Chakesar Valley, District Shangla, for the first time. Chakesar is a tehsil in district Shangla, located between 34° 47′ 8″ N and 72° 46′ 11″ E, with an altitude of 3722 feet (Figure 1). Chakesar valley is on a formation of a river delta in a valley of 5 km wide by 11 km long at the elevation of 3722 feet above sea level. The village is on suture zone / collision zone between the Indian continent and Eurasian continent Tehsil Chakesar is enclosed to the north by Alpuri, to the south by tehsil Martung, to the north-west by tehsil Puran, and to the west by tehsil Besham (Ashraf *et al.,* 2012)**.** Chakesar valley is in Sino Japanes region and reaches to the basin of SaharoSindian region (Hussain, 2012). Chakesar Valley is comprised of four union councils: Chakesar, Opal, Sarkool, and Bunerwal. The study area was divided into different localities which are Danakol, Doriband, Khadang, Ona, Gunagar, Shawawoo, Siknai, Gumrash (Figure 1).

Data collection
The present checklist focused on long-time fieldwork from March 2019 to March 2022. The primary method employed for data collection was randomized direct observation. Observations of avian species were made in their natural habitats in different localities of the study area. To complement direct observations, birds were sighted with aid of binocular (10× 30), As well as photography was also conducted using a mobile device (the Mi A3) equipped with zooming lenses (2×16). Photographs were taken to record visual information, including physical characteristics, plumage patterns, and distinctive features of the observed bird species. Audio recordings of bird songs and calls were made using audio recording devices.

Identification
All collected data, including observations and photographs, were analyzed for species identification. Authoritative field guides such as Birds of Pakistan: Helm Field Guides (2008), and expert ornithologists were consulted when necessary to validate species identification. Birds recorded songs were analyzed and compared with the e-Birds. Besides these, various anonymous sources helped in the identification of the species. Identification was also performed by studying the available literature in research papers (Ali *et al.,* 1983; Grimmett *et al.,* 2008; Altaf *et al.,* 2013; Altaf, 2016; Altaf *et al.,* 2018; Zaman *et al.,* 2022).

3. RESULTS AND DISCUSSION

The currents work is a preliminary survey, carried out to explore the avian fauna of Chakesar Valley from March 2019 to March 2022. The study resulted in a total of 38 bird species belonging to 20 families and 4 orders in the study area (Table 1). The orders that were reported in the study area are Passeriformes, Columbiformes, Coraciiformes, and Accipitriformes. Passeriformes was the dominant order, representing 85% of families (n = 17) and 91.8% of species (n = 34) (Figure 2). The family Muscicapidae was the dominant, representing the leading number of species (n = 6) (Figure 3) The area's major avifaunal composition consisted of resident birds, which made up 72.7% of the species (n=27) of the total birds (n=37) (Figure 4).

All the species in the study area were first reported. Passeriformes order was the dominant and represented by 34 species which *Acridotheres tristis* (Linnaeus, 1766), *Acridotheres fuscus* (Wagler, 1827), *Sturnia pagodarum* (Gmelin, JF, 1789), *Passer domesticus , Passer montanus* (Linnaeus, 1758), *Pycnonotus leucogenys*(Linnaeus, 1766), *Pycnonotus cafer* (Gray, JE, 1835), *Hypsipetes leucocephalus* (Gmelin, JF, 1789). *Phylloscopus xanthoschist* (Gray, JE & Gray, GR, 1847), *Phylloscopus spp, Terpsiphone paradisi* (Linnaeus, 1758), *Motacilla cinerea* (Tunstall, 1771) , *Motacilla alba* (Linnaeus, 1758), *Oriolus kundoo* (Sykes, 1832), *Emberiza lathami* (Gray, JE, 1831), *Phoenicurus fuliginous* (Vigors, 1831), *Phoenicurus leucocephalus* (Vigors, 1831), *Copsychus saularis* (Linnaeus, 1758), *Eumyias thalassinus* (Swainson, 1838), *Saxicola maurus* (Pallas, 1773), *Saxicola caprata* (Linnaeus, 1766), *Hirundo rustica* (Linnaeus, 1758), *Cecropis daurica* (Laxmann, 1769), *Hirundo smithii* (Leach, 1818), *Dicrurus macrocercus* (Vieillot, 1817), *Corvus splendens* (Vieillot, 1817), *Corvus macrorhynchos* (Wagler, 1827), *Dendrocitta vagabunda* (Latham, 1790), *Parus cinereus* (Vieillot, 1818), *Lanius schach* (Linnaeus, 1758), *Chloris spinoides* (Vigors, 1831 , *Prinia crinigera* (Hodgson, 1836), *and Lonchura punctulate* (Linnaeus, 1758). order Coraciiformes *was represented by two species viz., Halcyon smyrnensis* (Linnaeus, 1758)*, Alcedo atthis* (Linnaeus, 1758) while the rest two orders were represented by single species in each viz., order

Columbiformes containing *Spilopelia senegalensis* (Linnaeus, 1766)*,* order Accipitriformes by *Gyps himalayensis* (Hume, 1869). During the current study, *Emberiza lathami* was rarely sighted. The findings from this preliminary study will be valuable for researchers, ornithologists, and environmentalists working towards biodiversity conservation and habitat protection in the region. The greatest number of species were reported from Gumrash (34 species), followed by Doriband (32 Species) while the least number of Birds were reported from Siknai (21 Species).

Several Researchers have worked on avian fauna of Pakistan. According to one such study, 456 species have been reported in Khyber Pakhtunkhwa without any digital record (Shah, 2011). Public data reports 371 digitally documented species from the entire province as per (eBird, 2022). Similarly, Raja *et al.* (1999) conducted a comprehensive and long-term ornithological survey in Palas Valley, District Kohistan between May 1987 and December 1996, and reported a total of 157 species belonging to 47 families. According to Altaf *et al.* (2013) 64 bird species were recorded from Head Khanki, water works on Chenab River in District, Gujranwala, Punjab.In our current study we reported 37 species of birds in 20 families from Chakesar Valley for the first time. Sadam *et al.* (2021) reported 35 bird species from district Mardan in two major habitats (i.e., cropland and urban areas). In their another study they reported more species in agricultural areas near urban areas. He also reported bird species were more diverse and abundant in dense forests of Mardan Khyber-Pakhtunkhwa (Sadam *et al.,* 2021)

In a series of studies on the avian diversity of Swat, Pakistan, researchers have contributed valuable insights into the region's birdlife. Firstly, in Kalam Conservancy, Swat, Shah *et al.* (2014) conducted a comprehensive work from April 2012 to April 2013, aiming to study the spatial distribution, nidification records, and vegetation habitats for the pheasants in the study site. Moreover, during an ornithological field trip to Ushu Forest, Kalam, Swat in May 1983, Roberts (1991) reported 12 bird species belonging to six families, further enhancing our understanding of the avifauna in the region. Additionally, a preliminary study on the avian diversity of district Swat, Khyber Pakhtunkhwa, Pakistan was carried out from January to December 2013, resulting in the documentation of 138 species from 48 families (Pathan et al., 2014). In our current study we reported 37 species of birds in 20 families from Chakesar Valley for the first time.

Rehman *et al.* (2016) conducted a survey at various habitats, including riverbanks, roadside trees, agricultural fields, wetlands municipal dumping areas to document bird diversity and the threats they are facing in district Karak, Khyber Pakhtunkhwa, from June 2014 to July 2015. Their study reported 32 species belonging to 23 families. In a comprehensive survey of avian diversity, different regions of Chitral were explored to gather the data on both passerines and non- passerines, collectively 47 species were documented (Manzoor et al., 2017).In our current study we reported 37 species of Birds in 20 families under 4 orders of Birds ,all the species were recorded for the first time.In another study focusing on Buner, Shahabuddin et al. (2016) gathered information about bioecology of Chukar (*Alectoris chukar*) in Totalai Game Reserve during the period of 2013-2014.More recently, (Khan & Gabol, 2023) studied Bioecology, diversity and distribution of birds of District Bajaur and reported ,83 bird species belonging to 40 families and 15 orders.

In the current study we focused on the observation of avian fauna from Chakesar Valley, Shangla for a period of three years from March 2018 to March 2021.The study resulted in 37 species of birds in 20 families under 4 orders. The current study was a preliminary work on the avian fauna of the study area and will help in future for further study.

4. CONCLUSION

In conclusion, the study demonstrates a rich bird species composition in the Chakesar Valley of District Shangla, with 38 species reported. Due to the abundance of vegetation in this location, the bird fauna was spectacular. And therefore, it is suggested that further research in this matter should be in that direction.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

6. ACKNOWLEDMENT

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Figure 1. Study area Chakesar Valley (Source AC Chakesar Office)

Table 1 Checklist of Observed Avian species in Chakesar Valley, Shangla, Pakistan

S. No.	Order	Family	Species	Common Name	Country status
1	Passeriformes	Sturnidae	*Acridotheres tristis*	Common Myna	Resident
2			*Acridotheres fuscus*	Jungle Myna	Resident
3			*Sturnia pagodarum*	Brahminy Starlings	Summer Migrant
5		Passeridae	*Passer domesticus*	House Sparrow	Resident
6			*Passer montanus*	Eurassian Tree Sparrow	Resident
7		Pycnonotidae	*Pycnonotus leucogenys*	Himalyan bulbul	Resident
8			*Pycnonotus cafer*	Red-Vented Bulbul	Resident
9			*Hypsipetes leucocephalus*	Black Bulbul	Resident
10		Phylloscopidae	*Phylloscopus xanthoschist*	Gray-Hooded Warbler	Resident

11			*Phylloscopus spp*	Leaf warbler	
12		Monarchidae	*Terpsiphone paradisi*	Indian Paradise Flycatcher	Resident
13		Motacillidae	*Motacilla cinerea*	Gray Wagtail	Resident
14			*Motacilla alba*	White Wagtail	Resident
15		Oriolidae	*Oriolus kundoo*	Indian Golden Oriole	Summer Migrant
16		Emberizidae	*Emberiza lathami*	Crested Bunting	Summer Migrant
17		Muscicapidae	*Phoenicurus fuliginous*	Plumbeous Redstart	Resident
18			*Phoenicurus leucocephalus*	White-capped Redstart	Resident
19			*Copsychus saularis*	Oriental Magpie Robin	Resident
20			*Eumyias thalassinus*	verditer flycatcher	Summer Migrant
21			*Saxicola maurus*	Siberian Stonechat	Summer Migrant
22			*Saxicola caprata*	Pied Bush chat	Summer Migrant
23		Hirundinidae	*Hirundo rustica*	Barn Swallow	Summer Migrant
24			*Cecropis daurica*	Red-Rummped swallow	Summer Migrant
25			*Hirundo smithii*	Wire Tailed Swallow	Summer Migrant
26		Dicruridae	*Dicrurus macrocercus*	Black drongo	Summer Migrant
27		Corvidae	*Corvus splendens*	House crow	Resident
28			*Corvus macrorhynchos*	Large-billed Crow	Resident
29			*Dendrocitta vagabunda*	Rufous treepie	Resident
30		Paridae	*Parus cinereus*	Cinereous Tit	Resident
31		Laniidae	*Lanius schach*	Long-tailed Shrike	Resident

32		Fringillidae	*Chloris spinoides*	Yellow-breasted Greenfinch	Resident
33		Cisticolidae	*Prinia crinigera*	Himalayan Prinia	Resident
34		Estrildidae	*Lonchura punctulate*	Scaly-breasted Munia	Resident
35	Columbiformes	Columbidae	*Spilopelia senegalensis*	Laughing dove	Resident
36	Coraciiformes	Alcedinidae	*Halcyon smyrnensis*	White-Throated Kingfisher	Resident
37			*Alcedo atthis*	Common Kingfisher	Resident
38	Accipitriformes	Accipitridae	*Gyps himalayensis*	Himalayan vulture	Resident
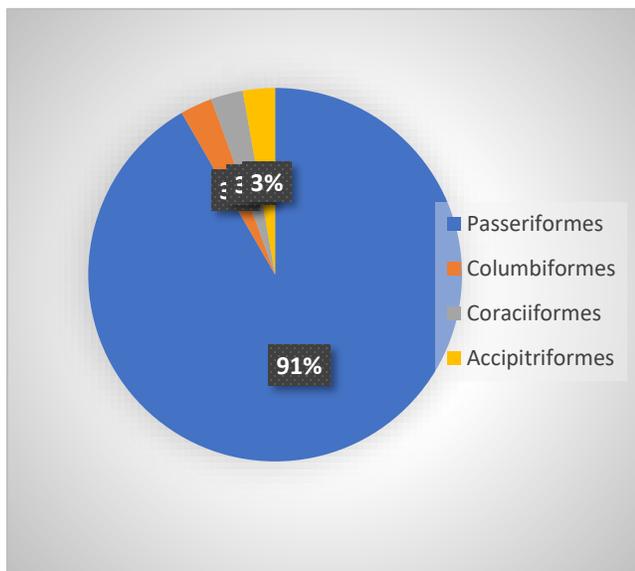
Figure 2. Percentage of Birds species in each order of the Chakesar Valley, Shangla
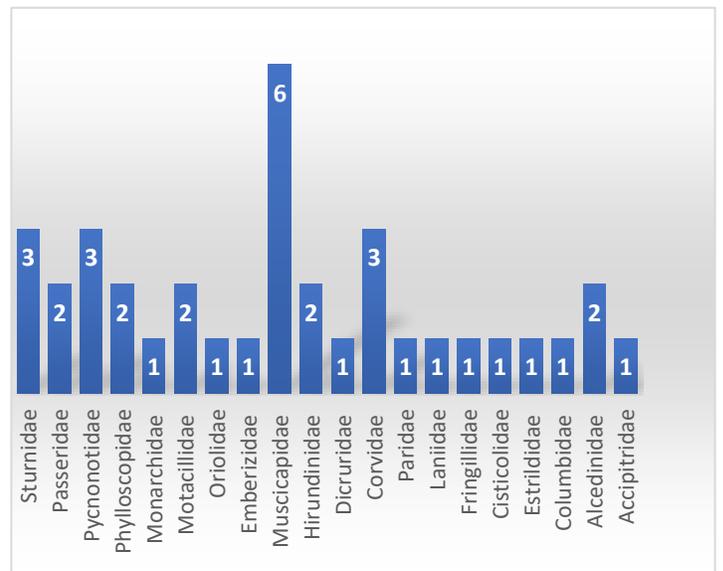
Figure 3. Number of Birds species in each family of the study area, Chakesar Valley, Shangla

