



Research Article

Mystery Behind Camel Mortalities in Khyber Pakhtunkhwa (Lakki Marwat), Pakistan

Rafiullah¹, Said Sajjad Ali Shah^{1*}, Muhammad Ilyas Khan², Anwar Ali³, Imtiaz Ali Shah¹ and Sohaib ul Hassan⁴

¹Veterinary Research Institute, Peshawar, Khyber Pakhtunkhwa, Pakistan; ²Veterinary Research and Disease Investigation Centre, Chitral, Pakistan; ³Livestock Research and Dairy Development Station, Dir Lower, Khyber Pakhtunkhwa, Pakistan;

⁴The University of Agriculture, Peshawar, Khyber Pakhtunkhwa, Pakistan.

Abstract | Camel is an important animal, possessing unique physiological characteristics and serving millions of poor people throughout the world. Mortality in the camel population was observed in Lakki Marwat district of Khyber Pakhtunkhwa in 2018, in which more than 60 camels died. Two dogs that consumed the meat of dead camels were also found dead in the study area. Different types of samples, i.e., tissue samples from dead camels (n=2) and blood samples from infected live camels (n=60) were collected from the study area for bacteriological, hematological, and parasitological studies, while for feed analysis, feed samples (n=20) were collected. Postmortem examination of dead camels revealed extensive hemorrhages in the small intestine and severe congestion of the colon and rectum. Among the hematological parameters, there was a significant reduction in hemoglobin level and hematocrit values and a significant increase in total leukocyte count, whereas all other parameters were in the normal range. Microbiological results revealed sporulated bacilli indicative of *Bacillus cereus*, which is an important cause of food poisoning, causing diarrheal and emetic illness. Analysis of feed samples showed a higher level of Aflatoxin B1 in spoiled Gram and Berseem fed to camels. From the results of the study, it can be concluded that recent mortality in camels might be due to food poisoning due to *Bacillus cereus* intoxication (Haemorrhagic diathesis) and a higher level of Aflatoxin B1 in the feed. Dog mortality may be related to indospicine toxicity due to feeding of indigoferous plants by camels.

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***Correspondence** | Said Sajjad Ali Shah, Veterinary Research Institute, Peshawar, Khyber Pakhtunkhwa, Pakistan; **Email:** sajjadsheikh0695@gmail.com

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Introduction

Camel, an even-toed ungulate having unique physiological characteristics, possesses humps i.e., Dromedary (single hump) and Bactrian (two

humps) (Faraz *et al.*, 2013). Camel has the potential to serve millions of poor people throughout the world by providing milk, meat, hair, and wool in harsh areas of the world and is extensively used for transportation in mountainous areas and deserts

(Ahmad *et al.*, 2010). The world's camel population has been estimated at 24 million and more than 95% of camels are in developing countries. Pakistan's camel population is about one million and Pakistan ranks 8th among camel-raising countries in the world (Anonymous 2006). Camels in Pakistan are used commonly by farmers for agricultural purposes, and the transportation of goods, and crops.

No doubt, the camel has gained much less attention and generally is ignored specie comparatively among the research institutes of the world and more specifically in Pakistan. Camel demands advanced research in various aspects i.e., production potential, disease diagnostics, and treatment, to meet the increasing demand of the community. Information and literature are scarce about the diseases and mortalities of camels in Pakistan. More than 100 camels died in different districts of Punjab, Pakistan in 2015 due to *Bacillus cereus* intoxication (Khan *et al.*, 2017a). Due to mismanagement and lack of awareness among the farmers (pastoralists) about disease diagnosis and treatment, most of the cases result in mortalities. Mortality in the camel population was observed in Lakki Marwat district of Khyber Pakhtunkhwa in 2018, in which more than 60 camels died. According to the local farmers, most of the camels died during working hours. Farmers were of the view that their camels died after feeding gram spoiled during the heavy rainfall in the area. Interestingly, two dogs who consumed the meat of the dead camels also died. Farmers said that camels were shivering, having frothy discharge from the mouth before death. Rigor mortis was observed in almost all of the cases. Postmortem of dead carcasses showed splenomegaly, hemorrhagic enteritis of the small intestine, and severe congestion of the large intestine i.e., colon. The present study aimed to properly address the cause of recent camel mortalities in the Lakki Marwat district of Khyber Pakhtunkhwa.

Materials and Methods

Study area

This study was conducted in Lakki Marwat district of Khyber Pakhtunkhwa. It is located at 32.5° N latitude and 70.7° E longitude with an altitude of 270.5 meters. Camel population is highest in the southern districts of Khyber Pakhtunkhwa and Lakki Marwat, there are more than 8000 heads (Anonymous 2006).