Table 2 Birds sighted in different localieties of Chakesar Valley, Shangla

S.No	Species	Doriband	Danakol	Gunanger	Khadang	Ona	Shawawoo	Siknai	Gumrash
1	*Acridotheres tristis*	+	+	+	+	+	+	+	+
2	*Acridotheres fuscus*	+	+	+	+	+	+	+	+
3	*Sturnia pagodarum*	+	-	+	-	-	+	-	+
5	*Passer domesticus*	+	-	-	-	+	+	-	+
6	*Passer montanus*	+	+	-	+	+	-	-	+
7	*Pycnonotus leucogenys*	+	-	+	-	-	+	-	+
8	*Pycnonotus cafer*	+	+	+	+	+	+	+	+
9	*Hypsipetes leucocephalus*	+	+	+	+	+	+	+	+
10	*Phylloscopus xanthoschist*	+	+	+	-	-	-	+	+
11	*Phylloscopus spp*	+	+	+	+	+	+	+	+
12	*Terpsiphone paradisi*	+	+	+	+	+	+	-	+
13	*Motacilla cinerea*	-	-	+	-	+	+	+	+
14	*Motacilla alba*	+	+	-	+	-	+	+	+
15	*Oriolus kundoo*	+	-	+	+	+	+	+	+
16	*Emberiza lathami*	+	-	-	-	-	-	-	-
17	*Phoenicurus fuliginous*	+	+	+	+	+	+	+	+
18	*Phoenicurus leucocephalus*	+	+	+	+	+	+	+	+
19	*Copsychus saularis*	+	+	+	+	+	+	+	+
20	*Eumyias thalassinus*	+	+	+	+	-	+	+	+
21	*Saxicola maurus*	+	-	+	-	+	+	+	+
22	*Saxicola caprata*	+	+	-	+	+	-	+	+
23	*Hirundo rustica*	+	+	+	+	+	+	-	+
24	*Cecropis daurica*				+	+	-	-	-

25	*Hirundo smithii*	+	-	+	-	+	+	-	+
26	*Dicrurus macrocercus*	+	-	-	-	+	+	-	+
27	*Corvus splendens*	+	+	+	+	-	+	+	+
28	*Corvus macrorhynchos*	+	-	-	+	-	+	-	+
29	*Dendrocitta vagabunda*	-	-	-	-	-	-	-	+
30	*Parus cinereus*	+	+	+	+	+	+	+	+
31	*Lanius schach*	+	-	+	-	-	+	-	+
32	*Chloris spinoides*	+	-	-	-	+	+	-	+
33	*Prinia crinigera*	+	+	-	+	+	-	-	+
34	*Lonchura punctulate*	+	+	+	+	+	+	+	+
35	*Spilopelia senegalensis*	+	+	-	+	-	-	+	+
36	*Halcyon smyrnensis*	-	-	+	+	-	-	-	-
37	*Alcedo atthis*	-	-	-	-	+	+	+	-
38	*Gyps himalayensis*	-	-	-	-	-	-	-	+
	Total reported Birds	**32**	**22**	**25**	**23**	**25**	**27**	**21**	**34**
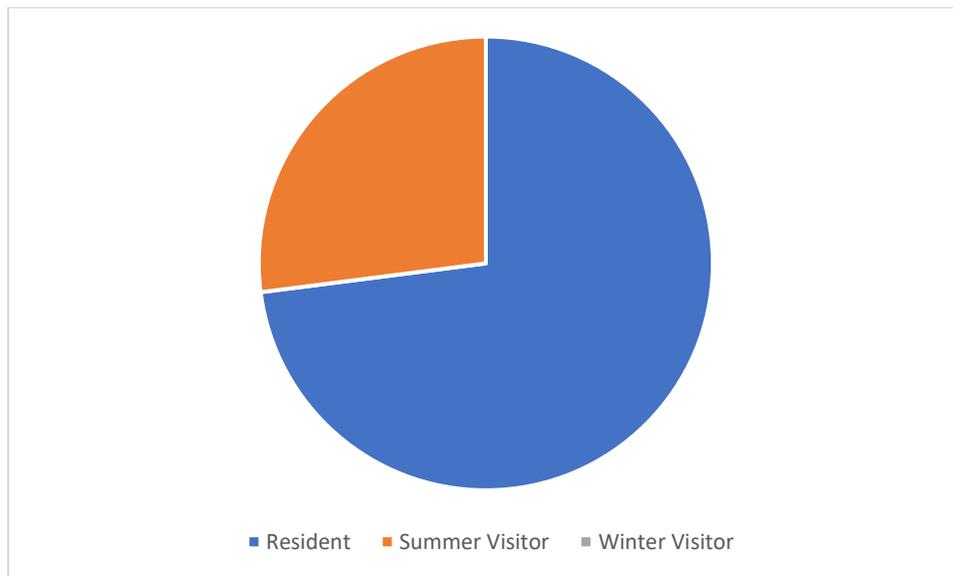
Figure 4. status of the birds in Chakesar valley Shangla

Figure 5. A, *Acridotheres tristis* ; B, *Acridotheres fuscus* ; C *Sturnia pagodarum* ; D, *Passer domesticus* ; E, *Passer montanus* ; F, *Pycnonotus leucogenys* ; G, *Pycnonotus cafer* ; H, *Hypsipetes leucocephalus* ; I, *Phylloscopus xanthoschist.*
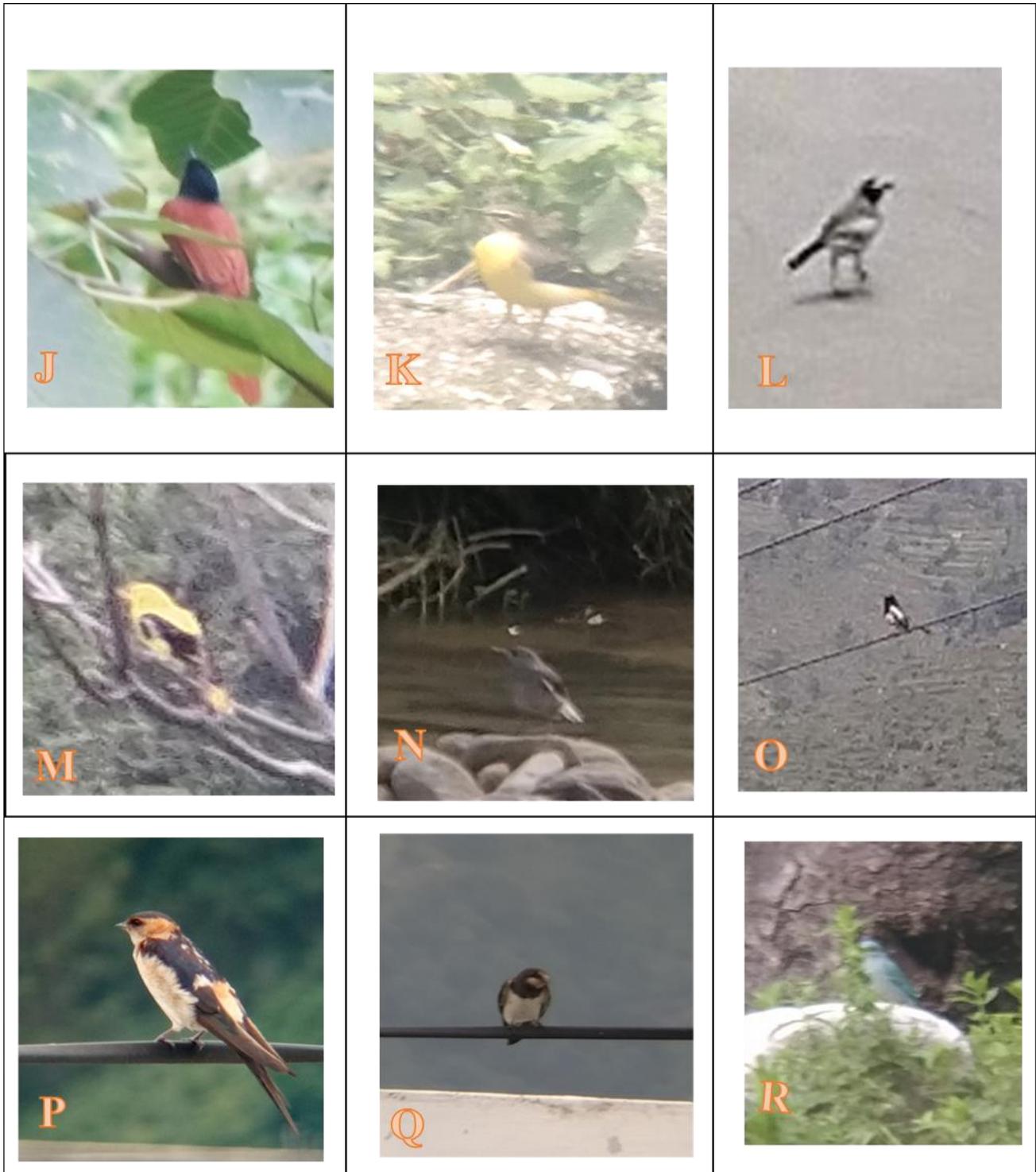
Figure 6. J, *Terpsiphone paradisi;* K, *Motacilla cinerea*; L, *Motacilla alba* ; M, *Oriolus kundoo* ; N, *Phoenicurus fuliginous* ; O, *Copsychus saularis* ; P, *Cecropis daurica* ; Q, *Hirundo rustica* ; R, *Eumyias thalassinus*

Figure 7. S, *Corvus splendens;* T, *Saxicola maurus;* U, *Saxicola caprata; V, Alcedo atthis* ; W, *Halcyon smyrnensis* ; X, *Lanius schach* ; Y, *Dicrurus macrocercus ;* Z, *Lonchura punctulate ;* XY, *Emberiza lathami*

SARCOPTIC MANGE (SCABIES) AND ITS SUCCESSFUL THERAPEUTIC MANAGEMENT IN RABBITS

JAVAID IQBAL MEMON[1], TEHMINA SOHAIL[1*], HINA IMRAN[1], RASHID ALI KHAN[1], SHAZIA SYED[2]

[1]Pharmaceutical Research Centre, PCSIR Labs Complex, Karachi.
[2]Department of Chemistry, University of Karachi, Karachi.

ABSTRACT

Ten rabbits were presented with clinical signs of alopecia, intense itching, erythema and dry crusty lesions on eyes, ears, nose, legs, genital areas and dorsal region of the body. Skin scrapings revealed *Sarcoptes scabiei* mites. Treatment was initiated with subcutaneous injections of ivermectin @ 400µg/kg body weight every 7th day on 3 occasions and multivitamins as supportive therapy at 10 drops once a day was given for early recovery. After 3 weeks of treatments, clinical examination revealed marked improvement of lesions, and skin scrapings were negative for mites. No relapse of infestation was observed in any of the rabbits under treatment during the 6 month observation period following the treatment.

1. INTRODUCTION

Dermatological problems are one of the most common clinical entities in domestic pets and fur bearing animals (Deshmukh *et al.,* 2010) and among them, sarcoptic mange is a highly contagious, non-seasonal, pruritic skin condition in rabbits and is caused by *Sarcoptes scabiei*. Overcrowded living conditions and poor hygiene are significant factors for infection (McCarthy *et al.,* 2004). *Sarcoptes Scabiei* is a deep burrowing mite in epidermis causing intense itching, alopecia, erythema, pruritus, crust formation, scale production, thickening and wrinkling on skin of affected areas (Singh *et al.,* 2017). The mite is very easily transmitted to other surrounding animals through close contact. Severe infection, especially in young and weak animals, causes high mortality (Bornstein *et al.,* 2001). It is the most obstinate, persistent, and zoonotically important contagious disease (Kumar *et al.,* 2002). Sarcoptic mange if left untreated causes significant morbidity and economic losses in livestock (Rehbein *et al.,* 2003; Walton *et al.,* 2004).

The avermectin drug group includes ivermectin, abamectin, doramectin, eprinomectin and selamectin which can be used to treat rabbits that are naturally infested with *Sarcoptes scabiei*. Ivermectin is used as a broad spectrum parasiticide in domestic animals and is also recommended for treatment of ear mange in rabbits.

2. MATERIALS AND METHODS

History And Clinical Findings:
Ten rabbits were presented in Animal house of PCSIR Labs Complex Karachi with the history of dullness, anorexia, skin lesions with intense itching in ears and nose. On clinical examination, erythema, alopecia, dry crusty lesions on eyes, ears, nose, legs, genital areas, and dorsal region of the body were observed (Fig.1). The conditions of the rabbits were weak, and their body coat was ruffled. Anamnesis revealed that rabbits were kept indoors in moist, dirty, and poorly ventilated houses. Rabbits were divided into two groups (5 in the control group and 5 in the treatment group).

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* Corresponding Author**:** tehminasohail23@gmail.com

Laboratory Examination and Diagnosis:
For confirmatory diagnosis, skin scraping examination was carried out as per the standard method (Soulsby *et al.,* 2001). Sample skin scrapings were collected aseptically from different affected sites in 10% solution of Potassium Hydroxide (KOH). The mixture was stirred, centrifuged and supernatant discarded, a few drops of sediment were placed on a slide for direct microscopic examination under 40x magnification. Examination of the sample revealed the presence of large numbers of sarcoptes species mange mites (Fig.2).

Therapeutic Management:
After confirmation by laboratory examination, control group was given placebo and affected rabbits were treated with injection Ivermectin @ 400µg/kg body weight subcutaneously for every 7th day on 3 occasions along with supportive treatment Syp: Polybion forte (Vit: B1, B2, B6, B12) @ 10 drops once a day was also prescribed to fasten up the recovery. Disinfection of nests and surrounding area of the shades with cypermethrin @ 2 ml/liter of water was also advised.

3. RESULTS AND DISCUSSION

Clinically, significant improvement was noted from the 7th day and rabbits recovered completely after 21 days. At the same time, after removal of crusts, clinical signs like alopecia and intense itching were also resolved completely (Fig. 3).

Adverse reactions in rabbits under treatment were analyzed by examination during the 10th and 21st day of presentation. The owner was also advised to keep the rabbits under observation for any signs of toxicity. All the rabbits were kept under observation for 6-month post treatment and no relapse of infestation was observed in any of the rabbits during the observation period. All the rabbits were free from any clinical observable adverse reaction due to treatment with Ivermectin throughout the period of study.

Clinical manifestations such as development of scale, scabs, crusts and alopecia along with a considerable number of *Sarcoptes Scabiei* below crusts as observed in present study were consistent with findings of (Chandey *et al.*, 2000; Oraon *et al.*, 2000; Kaplaywar *et al.*, 2017). The lesions observed in rabbits in the present outbreak are like those observed by (Kumar *et al.*, 1999). The leading outbreak of disease occurred during the post raining season. Similarly, high prevalence of mites during low atmospheric and high humidity was also reported by (Ravindaran *et al.*, 2000). Ivermectin, @ a dosage of 0.2-0.4mg/kg of body weight administered subcutaneously once every two weeks for 2-3 treatments is usually a simple, safe, and effective treatment (White *et al.*, 2003; Mitra *et al.*, 2014).

In the present case study, treatment was carried out with Ivermectin @ 400µg/kg body weight on 3 occasions as reported by (Kachhawa *et al.,* 2013; Mitra *et al.,* 2014; Singh *et al.,* 2017).

4. CONCLUSION

The present observations indicate that Ivermectin therapy coupled with supportive treatment and disinfection of rabbit nests and surrounding areas is effective in control of Sarcoptic mange in rabbits.
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5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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Figure 2. Showing mange mites under microscope.

Figure 1. Showing clinical signs on different body parts. (Day 1)

Figure 3. Showing the progress of recovery in a 21-day time span.

ANALYZING CADMIUM AND NICKEL LEVELS IN *LABEO ROHITA, CATLA CATLA*, AND *CIRRIHINUS MRIGALA* FROM LOCAL FISH MARKETS IN LAHORE, PAKISTAN

KHADIJA ILYAS[1], SANA SHAHBAZ[1*], MUHAMMAD MATEEN TAHIR[2], MUHAMMAD NAVEED[3], AMMARA RIAZ[4], MUHAMMAD NASEEM ULLAH[5], MUHAMMAD WAHEED[6]

[1]Department of Zoology, Lahore Garrison University, Lahore, Pakistan
[2]Key Laboratory of Molecular Epigenetics, Ministry of Education and Institute of Cytology and Genetics, Northeast Normal University, Changchun 130024, China
[3]Key Laboratory of Geographical Processes and Ecological Security in Changbai Mountains, Ministry of Education, School of Geographical Sciences, Northeast Normal University, Changchun 130024, China
[4]Department of Life Sciences, Faculty of Natural and Applied Sciences, Khwaja Fareed University of Engineering and Information Technology
[5]Department of Zoology, Virtual University of Pakistan, Sahiwal campus
[6]Punjab school Education Department, Government Higher Secondary School Nangal Sahdan Muridke 39000, Sheikhupura, Pakistan

ARTICLE INFORMATION

ABSTRACT

Thirty-six (36) fish samples were collected from the different local fish markets of Lahore city to check the bioaccumulation of heavy metals. The mean Cd concentration ranged from (32.33 – 39.69ug/kg), (23.26 – 35.45ug/kg), (35.37 -45.06ug/kg) in gills (28.99-38.17ug/kg), (21.74– 30.07ug/kg), (33.92 – 43.61ug/kg) in muscles (26.12– 35.56ug/kg), (18,87– 31.32ug/kg), (31.05 – 41.01ug/kg) in liver of *Labeo rohita*, *Catla catla* and *Cirrihinus mrigala* respectively. The mean Ni concentration ranged from (44.93 – 52.86ug/kg), (45.65 – 52.86ug/kg), (52.30 -61.14ug/kg) in gills (43.48- 51.41ug/kg), (44.20– 54.62ug/kg), (50.86 – 59.69ug/kg) in muscles (40.48 – 48.81ug/kg), (41.56 – 48.81ug/kg), (48.21 – 57.08ug/kg) in liver of *Labeo rohita*, *Catla catla* and *Cirrihinus mrigala* respectively. The comparison between sampling markets revealed no significant difference among them and the results between fish species shows highest concentration of Ni (55.26ppb) in *Cirrhina mrigala*. The accumulation pattern of all the heavy metals in different organs of all the studied fish species was significantly lower ($p<0.05$) as compared to admissible concentration reported by WHO. Therefore, the fish of these areas do not pose serious threat to human health upon consumption.

1. INTRODUCTION

The major problem for the permanent stay of heavy metals in aquatic flora and fauna is due to its ability of bio concentration and biomagnifications which is responsible for the accumulation of heavy metals in living bodies at prominent level (Agah, 2021). Heavy metals including chromium, manganese, iron, cobalt, nickel, zinc, copper, and selenium etc. are very important to living bodies whereas lead, cadmium, mercury, and arsenic are dangerous for human health (Ozturk *et al.*, 2009).

Even essential trace metals are dangerous for health if their concentration is slightly greater than their reommended values (Sapozhnikova *et al.*, 2005). Fish is considered the major source of protein. All over the world human beings get twenty five percent (25%) of proteins from seafood (Benson *et al.,* 2007). According to rough estimate in 2004, humans consumed almost 75%, that is 105.6 million tons of world level fish production (Rakib *et al*., 2021). It has been predicted that fish consumption in developing countries will increase by 57 percent, from 62.7 million tons in 1997 to 98.6 million in 2020 (Carraso *et al*., 2008).

Omega-3 fatty acids, eicosapentenoic acid (EPA) and docosahexenoic acid (DHA) are richly found in Fish, shellfish and prawns. This reduces heart diseases, cholesterol level, and preterm delivery in humans (Agah, 2021). The wastewater effluents discharged from factories is responsible for Minimata disease which is because of eating mercury contaminated seafood (Carraso *et al.*, 2008). Ita-Ita disease in humans of Japan was also considered because of consuming cadmium contaminated seafood which was the result of digging in mines in the proximate area (Benson *et al.*, 2007).

Acute heavy metals toxifications can destroy brain and heart functioning and GIT track including liver and endocrine gland in human beings. Chronic expose of Pb (II) may lead to the death (Benson *et al.*, 2007). As the ions of trace metal in human beings is mainly due to consumption of heavy metal polluted diet. Thus, the heavy metal analyses of eatables consumed by humans are performed on regular basis. Because fish is present at the apex level of aquatic food chain, so large number of heavy metals are biomagnified making it dangerous to eat (Qadir & Malik, 2011).

2. MATERIALS AND METHODS

The research was conducted at Lahore Garrison University Lahore and some part of the Experimental work was performed at the Lab of the LCWU (Lahore College for women university, Lahore).

Study Area
Thirty-six (36) fish samples were collected from the different local fish markets of Lahore city to check the bioaccumulation of heavy metals. Due to the intensive agricultural conditions, unchecked industrial waste, global warming, heavy traffic, and urbanization, these fish markets were the most polluted areas. Due to the high contamination of industrial waste, heavy metal is exposed to be present in large quantity, so these sites are selected for study (Iqbal *et al.*, 2013).

Collection of fish
During March 2021, a total of thirty-six (36) samples of *Labeo rohita*, *Catla catla and Cirrhina mrigala* were collected from different local fish markets (Bhatti Chowk, Shahdara and Do Moria Pull) of Lahore city. These fish samples were transferred to the lab, clean with water and then stored at -20 ℃. During sample collection, the weight of each fish sample was recorded by using weight machine.

Dissection of sample
Fish were dissected under laminar air flow by using sterilized apparatus and different tissues like muscles, gills and liver were separated and washed with de-ionized water. Dissection of fish sample was done and separated the desired parts as liver, Gills and Muscle. The muscle sample was obtained by careful removal of operculum, epidermal and subcutaneous tissues. The liver was isolated by separating it through connective tissues surrounding the liver. Washing was done by distilled water. After washing samples were labelled by specific tag. Dissected samples were stored at 20º C for further analysis (Kvach *et al.*, 2016).

Instrumentation
Inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin Elmer Optima 7000DV) was used for analysis of heavy metal concentration in fish and water samples. Using ICP-OES, samples were subjected as acid solutions to be nebulized and formed a fine aerosol that was transported into the plasma. Then, it undergone desiccation, vaporization to molecular gases, and dissociation into atoms that could be ionized. Dilutions were made from stock solution and standard solution for the detection of metals was prepared (Sharma, 2020).

Glassware and reagents
De-ionized water was used during the whole working in lab for the washing purpose of all the apparatus. Samples bottles were rinsed with HNO_3 before the collection of samples. To avoid any contamination all glassware was washed with HNO_3 several times (Voegborlo & Adimado, 2010).

Preparation of fish tissues for Wet Digestion
1- Liver, kidney, muscles, and gills of fish were thawed and weighed in measuring balance. Each sample of 2g was taken and chopped. It was chopped with a sterilized steel knife and mixed properly to achieve homogeneity.
2- In china crucible10ml of 1:1 HNO3 was taken and mixed with fish solid sample and allow to heat on hot plate at 90 to 95 ℃ and for 10 to 15 minutes it was refluxed.
3- On the cooling of sample 3ml of 30% hydrogen peroxide (H_2O_2) and2ml of deionized water was added. Samples were placed again onto the hot plate and allowed to heat until the effervescence stops.
4- Again1ml of 30% H_2O_2 was added and warmed until the effervescence stop and cooled at room temperature.
5- By using the syringe filter, Nalgene 0.25 nm the digested samples of fish tissues were filtered. Filtrate was added to a new glass vial and stored at room temperature until analytical procedures could be done (Idera *et al.,* 2015).

Analysis of Samples for Determination of Metal ions
The above-mentioned samples (filtrates) were sent to lab of Microbiology and genetics LCWU, Lahore, for heavy metal determination by Cadmium (Cd), Nickel (Ni), by using "inductively coupled plasma optical emission

spectrometry (ICP-OES, Perkin Elmer Optima 7000DV)" to estimate the heavy metal contents in trace quantities (Mensoor & Said, 2018).

Statistical Analysis

By using statistical software (SPSS) for social sciences, statistical analysis of the data was performed. One way ANOVA and Microsoft Excel 2019 were performed for the determination of significant difference between the values.

3. RESULTS AND DISCUSSION

Concentration of different heavy metals in fish sample

The concentration of different heavy metals Cd, and Ni were detected in different organs of fish collected from three different local fish markets. The mean concentration of different heavy metals studied in current investigation in different organs of different fish species are presented in tables below. The accumulation pattern of all the heavy metals in differential organs of all the studied fish species were significantly lower ($p>0.05$) as compared to admissible concentration reported by WHO/FAO.

Cadmium Metal Results

The result of Cd concentration in three different organs of three different fish species are listed in Table 1 to Table 9. The mean Cd concentration ranged from (39.69 – 32.33ug/kg), (35.45 – 23.26ug/kg), (45.06 -35.37ug/kg) in gills (38.17- 28.99ug/kg), (30.07 – 21.74ug/kg), (43.61 – 33.92ug/kg) in muscles (35.56 – 26.12ug/kg), (31.32 – 18.87ug/kg), (41.01 – 31.05ug/kg) in liver of *Labeo rohita*, *Catla catla* and *Cirrihinus mrigala* respectively.

Nickle Metal Results

The result of Ni concentration in three different organs of three different fish species are listed in Table 10 to Table 18The mean Ni concentration ranged from (44.93 – 52.86ug/kg), (45.65 – 52.86ug/kg), (52.30 -61.14ug/kg) in gills (43.48- 51.41ug/kg), (44.20– 54.62ug/kg), (50.86 – 59.69ug/kg) in muscles (40.48 – 48.81ug/kg), (41.56 – 48.81ug/kg), (48.21 – 57.08ug/kg) in liver of *Labeo rohita*, *Catla catla* and *Cirrihinus mrigala* respectively.

ANOVA Results

The collected data from heavy metal analysis were subjective to descriptive statistical analysis followed by one-way ANOVA P<0.05 was considered indicating statistical significance.

Comparison of fish market

Comparison of Cd level in fish market

Results of descriptive statistics and ANOVA of Cd in fish market are summarized in table 3.19 and 3.20, respectively. Mean concentration of Cd did not vary significantly between the three markets. Cd level between

three market has following increasing order Bhaati Chowk < Shahdara < Do Moria Pull.

Comparison of Fish Species

Comparison of Ni level in fish market

Results of descriptive statistics and Anova of Ni in fish market are summarized in table 3.21 and 3.22, respectively. Mean concentration of Ni did not vary significantly between the three markets. Ni level between three market has following order Bhaati Chowk < Shahdara < Do Moria Pull.

Comparison of Cadmium level in fish species

Descriptive statistics and ANOVA results of cadmium in fish species are given in table 3.23, 3.24 respectively. Statistical analysis of Cd concentration is found to be non-significant among the three fish ($P < 0.05$). Decreasing order of cadmium concentration in fish species was as: *Cirrhina mrigala* > *Labeo rohita* > *Catla catla.*

The heavy metal concentration in different organs of Fish is shown its availability in a body of water can cause carcinogenic effects on aquatic biota and human health (US EPA) (Hadyait et al., 2019). The presence of these metals has many potential toxins. It has caused irregularities such as reduced development as well low growth rate and very low concentration of these metals like Cd can cause bone ossification (Mehana et al., 2020).

The present study was conduct to evaluate the concentration of heavy metals from thirty six (36) samples of three edible fish *Labeo rohita* (Rahu), *Catla catla* (Thela), *Cirrhina mrigala*(Mori) collected from three local fish market of Lahore (Bhatti Chowk, Shahdara, Do Mori Pull) (Hamid et al., 2016).

Heavy metals Cadmium (Cd), Nickel (Ni), were analyzed by the ICP-OES. Descriptive statistics and ANOVA results for cadmium and nickel in fish markets are summarized in table 3.1 to 3.26 respectively (Mehana et al., 2020). Fish markets have following decreasing order: Do Moria pull > Shahdara > Bhatti Chowk for Cadmium and Nickel. However, these results did not reveal any significant difference between different sampling sites (Green & Planchart, 2018).

Result for Nickel (Ni) metal concentration in three fish species are given in table 10 to table 18. The mean concentration for Ni ranged from 40.84ppb- 61.14ppb. Results for Cadmium (Cd) metal concentration in three fish species. The Cd concentration ranged from 18.87 ppb- 45.06ppb (Paschoalini & Bazzoli, 2021). The concentration of nickel also found highest in the gills as compared to other studied organs of the fish (Mehana et al., 2020). The lowest concentration was observed in the flesh of the fish. The same trend of bioaccumulation of

nickel was also observed highest in *C. catla* followed by *C. carpio* and *L. rohita*. Our study was like already reported literature by Gupta *et al.*, 2009. The sampling site Do Moria pull showed the highest level of nickel as compared to other sites (Akoto et al., 2014).

Comparison of Nickel level in fish species
Descriptive statistics and ANOVA results of Nickel in fish species are given in table 3.25, 3.26 respectively. Statistical analysis of Ni concentration found a significant difference among the three fish (P < 0.05). Decreasing order of cadmium concentration in fish species was as: *Cirrhina mrigala > Catla catla > Labeo rohita.*

4. CONCLUSION

Current research has shown that the focus of heavy metals, cadmium, and nickel in the Gills, liver, and Flesh of three different species collected from different local fish markets of Lahore. The concentration of heavy metals did not exceed the permissible limits set by the studied heavy metals by regulations. Therefore, the fish of these areas did not pose serious threat to human health upon consumption. But it is suggested that precautionary measurements should be adopted so that the metals accumulation did not exceed permissible limits in fish for human consumption. Moreover, Fish has potential health benefits due to its high nutrient value. , however, it is essential that the levels of heavy metal do not exceed the permitted limits. The need for ongoing communication between health institutions, public organizations, and private businesses about the risks and benefits of fish consumption demonstrates both the need to examine the underlying assumptions that guide the development of public policy and the obligation to control the quality of fish and balance its benefits and risks.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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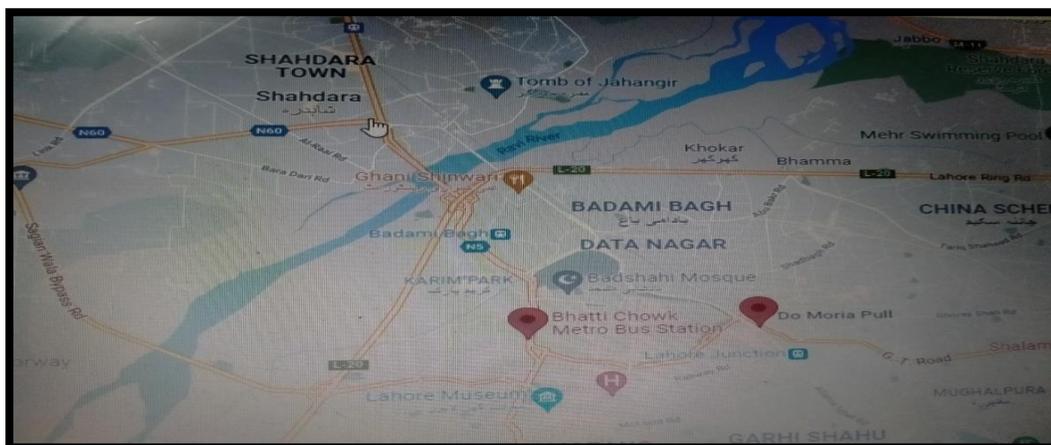
Figure 1. Map of Lahore showing sampling sites

Figure 2. ICP-OES instrument for metal detection

Figure 3. Sample preparation for metal analysis

Table 1. Concentration of Cd in gills of *Labeo rohita* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	30.91	34.63	38.62
2	34.04	33.04	41.101
3	30.41	30.51	38.74
4	33.971	34.37	40.321
MEAN	32.33275	33.1375	39.6955
SD	1.682242	1.632473	1.053134

Table 2. Concentration of Cd in gills of *Catla catla* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria pull
1	23.51	28.51	34.72
2	25.84	32.52	36.92
3	21.41	28.64	37.95
4	22.301	30.651	32.221
MEAN	23.26525	30.08025	35.45275
SD	1.662902	1.644547	2.20058

Table 3. Concentration of Cd in gills of *Cirrhinus mrigala* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	35.72	41.72	44.52
2	35.92	41.71	44.91
3	37.643	41.223	44.623
4	32.221	43.011	46.211
MEAN	35.376	41.916	45.066
SD	1.968984	0.663349	0.676337
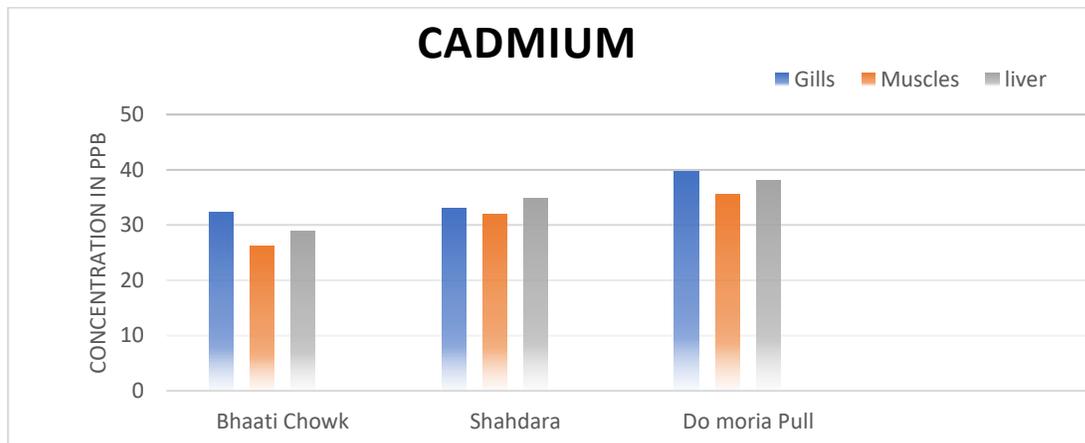
Figure 4. The accumulation of Cadmium (Cd) in organs (liver, gills, muscles) of fish sample *Labeo rohita* taken from different sampling site.
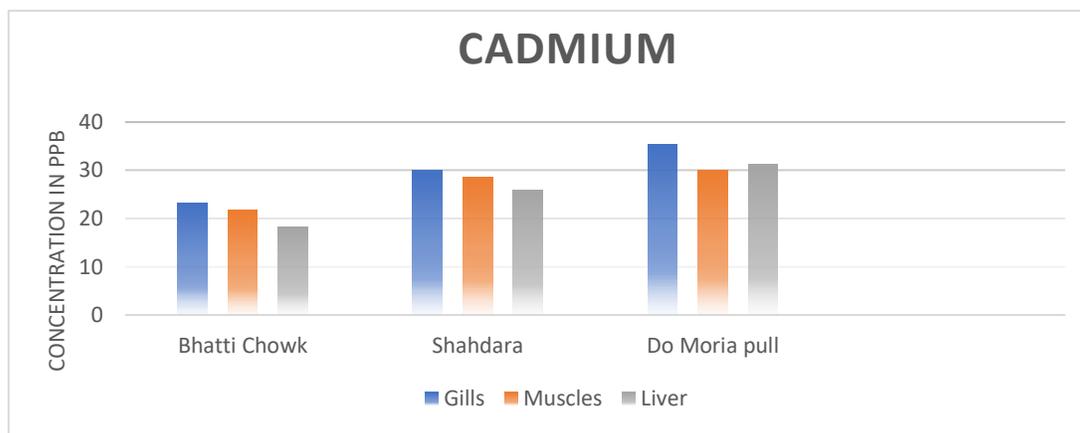
Figure 5. The accumulation of Cadmium (Cd) in organs (liver, gills, muscles) of fish sample *Catla catla* taken from different sampling site.
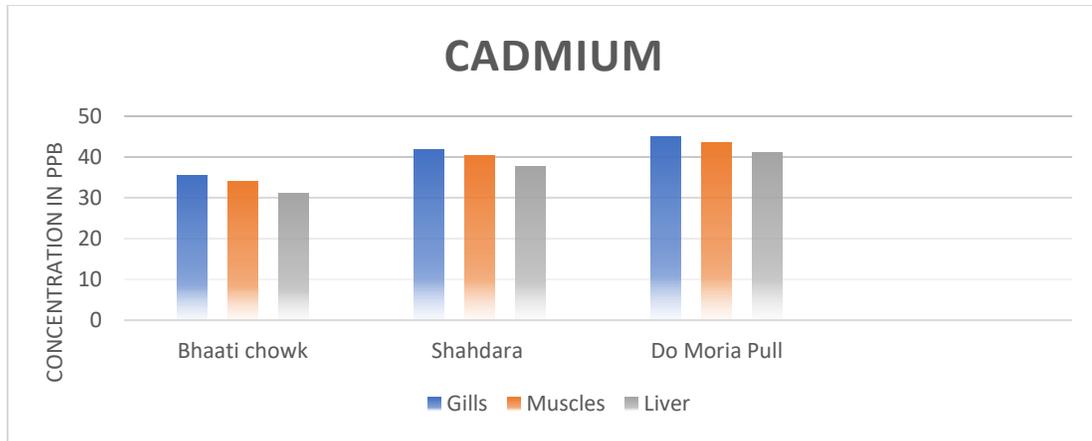
Figure 6. The accumulation of Cadmium (Cd) in organs (liver, gills, muscles) of fish sample *Cirrihinus mrigala* taken from different sampling site
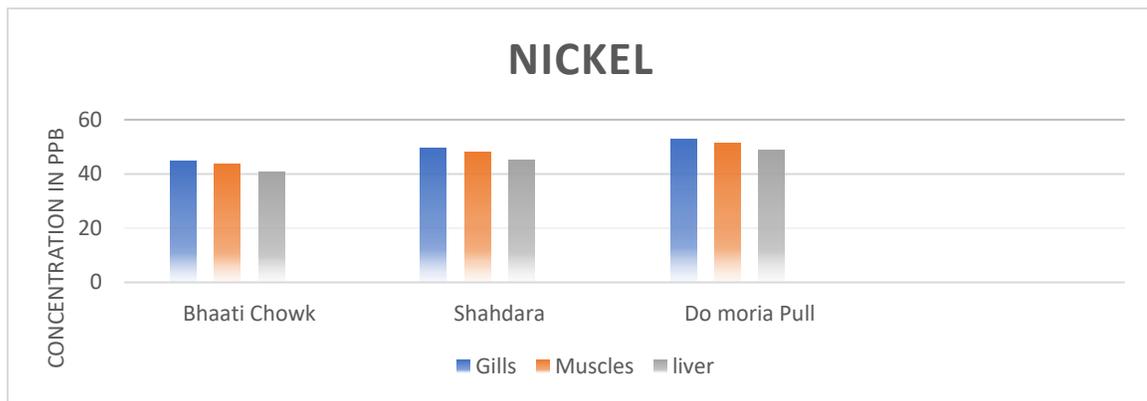
Figure 7. The accumulation of Nickel (Ni) in organs (liver, gills, muscles) of fish sample *Labeo rohita* taken from different sampling site.
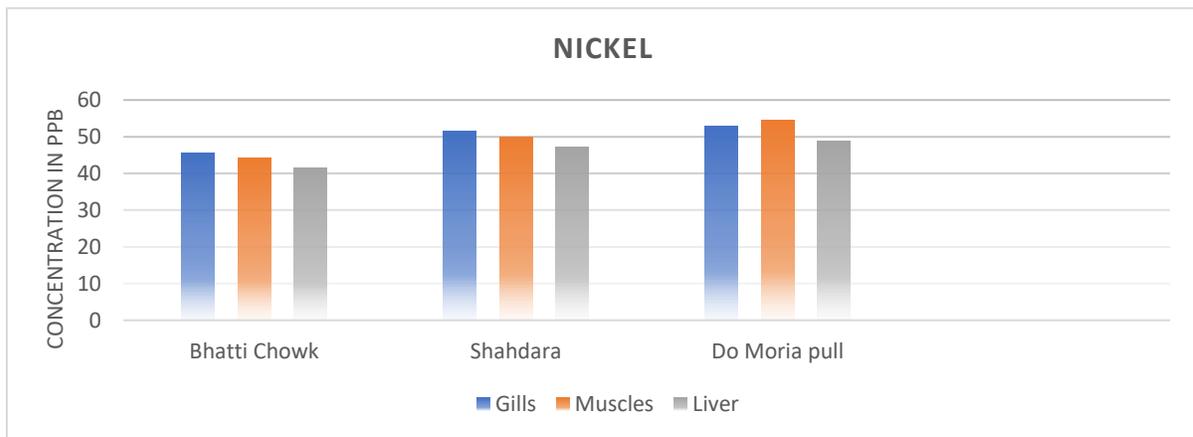
Figure 8. The accumulation of Nickel (Ni) in organs (liver, gills, muscles) of fish sample *Catla catla* taken from different sampling site.
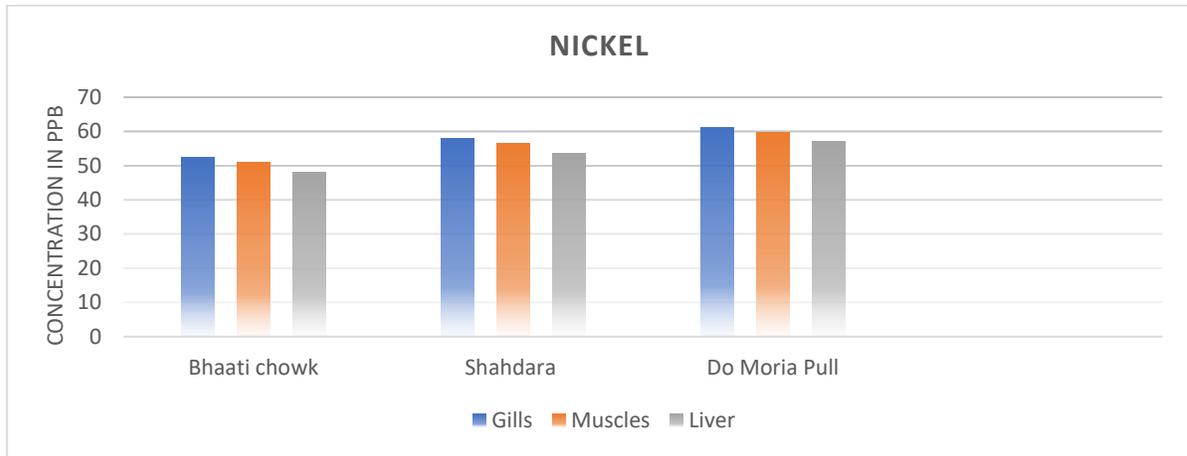
Figure 9. The accumulation of Nickel (Ni) in organs (liver, gills, muscles) of fish sample *Cirrihinus mrigala* taken from different sampling site.

Table 4. Concentration of Cd in muscles of *Labeo rohita* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	28.11	33.23	37.22
2	29.55	35.501	38.701
3	27.23	34.34	37.44
4	31.1	36.12	39.32
MEAN	28.9975	34.79775	38.17025
SD	1.469479	1.107958	0.87176

Table 5. Concentration of Cd in muscles of *Catla catla* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	22.11	27.11	29.33
2	23.44	30.12	30.12
3	20.11	27.34	31.1
4	21.3	29.65	29.76
MEAN	21.74	28.555	30.0775
SD	1.212167	1.342805	0.653237

Table 6. Concentration of Cd in muscles of *Cirrhinus mrigala* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	33.32	39.32	42.12
2	34.52	40.31	43.51
3	36.65	40.23	43.63
4	31.22	42.01	45.21
MEAN	33.9275	40.4675	43.6175
SD	1.96613	0.971761	1.094335

Table 7. Concentration of Cd in Liver of *Labeo rohita* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	25.51	31.6	35.02
2	25.91	34.141	35.801
3	25.45	29.78	33.45
4	27.64	32.69	37.98
MEAN	26.1275	32.05275	35.56275
SD	0.890965	1.591952	1.632341

Table 8. Concentration of Cd in Liver of *Catla catla* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	19.51	25.48	31.12
2	19.8	28.76	31.62
3	18.33	22.78	32.66
4	17.84	26.22	29.88
MEAN	18.87	25.81	31.32
SD	0.810401	2.130751	0.9999

Table 9. Concentration of Cd in Liver of *Cirrhinus mrigala* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	30.72	37.69	39.92
2	30.88	38.95	40.61
3	34.87	35.67	39.64
4	27.76	38.58	43.87
MEAN	31.0575	37.7225	41.01
SD	2.527552	1.270421	1.688535

Table 10. Concentration of Ni in Gills of *Labeo rohita* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	45.61	49.83	53.03
2	46.93	47.83	50.93
3	44.863	50.313	53.513
4	42.331	50.003	53.993
MEAN	44.9335	49.494	52.8665
SD	1.674936	0.97617	1.168731

Table 11. Concentration of Ni in Gills of *Catla catla* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	46.83	50.89	53.03
2	46.72	50.4	50.93
3	47.753	51.193	53.513
4	41.301	53.341	53.993
MEAN	45.651	51.456	52.8665
SD	2.54331	1.124487	1.168731

Table 12. Concentration of Ni in Gills of *Cirrhinus mrigala* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	53.51	60.62	63.52
2	51.63	55.62	57.82
3	49.893	57.423	60.823
4	54.201	58.101	62.401
MEAN	52.3085	57.941	61.141
SD	1.682272	1.792903	2.143444

Table 13. Concentration of Ni in Muscles of *Labeo rohita* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	43.21	47.43	50.63
2	45.53	46.43	49.53
3	43.87	49.32	52.52
4	41.33	49.002	52.992
MEAN	43.485	48.0455	51.418
SD	1.504153	1.175577	1.403332

Table 14. Concentration of Ni in Muscles of *Catla catla* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	44.43	48.49	55.1
2	45.32	49	53.2
3	46.76	50.2	53.1
4	40.3	52.34	57.1
MEAN	44.2025	50.0075	54.625
SD	2.401608	1.482858	1.636116

Table 15. Concentration of Ni in Muscles of *Cirrhinus mrigala* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	51.11	58.22	61.12
2	50.23	54.22	56.42
3	48.9	56.43	59.83
4	53.2	57.1	61.4
MEAN	50.86	56.4925	59.6925
SD	1.563378	1.459578	1.979992

Table 16. Concentration of Ni in Liver of *Labeo rohita* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	40.31	45.1	48.43
2	42.09	44.53	46.63
3	41.54	46.42	48.53
4	39.43	45.012	51.652
MEAN	40.8425	45.2655	48.8105
SD	1.039408	0.700978	1.806389

Table 17. Concentration of Ni in Liver of *Catla catla* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	41.53	46.16	48.43
2	41.88	47.1	46.63
3	44.43	47.3	48.53
4	38.4	48.35	51.652
MEAN	41.56	47.2275	48.8105
SD	2.140432	0.77799	1.806389

Table 18. Concentration of Ni in Liver of *Cirrhinus mrigala* (ppb)

Sample	Bhaati Chowk	Shahdara	Do Moria Pull
1	48.21	55.89	58.92
2	46.79	52.32	53.52
3	46.57	53.53	55.84
4	51.3	53.11	60.06
MEAN	48.2175	53.7125	57.085
SD	1.887715	1.33012	2.572795

Table 19. Descriptive statistics of Cd in fish market

Sampling Sites	N	Mean	S. D	Minimum	Maximum
Bhaati Chowk	12	27.966	5.695226	17.84	37.643
Shahdara	12	33.83769	5.310839	22.78	43.011
Do Moria Pull	12	37.77469	5.03452	29.33	46.211
Total	36	33.1928	5.346862	23.31667	42.28833

N= Number of fish

Table 20. ANOVA of Cd in fish market

	Sum of squares	Degree of freedom	Mean square	F	Significant
Between groups	1754	2	877.1		
Within groups	3096	105	29.48	29.75	0.7669
Total	4850	107			

Significant $p < 0.05$

Table 21. Descriptive statistics of Ni in fish market

Sampling Sites	N	Mean	S. D	Minimum	Maximum
Bhaati Chowk	12	45.7845	4.174639	38.4	54.201
Shahdara	12	51.07133	4.202806	44.53	60.62
Do Moria Pull	12	54.14617	4.53689	46.63	63.52
Total	36	50.334	4.304778	43.18667	59.447

N= Number of fish

Table 22. ANOVA of Ni in fish market

	Sum of squares	Degree of freedom	Mean square	F Value	Significant
Between groups	1288	2	643.9		
Within groups	2004	105	19.09	33.73	0.0778
Total	3292	107			

Significant $p < 0.05$

Table 23. Descriptive statistics for Cadmium in fish species

Fish species	N	Mean	S. D	Minimum	Maximum
Labeo rohita	12	33.4256	4.24846	26.12	39.69
Catla catla	12	27.2389	5.24444	18.87	35.45
Cirrhina mrigala	12	38.9011	4.69510	31.05	45.06
Total	36	33.1885	6.66094	18.87	45.06

N= Number of fish

Table 24. ANOVA of Cadmium in fish species

	Sum of squares	Degree of freedom	Mean square	F Value	Significant
Between groups	612.79	2	306.39		
Within groups	540.78	24	22.53	13.59	0.1876
Total	1153.57	26			

Significant p< 0.05

Table 25. Descriptive statistics for Nickel in fish species

Fish species	N	Mean	S. D	Minimum	Maximum
Labeo rohita	12	47.23	3.8970	40.84	52.86
Catla catla	12	48.48	4.2415	41.56	54.62
Cirrhina mrigala	12	55.26	4.8783	48.21	61.14
Total	36	50.33	5.3643	40.84	61.14

N= Number of fish

Table 26. ANOVA of Nickel in fish species

	Sum of squares	Degree of freedom	Mean square	F Value	Significant
Between groups	336.33	2	168.16		
Within groups	411.85	24	17.16	9.800	0.001
Total	748.18	26			

Significant p< 0.05

COMPERATIVE STUDY ON ANESTHETIC EFFECT OF XYLAZINE-KETAMINE AND DIAZEPAM-KETAMINE IN RABBIT MODEL

MUHAMMAD YOUSIF JAKHRANI*, ALI MUJTABA SHAH, MUHAMMAD FAROOQUE HASSAN, AYESHA LAGHARI, HABIBULLAH JANYARO, AZHAR HYDER KAZI, KHUSHBOO SOOMRO, ANEES AHMED BURIRO

Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand

ABSTRACT

This study was conducted to investigate the anesthetic effects of Xylazine-ketamine and Diazepam-ketamine in rabbits. Twelve adult white New-Zealand rabbits weighing 1.3+0.2 kg, were divided into two groups. A (Xylazine 5mg/kg – ketamine 35mg/kg) and B (Diazepam 5mg/kg – ketamine 35mg/kg). However, the following parameters were observed: analgesic effects (onset and duration) and clinical-parameters (means of heart-rates/BPM, respiratory-rates/BPM and rectal temperature. The onset was significantly $p<0.05$ increased and the duration was significantly $p>0.05$ decreased in A. The heart-rate and the respiratory-rate was significantly $p<0.01$ decreased in A. But the rectal temperature was decreased non-significantly $p>0.05$ in both groups. General observations: in Group A, urination, nasal-discharge, salivation and lacrimation were present, in Group B, defecation, corneal-reflex and pedal-reflex were observed at their peak. It was concluded that the xylazine-ketamine is used for clinical/surgical procedures, while diazepam-ketamine may further be investigated by using other analgesics with this combination.

1. INTRODUCTION

The rabbits can be anesthetized by using injectable general anesthetic agents because the inhalant anesthetic delivery need expensive apparatus and can cause practical problems (Flecknell, 2009) However, General anesthetic agents like ketamine can be used as anesthetizing agent (Hall et al., 2003). Ketamine is a dissociative general anesthetic agent and it is made up of two isomers (Hady et al., 2017). Whereas, ketamine induces anesthesia of voluntary and involuntary accidental stages (I and II) but not surgical stage (III) anesthesia because it is a Gamma amino butyric acid (GABA) binding inhibitor (Muhammad et al., 2014). unlike the other General anesthetic agents, ketamine enhances cardiac rhythm, which ultimately increases heart beats per minute (Avsaroglu et al., 2003). Furthermore, analgesic effects produced greater than anesthetic effects with poor muscle relaxation and increased muscle tone (Annetta et al., 2005), muscular hyper-tonicity, myoclonus and convulsions when used as a single agent (Henke et al., 2005).

These effects can be reduced when ketamine is used with other sedative agents like alpha-2 agonists and benzodiazepine (Özkan et al., 2010). Xylazine, is a potent sedative drug of the alpha-2 agonist group, which inhibits release of cathecolamines and dopamine, and enhance the alpha-2 adrenergic receptor in the nerve endings of cerebral presynaptic clefts (Jakhrani et al., 2019), the analgesic and sedative effects is produced and delays nerve conduction in the CNS resulting in relaxation of striated muscles (Özkan et al., 2010). Xylazine induces a state of drowsiness with a high dose-dependent degree of analgesia, generalized muscle relaxation of central origin and cardiopulmonary depression (DeRossi et al., 2003). Moreover, xylazine may decrease heart rate, respiratory rate and induce hypotension, the severity depends upon the dose and species (Amarpal et al., 2010). However, there are some adverse effect like hypoxaemia associated with cardiopulmonary suppression is noticed when it is used alone (Udegbunam & Adetunji, 2007). Xylazine is mostly administered in painful surgical anaesthesia in order to obtained safe and effective anaesthetic applications (Celestineet al., 2014).

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* Corresponding Author: dr.jakhranibaloch@gmail.com

Diazepam is a benzodiazepine which is also a sedative (a potent hypnotic) drug that is used for muscle relaxation (Oguntoye & Oke, 2014). Unlike other sedative drugs diazepam does not affect the heart rate due to its to its slow metabolism but induce hypotension due to its solvent (propylene glycol) nature (Koshy et al., 2003). While the sedative effect of benzodiazepine depends on the species and sometimes individual dependent. its solvent (propylene glycol) may produce hypotension (Oguntoye & Oke, 2014). Moreover, it has been experimentally observed that mostly rabbits are anesthetized as compared to other species, but the anesthetic mortality rate is greater than dogs and cats (Brodbelt, 2009). Therefore, this experiment was conducted to compare efficacy and safety of intramuscular (im) administration of xylazine-ketamine combination (XK) and diazepam-ketamine comination (DK) for clinical use.

2. MATERIALS AND METHODS

Animal Ethical approval
The present research was conducted as per the rules and regulation of the Animal care and management SBBUVAS, Sakrand.

Experiment Design
In the present study a total 12 adult healthy White New Zealand rabbits weighed 1.3 ± 0.2 kg (Mean ± SEM) of either sex were used in this study. This experiment was conducted at Department of Veterinary Surgery SBBUVAS Sakrand. All the rabbits were housed in individual cages. The feed and water were provided ad libitum.

Research protocol
All the rabbits were randomly divided into two groups, the groups consist of different anesthetic protocols (A and B treatment) with one-week interval. In treatment A all rabbits were treated with xylazine (Xylaz ®, Farvet laboratories Handelweg 25-5531 AE BLADEL the Netherlands, 20mg/ml) dose rate of 5mg/kg body weight 30 minutes before induction with ketamine (Ketasol ®, indus Pharma (Pvt) Ltd, 65/27, Korangi Industrial Area, Karachi-Pakistan, 50mg/ml) 35mg/kg body weight. Whereas, in the treatment B, all rabbits were treated with diazepam ((Diazomil ®, Mediate Pharmaceutical (Pvt) Ltd, 150-151, Sector 24, Korangi Industrial Area, Karachi-Pakistan, 5mg/ml) 30 minutes before induction with ketamine (Ketasol ®, indus Pharma (Pvt) Ltd, 65/27, Korangi Industrial Area, Karachi-Pakistan, 50mg/ml) 35mg/kg body weight. These anesthetic agents were administered through the intramuscular route.

All the rabbits were monitored after administration of ketamine through analgesia (onset and duration) and physiological parameters (rectal temperature, heart rate and respiratory rate). Analgesia: The analgesia was accessed by pinching of paws with artery forcep (closed to 1st ratchet) after every 5 minutes.

Calculation of anesthetic indices
The following parameters were recorded and calculated as the anesthetic indices: Induction of analgesia was assessed as the period between the induction of anesthesia (ketamine) up to absence of painful stimuli on pinching of hind paws with hemostatic forceps close to the first ratchet. However, the paws of rabbits were pinched after interval of each 10 minutes till the response on pinching of hemostatic forceps to the paw. The whole period is treated as the duration of analgesia.

Onset of analgesia: Time interval between injection of anesthetic agents and loss of pedal reflexes.

Duration of Analgesia: Time interval between loss pedal reflexes to recovery of pedal reflexes.

Physiological parameters
In this study, physio-clinical parameters (heart rates, respiratory rates, and rectal temperature) of all the experimental animals were recorded after every 10 minutes from administration of ketamine till the end of experiment. Furthermore, Heart rate (in beats / min) was calculated by auscultation by placing a stethoscope on the left side over the intercostal space of 2nd and 5th rib. While the respiratory rate (in breaths / min) was recorded by the observing thoracic movement during each respiration (inspiration and expiration) and finally the digital clinical thermometer was inserted into the rectum for determination of rectal temperature (^{0}F)

Statistical Analysis
All the data were statistically calculated as means ± standard error of means (SEM) of 12 rabbits. The means of analgesic parameters (induction and duration of analgesia) and physiological parameters (heart rates, respiratory rates and rectal temperature) were compared using analysis of variance (ANOVA) and followed by LSD test when a significant difference was indicated through computer software SPSS computer software (version 25 for Windows; SPSS, Chicago, USA). The significant level was showed at $P < 0.05$, with a trend that was declared at $0.05 < P < 0.10$ (Dawson and Trapp, 2004).

3. RESULTS

Analgesics effect
The results of effect of xylaxine ketamine and diazepam ketamine is presented in Table 1. The results showed that the onset of analgesia in group A was significantly

p<0.05 increased compared to group B. While the duration of analgesia was significantly p<0.05 increased in Group B compared to group A.

Heart rate

Pre-treatment (Control) mean heart rates were 184.8±0.90 and 187.2±0.30 beats per min. in with the intramuscular administration of xylazine-ketamine and diazepam-ketamine respectively. These values were not significant from each other in all rabbits (Table-2).

The heart rate decreased in group A. This decrease was statistically significant between the groups and within the group till 40 minutes after administration and showed the maximum decrease of heart rate 40 minutes and slowly increased till the end of experiment. However, the values showed significant difference from the baseline. While the heart rate in group B decreases non-significantly within group till the end of experiment but these values are significantly different between groups.

Respiratory rate

Pre-treatment (Control) mean respiratory rates were 181.0±2.32 and 185.2±0.76 beats per min. in with the intramuscular administration of xylazine-ketamine and diazepam-ketamine respectively. These values were not significant from each other in all rabbits (Table-3).

The respiratory rate decreased in Group A. This decrease was statistically significant between the groups and within the group up to 40 minutes after administration and showed the maximum decrease of heart rate 40 minutes and slowly increased till the end of experiment. However, the values showed significant difference from the baseline. While respiratory rate was also decreased in group B significantly within group upto 40 minutes after administration and showed the maximum decrease of heart rate 40 minutes and slowly increased till the end of experiment. However, the values showed significant difference from the baseline.

Rectal temperature

Pre-treatment (Control) mean rectal temperature were 104.4±0.00 and 104.6±0.25 ^{0}f with the intramuscular administration of xylazine-ketamine and diazepam-ketamine respectively. These values were not significant from each other in all rabbits (Table-4).
The rectal temperature was decreased in Group A and B till the end of experiment. This decrease was statistically non-significant between the groups and within the group.

General observation

General observations in rabbits after administration of xylazine-ketamine (treatment A) and diazepam-ketamine (treatment B) were examined and presented in Graph-1. The data indicated that in treatment A the following

observations viz. urination, nasal discharge, salivation and lacrimation were present. While, in treatment B, the following observations i.e., defecation, corneal reflex and pedal reflex were observed at peak.

4. DISCUSSION

Ketamine is a dissociative General anaesthetic agent that can be used alone or combined with other per-anaesthetic drugs in order to preoperative sedation, onset of anaesthesia and intraoperative anaesthesia maintain the duration of anaesthesia, epidural anaesthesia and also used for postoperative analgesia (Dawson & Trapp, 2004). Some pre-anaesthetic agents can be used for safe and effective anaesthesia like diazepam and xylazine because they reduce the heart and respiratory rates, induce the hypotension and counteract the adverse effects of ketamine such as: increased heart rate, respiratory rate and poor muscle relaxation (Molaei et al., 2010). Ketamine is important to maintain and stimulate respiratory system, induce broncho-dilation and maintain lung esidual capacity (Dzikiti et al., 2007). Ketamine increase the secretions that is undesired effect because it may cause respiratory distress (Biswas et al., 2017). These respiratory secretions can be reduced by inducing anti-muscarinic agents i.e; atropine (Von Ungern et al., 2007; Singh et al., 2005). Benzodiazepines like; diazepam reduce heart rate effects of ketamine depressing the cardiovascular system, likewise, alpha-2 agonists i.e; xylazine also subside cardiopulmonary activity, that was enhanced by administration of ketamine (Hall et al., 2001).
The cardiopulmonary activity and catecholamine levels is altered depend upon the degree of stress when ketamine is used with these sedative agents (Aydİlek et al., 2007). Similar to the findings of other studies, our results showed that clinical findings such as heart rates and respiratory rates reduced from baseline values after induction. The sympathetic effects of ketamine, higher values of the three hemodynamic parameters in the ketamine-diazepam group can be attributed to relatively lesser contribution of diazepam to the induction of anesthesia compared to xylazine (Ismail, 2016). In combination with benzodiazepines and an alpha-2 agonist xylazine, an increase in the quality of analgesia, sedation, anesthesia and recovery, alleviation of anxiety and a decrease in the unwanted ketamine have been demonstrated (Morse et al., 2004). Group B showed prolonged induction and inadequate depth of intraoperative anaesthesia were observed in our study, on other hand group A produced desired anaesthetic effects. As compared to group B, group A produced prolong duration of anaesthesia, adequate depth of surgical anaesthesia and satisfaction at every stage of anaesthesia have been observed (Brohi et al., 2019). The analgesic effects of group A in our study is resembles with the

results of consistent with other studies (Kazemi et al., 2002; Kiliç, 2004). However, our study indicates anaesthetic effect of group A is useful for painful surgical intervention and group B showed lack of analgesia. These findings were agreement with the results of Kazemi and Sumitra (Kazemi et al., 2002; Sumitra et al., 2004).

5. CONCLUSION

In conclusion, both groups (A and B) produced anaesthetic and analgesic effects but only group A provided adequate surgical analgesia for many painful procedures. However, some mild complications were also produced (decreased heart rate) bradycardia, dysphagia (decreased respiratory rate) and salivation (increased secretion of saliva in mouth) after xylazine and ketamine combination, but this can be overcome by addition of atropine injection prior administration of anaesthesia. While diazepam ad ketamine combination cannot produce surgical anaesthia for painful procedures, because this combination cannot produce adequate analgesia for painful surgical procedures. Therefore, it is recommended that the xylazine and ketamine combination can be used in clinical painful surgical interventions.

6. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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EFFECT OF DIARRHEA ON SERUM ELECTROLYTES AND TRACE ELEMENTS IN SHEEP AND GOATS

SOBIA MUMTAZ[1,2], MUHAMMAD KASHIF IQBAL[3], MUHAMMAD ARIF RIZWAN[3], MUHAMMAD KALEEM[3], BABAR MAQBOOL[4], MUHAMMAD NOMAN[5]

[1]Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Lahore, Pakistan
[2]Livestock and Dairy Development, Government of Punjab, Lahore
[3]Institute of Continuing Education & Extension, Cholistan University of Veterinary and Animal Sciences, Bahawalpur
[4]Faculty of Veterinary & Animal Sciences, University of Agriculture, Dera Ismail Khan
[5]Centre for Biotechnology and Microbiology, University of Swat, Swat

ARTICLE INFORMATION

ABSTRACT

Diarrhea leads to deprivation of health and decrease in production potential of an animal. The main objective of this study was to evaluate the serum electrolytes and trace elements in diarrheic sheep and goats. For this study, a total of 100 diarrheic animals were included, comprising 50 sheep and 50 goats experiencing clinical diarrhea, regardless of the cause from different livestock farms situated in peri urban areas of Lahore were examined. The results showed that the levels of serum Sodium (Na) and Potassium (K) were increased significantly ($P < 0.05$) while serum Calcium (Ca) level was decreased significantly ($P < 0.05$) in diarrheic group as compared to the control group. There is no significant change in serum Iron (Fe) and Copper (Cu) levels in diarrheic group as compared to the control group. Serum copper and Pack cell volume (PCV) had a significant correlation in case of diarrheic animals. It was concluded that diarrhea have a significant correlation with serum electrolytes and trace elements. This study will help to veterinary practitioners in field regarding diagnosis and treatment of diarrhea.

1. INTRODUCTION

Diarrhea leads to deprivation of health and decrease in production potential of animals (Ijaz *et al.*, 2019). Diarrhea means increase in frequency, fluid quantity and volume of fecal excretion. Animals suffering from diarrhea may have blood, mucous, foul smell and abnormal color of feces unlike healthy animals. Multiple factors causing diarrhea include environmental factor, improper animal hygiene, nutritional, excessive amounts of milk or milk replacer and infectious causes like rotaviruses, corona viruses, entero-toxigenic E. coli (Kaba *et al.,* 2006). Overfeeding, overpopulation, cold temperature, bad hygiene, artificial feeding and colostrums deprivation are all predisposing factors which can be important in the complex etiology of diarrhea (Hemashenpagam *et al.,* 2009).

During diarrhea, dehydration occurs due to loss of water and electrolytes from the gastrointestinal tract and in severe form it leads to death of the animal. Dehydration in animals occurs when extracellular and intracellular fluid, blood volume, water or electrolyte concentration decreases. Severe deficiency of electrolytes in diarrheic ruminants might be the reason of death in serious cases. Even though it is well understood that serum concentration of sodium (Na) and potassium (K) are necessary to rise to the normal level with reference to the fluids used for therapy of diarrhea (Groutides & Michell. 1990). Untreated changes in serum concentrations of Ca, Mg and some trace elements may be the cause of some death or post diarrhea complications such as growth retardation (Tajik & Nazifi, 2012).

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* Corresponding Author**:** dr.sobe786@gmail.com

Some macro minerals and trace elements are classified as essential mineral elements. This classification is based on the concentration needed in the diet and in animal tissues (Herdt & Hoff, 2011), and does not reflect the importance of the mineral (Rankins & Pugh, 2012). Certain macro minerals are required in large quantities and include calcium, chloride, magnesium, phosphorous, potassium, sodium and sulphur. Essential micro minerals or trace elements include cobalt, copper, iodine, iron, manganese, molybdenum, selenium and zinc. These elements are required in smaller quantities (Fraga, 2005). Trace elements (Copper, Iron and Cobalt) take part in growth, reproduction and productivity of household animals. Loss of minerals suffers major losses mutually in production and economy (Graham 1991; Jones *et al.,* 1990). The major clinical symptoms of trace element deficiencies in animals are diarrhea, anemia, loss of hair, depigmentation, growth disorders in bones, difficulty in walking, scurf in skin, hyperkeratosis, parakeratosis, lack of appetite, fertility disorders, decrease in reproductively, young animal growth disorders, tetany, decrease in protein synthesis, immune deficiencies, abortus related to non-infectious reasons and pica (Saleh, 2019). Elements required in less quantity are essential to enhance resistance against ailment in animals. However, decrease in concentration of trace elements like cobalt, copper, iron, iodine, manganese, selenium, or zinc leads to decrease reproductive rate and lactation (Arshad *et al.,* 2020). While milk fever (hypocalcaemia), grass tetany (hypomagnesemia) and abomasum displacement are due to electrolyte imbalance and usually occur at early stage of milk production which leads to considerable economic losses (Graham, 1991). Therefore, these minerals like calcium-phosphate have great value in both adult and growing ruminants (Saleh, 2019).

Keeping in view the significance of electrolytes and other trace elements in diarrhea, this study was designed to determine the serum concentration of electrolytes and trace elements (Ca, Cu, Na, Li, K and Fe) in diarrheic sheep and goat.

2. MATERIALS AND METHODS

Experimental design
In this study, a total of 100 diarrheic animals were included, comprising 50 sheep and 50 goats experiencing clinical diarrhea, regardless of the cause. Additionally, a negative control group of 10 animals was established, consisting of 5 sheep and 5 goats.

Collection of Samples
Blood samples
Blood sample (5ml) was collected from the jugular vein of each of the animal using disposable syringes under aseptic conditions. After collection, blood samples were carefully transferred to EDTA coated vacutainer (purple-topped) for analysis.

Serum samples
The blood samples were allowed to clot and then clotted blood was centrifuged at 3500rpm for 5 minutes. After centrifugation, the supernatant, clear straw-colored fluid (serum) was aspirated into eppendorf with the help of pasture pipette. The serum samples thus collected were stored at -20°C until analysis was performed (Ijaz *et al.,* 2019). All serum samples were prepared by wet digestion following the procedure was 0.5 ml serum sample was digested with 10ml concentrated nitric acid in a 100ml digestion flask at low temperature for about 15-20 minutes until the contents were clear and then with 5ml perchloric acid for 15 minutes. The solution in the flask was heated vigorously till 2-3ml colourless material was left. After cooling the contents were diluted up to 20ml with redistilled water in a volumetric flask and preserved for the analysis of minerals (Akhtar *et al.,* 2007).

Serum electrolyte and trace element analysis
Concentrations of serum electrolytes and trace elements Ca, Cu, Na, Li, K and Fe were estimated through atomic absorption spectrophotometer. Acid digestion was used to prepare samples for chemical analysis of Ca, Cu, Na, Li, K and Fe. These levels were determined by using Atomic Absorption spectrophotometer (Varian AA-1275). The air-acetylene gas mixture was used as fuel for flame production. For each element, a specific hollow cathode lamp was used. The properly diluted standard solutions were individually aspirated, and absorbance was recorded to draw calibration curve for each mineral. Subsequently, concentrations of each element (ppm) in samples were determined by taking absorbance and comparing it with calibration curve.
Absorption of blanks was also determined to rule out any contamination in reagents and glassware and their values were subtracted from samples.

Blood Parameters
Hematology was performed through hematological analyzer. (Diatron company: abacus model)

Statistical Analysis
Analysis of variance tests (ANOVA) was used to compare serum electrolytes, trace elements and clinical signs after diarrhea and t-test was used to compare diarrheic and non-diarrheic sheep with serum electrolytes and trace element. Duncan's test applied for significant variables.

3. RESULTS

Serum samples were obtained from 100 diarrhea (n=50 sheep; n=50 goat) suffering from clinical diarrhea irrespective of cause of diarrhea was included in this

study. In the serum sample of diarrheic as well as non-diarrheic group of both sheep and goat, the concentration of sodium, potassium, calcium, copper and iron were evaluated. The results of the measurement of the serum electrolytes and trace elements of sheep are shown in Table 1.

The serum values of Sodium and Potassium and were increased significantly (P<0.05) while Calcium was decreased significantly (P<0.05) in diarrheic sheep as compared to non-diarrheic group (table 1). Trace elements such as Copper and Iron are required in a minute concentration for various physiological functions. Serum copper and iron were also measured in both diarrheic and non-diarrheic groups of sheep. The serum values of copper and iron were also decreased non-significantly (P>0.05) in diarrheic sheep as compared to non-diarrheic (table 1).

In diarrheic goats, the serum values of Sodium and Potassium were increased significantly (P<0.05) whereas Calcium was decreased significantly (P<0.05) as compared to non-diarrheic group (table 2). Serum copper and iron values were also measured in both diarrheic and non-diarrheic groups of goats. The serum copper and iron concentration were also decreased non-significantly (P>0.05) in goats having diarrhea as compared to non-diarrheic group (Table 2).

The values of PCV were significantly upregulated as compared to normal range in both sheep and goat due to diarrhea. This condition leads toward dehydration and consequently PCV (%) increased. Serum copper and PCV had a significant correlation in the case of diarrheic sheep and goat. Copper deficiency is a significant feature of anemia so that serum copper concentration has a direct link with PCV. Iron also has a strong correlation with copper because it is required for utilization, mobilization and absorption of iron in animal body.

4. DISCUSSION

Neonatal diarrhea is a significant cause of economic loss in ruminants. The etiology of diarrhea can be infectious or non-infectious (Elhassan *et al.,* 2011). Overfeeding, overpopulation, cold temperature, bad hygiene, artificial feeding, and colostrum deprivation are all predisposing factors which can be important in the complex etiology of the disease (Hemashenpagam *et al.,* 2009). There are different causes of diarrhea due to some infectious agents like viruses, bacteria or fungi (Cha£upnik *et al.,* 2012). In early age, groups of animals are more prone to diarrhea than adults. Causes of diarrhea may include uptake of toxins in small ruminants, as well as grazing at green or damp fodder in sheep and goats. Effects of diarrhea in sheep and goat is anorexia, lethargy, depression, acidosis,

dehydration, and gastroenteritis (Lorenz, 2003; Lorenz, 2007; Gidudu *et al.*, 2011).

In the present study, diarrheic ruminants without any treatment were clinically examined and their fecal consistency, age and days between disease onset and sampling were recorded. Alteration occurs in serum level of Na and K during diarrhea. In our study, the serum level of Na and K increase while Ca level decreases in small ruminants in case of diarrhea, along with significant co-relation of PCV and copper. The findings of our study congruent with the findings of Fazal *et al.,* (2019) who reported increased Na and K levels in diarrheic calves. In our study Na and K exhibiting the non-significant difference when co-relate with breed, age, and sex. Although Na level significantly different when co-related with sex in our study. Serum copper concentration had a significant correlation with PCV. In present study electrolytes were quantified with correlation of different breeds, age and sex in sheep as well as co-relations of Cu and PCV that relates with the study of (Groutides & Michell, 1990). These findings are also significant when the co-relation between PCV and copper was measured. There is also a little variation in the level of other electrolytes in serum such as calcium (Ca). From present study Ca level in serum of diarrheic sheep shows the significant difference between breeds, non-significant with age as well as non-significant difference with sex. Laboratory tests are required for proper diagnosis of diarrhea. Hypocalcaemia has been diagnosed as a cause of hypothermia in ruminants, which may explain the observed relationship between Ca and diarrhea in the current study which shows that decrease in Ca in diarrheic animals. Diarrheic sheep and goats also show a significant difference between breeds, non-significant in age groups and non-significant in groups classified based on sex. Changes in trace elements were also studied during diarrhea. Concentration of Fe was also measured in diarrheic sheep and goats during our study. The results showed that Fe has non-significant difference when compare with breed, age and sex. Similarly results of my study showing that the Cu level in serum has significant relation with the value of PCV in sheep. The results of present study also correlate the results of Fazal *et al.,* (2019).

In a previous study, a comparison between the concentrations of Cu and Zn in case of ruminants suffering with diarrhea to the non-diarrheic ruminants has been done and findings show the decrease in value (Arora *et al.,* 2007). The results of current study showed that the serum concentration of Fe and Cu had no significant correlations with clinical signs. Serum copper concentration had a significant correlation with PCV (P<0.05). The results of my study matched with the findings of the study of Rucker *et al.* 2008 as it indicated

that serum Cu level has significant correlation with PCV. Packed cell volume (PCV) is demonstrated as an index for the estimation and quantification of hydration status in the calves (Constable *et al.* 1998). PCV is the best indicator for the diagnosis of changes in hydration status (Haenlein & Anke, 2011) so there is decrease in hydration status of small ruminants which is coincide with the study of Elhassan *et al.*, (2011). Higher PCV means more severe dehydration, which is due to the prolonged water loss (Sadeghian *et al.*, 2011).

5. CONCLUSION

Trace elements and serum electrolytes take part in growth, reproduction, and productivity of household animals. Loss of minerals suffers major losses mutually in production and economy. This study highlights the importance of electrolyte and trace elements in diarrheic sheep and goats. This study will help veterinary practitioners in the field regarding diagnosis and treatment of diarrhea.

6. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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Table 1: Serum concentrations of electrolytes and trace elements in diarrheic and non-diarrheic sheep

Elements	Diarrheic sheep	Non-diarrheic
Sodium	2972.78±29.9	2433.78±24.9
Potassium	277.848± 12.8	241.4± 11.35
Calcium	68.1± 2.69	118.6± 8.65
Copper	4.44± 1.4	5.8± 1.46
Iron	4.46± 0.50	4.2±0.40

Table 2: Serum concentrations of electrolytes and trace elements in diarrheic and non-diarrheic goats

Elements	Diarrheic goats	Non-diarrheic
Sodium	2833.78±29.9	2572±76.1
Potassium	279.848± 14.8	241.4± 9.3
Calcium	88.1±8.6	118.6± 9.6
Copper	4.6± 0.4	4.8± 0.8
Iron	4.5± 0.30	4.4±0.5

EVALUATING THE SHELF LIFE OF *APIS MELLIFERA* HONEY COLLECTED IN SOUTH PUNJAB, PAKISTAN

SAMINA QAMER[1*], ABIR ISHTIAQ[2], BILAL AHMAD[2], ZULFIQAR HAIDER[3], NAVEED AHMAD KHAN[4], SHAKILA NAZ[5], HAZRAT USMAN SHERANI[3], MUHAMMAD SHAHJAHAN[5], MUHAMMAD ADRESS[5]

[1*]Department of Zoology, Rawalpindi Women University, Satellite Town, Rawalpindi
[2]Department of Zoology, Islamia University Bahawalpur, Bahawalnagar Campus.
[3]Department of Zoology, Government College University, Faisalabad
[4]Institute of Pure and Applied Biology, Zoology Division, Bahauddin Zakriya University, Multan
[5]Department of Zoology, Government College University Faisalabad, Layyah Campus

ABSTRACT

The study aimed to assess the shelf life of multi-floral honey collected from the southern region of Punjab Province, Pakistan, over 12 months. Fifty honey samples were analyzed for various physiochemical parameters every four months at room temperature. Initial values included pH, free acidity, electrical conductivity, diastase number, invertase number, HMF, proline content, fructose, glucose, and sucrose percentages. After 12 months, the pH, free acidity, electrical conductivity, diastase, invertase, glucose, and fructose values decreased, while HMF content, proline content, and sucrose percentage increased. These findings suggest that honey composition changes during storage, shortening its shelf life. Fresh A. mellifera honey is recommended for consumption within 6 to 8 months to maintain its quality.

1. INTRODUCTION

Honey is a nutritious substance which is obtain from floral sources by bees (da Silva et al., 2016; Dong et al., 2018). It's chemically composed of mono and dissaccrrides carbohydrates like fructose, glucose, maltose and sucrose (Sakač et al., 2019, Sajid et al., 2022). However, minerals, Protein, organic acid, amino acid, vitamins and aromatic substance are minor components found in honey (Amiry et al., 2017; Čanadanović-Brunet et al., 2014; Sakač et al., 2019). A further two hundred more substances are testified in honey (Ferreira et al, 2009). Due to presence of these components, that can act as antibacterial and anti-inflammatory chemicals (Liu et al, 2013). The quality of honey depends on various components including floral sources, climate condition, processing, Post harvesting handling and conditions for storage (Khan et al., 2016; Kaygusuz et al., 2016; Ansari et al, 2018).

Different types of chemical reactions caused changes in honey, like HMF is formed during sugar dehydration in acidic medium formed HMF, which is also used as honey freshness indicator parameter. The extracted fresh honey is usually present in liquid form, however, with the passage of time during storage honey may start crystallizing depending upon the nectar source and Glucose %. It is less valuable in market, so heat is used to liquefy the crystalline honey. As a result, honey lose their original chemical composition. The limited sources and excesses amount of honey price has provided an increased interest in adulteration (Barra et al., 2010; da Silva et al., 2016). Honey quality parameters are valuable for identifying these likely adulterations and to confirm hygiene conditions for handling and storing honey (Puscas et al., 2013). The aim of the present research was to evaluate the shelf life of fresh *A.mellifera* honey samples stored at room temperature for one year as per the International Honey Commission.

*Corresponding Author**:** samina.qamer@f.rwu.edu.pk

2. MATERIALS AND METHODS

Honey Collection

Fifty samples of honey were directly collected from beekeepers from the different areas/ districts such as Muzaffargarh, DG Khan, Multan and Bahawalpur of South Punjab Pakistan during the year 2021-2022. Plastic bottles and sealed glass were used to store fresh honey samples, then labeled and dated. In the laboratory samples were stored at room temperature, till analysis was completed. No preservatives or heating agents are done at any stage.

Honey quality tests

All physicochemical parameters were determined according to the European Honey Commission methods Bogdanov et al., (1999). pH and EC meter were used to determine Honey pH and Electrical Conductivity, 10g honey in 75ml deionized water. Same solution was used for the titration of free acidity AOAC (1975). Proline content was estimated according to Ough, (1969). For HMF determination Winkler method was used (Winkler, 1955). Honey Diastase and Invertase activity were determined according to the procedure of Siegenthaler (1977) and Schade et al. (1958). Reducing sugar (fructose and glucose) and apparent sucrose was determined by using Lane and Eynon procedure as described in AOAC (2000).

Statistical analysis

One way Analysis of variance (ANOVA) with P value at 0.05 significance level was used to analyze the data by using latest version of statistical software (SPSS-2001).

3. RESULTS AND DISCUSSION

Fig:1 shows the effect of one year storage on various quality parameters of 50 honey samples. According to Terrab et al., (2002) pH is an important factor that enhances honey quality and shelf life (Carreck, 2012; Dietemann et al., 2013). The current results showed that honey samples were more acidic as the average value of pH lies between 3.69-5.15. Sajid et al., (2022) also observed acidic pH (4.28) in *A.mellifera* Pakistani honeys. Laredj and waffa (2017) and Lokossou et al., (2017) reported Algerian and Burkina Fasan honey's pH in the range of 4.17-4.20 and 5-5.8, respectively. The upper value of 5-5.8 by Lokossou et al., (2017) and lower 3.67 by Boussaid et al., (2018) (3.67- 4.11) in Tunisian honey, 4.35 in Pakistani honey from same species (Sajid et al., 2020) seems related to present determined pH. More acidic honey or low pH of honey significantly support to the antimicrobial property of honey (Sajid et al 2020). Initially average free acidity was 21.54, which amplified to 40.80 meq/Kg by the end of 12 months, although was still within international limits (≤50 meq/kg). Acidity

reported by Ciappini et al., (2016) 19.5-22.3 meq/Kg in Argentine honey, Alqarni et al. (2016) 55.5–145.5 meq/kg, Azonwade et al., (2018) 35.7-40.5 meq/kg in Benin honey, El-Haskoury et al. (2018) 16.50–59.50 meq/kg and was 33–46.5 meq/kg in Pakistani honey by Sajid et al., 2020. The honey acidity varies due to different floral sources and yielding seasons.

Similarly, EC ranges found by Gular et al. (2017) 0.11-0.25ms/cm in Tunisian honey, 0.11–0.61 mS/cm by Sajid et al., (2020) and 0.27mS/cm in 2022 in Pakistani honeys, seems related to the current research values of 0.13-0.27 meq/kg. Anhwange, et al., (2015) 0.86- 0.91mS/cm in Nigerian honey, Bousaid et al. (2018) (0.39–0.89 mS/cm), Lokossou et al. (2017) (0.37–1.43 mS/cm) reported higher EC than the present study. Variation in Electrical conductivity of honey depends on different botanical sources explored by honeybees as nectar.

According to the European Union Honey Directive, certain constituents must be determined for honeys proposed for human consumption. Diastase enzyme activity (diastase number) is one of them. In general diastase activity should not be less than 8. Heating the honey degrades the enzyme, which is why the EU directive states minimum values. Diastase in honey converts starch to short-chain sugars and the enzymes' activity hints at possible heating and/or poor storage conditions. It originated both from plant and honeybees salivary and hypophyrngeal gland secretions. It is quite a stable enzyme so considered as storage indicator parameter. It naturally deteriorates over time and also with storage. A decline from 39.05 DN to 19.19DN was observed for 12 months storage period, the limit was within the Codex range (≥8DN) yet. Moloudian et al. (2018) also recorded reduction in diastase activity from 28.68DN to 17.75DN in Iranian honey, this trend is similar to the current study. Sajid et al., (2020) measured 26.97–43.47(DN) in Pakistani fresh honeys from various floral origin. Anhwange, et al., (2015) determined diastase range below (7.61DN) and just above the minimum limit (10.18 (DN) in Riyom, Plateau State honey.

Invertase is another essential enzyme which is liable to convert nectar and honeydew into honey. It has high sensitivity towards temperature increase. Therefore, in some European countries invertase activity determination is also used as a parameter related to the freshness of honey, to its warming or storing condition. Boussaid et al., (2018) and Lichtenberg-Kraag, (2012) stated invertase range of 46.25-184.68 (IN) and 33.76-86.95(IN) in Tunisia and German honey, respectively, which is closely related to the current IN values (27.70-67(IN). Below the IHC limit, invertase activity (23.91-0.02(IN) was found by Parvanov et al., (2012) in Bulgarian honey.

Honey freshness and purity depends on HMF (Al-Ghamdi et al., 2019) which is an aldehydic compound formed by the dehydration of some sugars, mostly fructose (Rosatella et al., 2011; Chris & Cornelia, 2014) and its levels should not exceed 40mg/kg. It is also temperature and storage conditions sensitive (Al-Ghamdi et al., 2019). In the present study higher (43.62mg/kg), yet with IHC limit, HMF content were measured after12 months storage compared to initial 14.68mg/kg. Ciappini et al., (2016) was described HMF range from 12.07 to 27.43 mg/kg in Tunisia honey. Comparative to current findings low HMF values have been recorded in Argentine honey (6.7-7.2meq/Kg) and Indian honey (3.87-4.64mg/kg), respectively, by Boussaid et al., (2018) and Kirvak & Karababa (2017). Chris and Cornelia (2014) treated *Acacia* and *lime* honey under various storage conditions and later tested its freshness by measuring HMF content. Similarly, Sajid et al. (2019) kept multi-floral honey at different temperatures and then checked its freshness by estimating HMF content. Sajid et al., in 2020 and 2022 found averagely 30.85mg/kg and 29.2mg/kg HMF in fresh Pakistani honeys, respectively.

Proline is predominant non-essential amino acid making up almost 50% to 85% of total amino acid content. Its origin is mainly honeybee's salivary gland secretion mixed with nectar to convert it in honey Proline content are usually applied as honey maturation criterion and it should not be less than 180mg/kg in honey as per IHC standard (Qamer et al., 2007; de Slvia et al., 2016). In present study Proline content after 4 months storage at room temperature was 469.82mg/kg, however, it dropped up to 148.30mg/kg level after 12 months storage which was less than minimum limit set by IHC. Sajid et al., (2022) reported an average of 235.32mg/kg Prolin content in *A.mellifera* honey without storing or any other treatment. Aazza et al. (2018) and Nayik and Nanda (2015) investigated the proline content between 256.46-924.98mg/Kg and 205.3-551.74mg/Kg.

As far as monosaccharides and sucrose are concerned, Fructose (42%), Glucose (38%) and Sucrose (2.25%) were found well within limits set by IHC even after 4-month storage. Anyhow, lowering in monosaccharides and raising in sucrose (6.76%) beyond the standard limits was observed in next 8 months. Sajid et al., (2022) determined fructose (38.97%), glucose (32.10%) and sucrose (2.25%) values fairly below the maximum border line set by IHC in fresh *A.mellifera* honey collected from Punjab province. Guler et al., (2017) and Khaliq & Swaileh, (2017) were reported reducing sugar concentration range from 68.41-68.77g/100g and 68.9–83.6g/100g in Turkish and Palestine honeys without storage, respectively. Khan et al., 2016 documented minimum 32.45% in *Zizpus spp* honey and maximum 38.03% Fructose in *Clover* honey from Punjab and

Khyber Pakhtunkhwa, respectively, from Provinces of Pakistan. Similarly, lowest (24%) from *Zizpus spp* and highest (32%) glucose in *Clover* and *Sunflower* was found from Punjab and Khyber Pakhtunkhwa honey samples, respectively. In the same way least sucrose (1.12%) was recorded in Acacia honey collected from Punjab and Khyber Pakhtunkhwa. Although he found more than 6% and 7% in *clover* and *Eucalyptus* honeys from Punjab Province and 9.7% sucrose in currant bush honey collected from Khyber Pakhtunkhwa. The maximum level of sucrose should not exceed 5% in honey as per recommendation of IHC. Khaliq and Swaileh, (2017) reported the sucrose% between 1.1-6.9g/100g in Palestine honey. While Guler et al., (2017) had sucrose concentration (0- 3.05g/100g) well within limits in Turkish honey.

4. CONCLUSION

From the above results it is evident that there took place some irreversible changes after one year storage at room temperature that shortens the freshness and shelf life of Pakistani honey produced by *A.mellifera*. Therefore, it is safe to consume honey within six months without compromising its quality.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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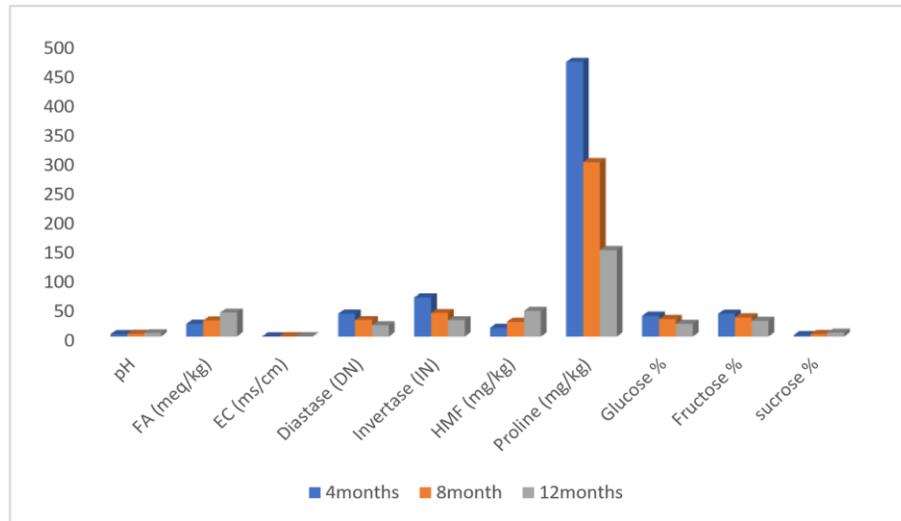

Fig.1. Graphic representation of Change in quality parameters of honey during 12th month's storage

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References:

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Sultana, R. (2019). A new genus *Schizocomicus* (Schizodactyloidea: Ensifera) from Sindh Pakistan. *Pakistan Journal of Zoology*, *51*(5), 1693-1697.

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Reference entry (Two authors):
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Sultana, R., Kumar, S., Samejo, A. A., Soomro, S., & Lecoq, M.(2021). The 2019–2020 upsurge of the desert locust and its impact in Pakistan. *Journal of Orthoptera Research*, *30*(2), 145–154

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