Sampling

Samples were collected during our visit to the study area. Blood samples (n=60) were collected from camels (infected animals) in the study area for hematological study, microbiological and parasitological examination. Moreover, Gram and Berseem samples (n=20) were also collected for the detection of mycotoxins. Postmortem examination of dead camels (n=2) and two dogs (dogs that were found dead after consumption of camel meat) was conducted. Tissue samples (liver, lung, heart, intestine, spleen) and intestinal contents of camels were also collected for bacteriological study.

Hematology

Anticoagulant-added whole blood samples were processed through an automatic hematology analyzer (Urit Vet 2900) for the estimation of the total erythrocytic count, hemoglobin, hematocrit, total leucocytic count, and erythrocytic indices (Shah *et al.*, 2017).

Microbiology

For microbiological examination, samples were sent to the National Veterinary Laboratory (NVL), Islamabad. Samples were cultured for growth on Nutrient agar and McConkey agar, for penicillin sensitivity, and for hemolysis on Blood agar. BHI (Brain heart infusion) broth culture was heated at 75°C to kill all the vegetative bacteria and was cultured again on Nutrient agar and MacConkey agar and incubated at 37°C for 24 hrs. Again sporulated bacilli were observed on gram staining of Nutrient agar culture while no growth on MacConkey agar was observed. The culture was also tested for sensitivity to penicillin but was not sensitive (Quinn *et al.*, 2002).

Parasitology

For parasitological examination, blood and fecal samples were processed at the Parasitology section, Veterinary Research Institute, Peshawar for hemo-parasites. Thin blood smears were prepared, fixed with methanol, and stained with Giemsa for 15 min. Stained smears were observed under a microscope at the oil immersion lens i.e., 100× objective (Khan *et al.*, 2017b). Fecal samples were processed through the Floatation technique and observed under 4x and 10x objectives.

Mycotoxin detection

The feed samples (Gram, Berseem) collected at the

study area were processed at the Center of Animal Nutrition (CAN), Veterinary Research Institute, Peshawar for the detection of mycotoxin. Aflatoxin was determined in the feed samples through Thin Layer Chromatography (TLC) by following the standard protocol of [Castro and Vargas \(2001\)](#).

Statistical analyses

The result of hematological values of infected and non-infected camels was arranged in MS Excel and the Student t-test was applied for hematological variations and means were compared by Duncan's multiple ranges (DMR) at $P \leq 0.05$ level of significance.

Results and Discussion

Postmortem examination

Necropsy of dead camels revealed extensive hemorrhages in the small intestine and severe congestion of the colon and rectum. Unclotted blood was present in the buccal cavity, trachea, and nose along with hemorrhages in the lungs and liver. Severe hepatotoxicity (liver was small in size, hemorrhagic, and nodular) was observed in dogs that died after eating camel meat.

Hematology

Results of complete blood count show that total erythrocytic count (TEC) were in the normal range whereas hemoglobin and hematocrit value was decreased in infected animals and was statistically significant ($P < 0.05$). In erythrocytic indices, only mean corpuscular volume (MCV) values were decreased significantly ($P < 0.05$) while mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were in the normal range. A significant increase in total leukocyte count was observed in infected animals as compared to non-infected animals ([Table 1](#)).

Microbiological examination

Microbiological results revealed sporulated bacilli on gram staining while animal pathogenicity tests and hemolysis on blood agar were negative. The possibility of *Bacillus anthracis* was ruled out at this stage because all the characteristics observed were related to *Bacillus cereus*.

Parasitological examination

Fecal samples and blood samples were negative on microscopy for intestinal parasites and haemo-

parasites, respectively.

Table 1: Complete blood count of infected and non-infected camels.

| Parameters | Non-infected | Infected | p-value |
|----------------------------------|------------------------------|------------------------------|---------|
| TEC ($\times 106/\mu\text{l}$) | 7.5 ^a ± 0.21 | 5.4 ^a ± 0.03 | 0.47 |
| Hb (g/dl) | 11.2 ^a ± 0.3 | 9.3 ^b ± 0.2 | 0.04 |
| Hct (%) | 38 ^a ± 0.82 | 30 ^b ± 0.76 | 0.00 |
| MCV (fl) | 55 ^a ± 0.53 | 50.6 ^b ± 0.41 | 0.00 |
| MCH (pg) | 14.6 ^a ± 0.04 | 16.6 ^a ± 0.1 | 0.24 |
| MCHC (g/dl) | 29 ^a ± 0.07 | 30 ^a ± 0.3 | 0.31 |
| TLC ($\times 103/\mu\text{l}$) | 12.3 ^b ± 0.46 | 16.6 ^a ± 0.5 | 0.01 |

Different superscripts along the row indicate significance at $P < 0.05$. TEC, Total erythrocytic count; Hb, Hemoglobin; Hct, Hematocrit; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; TLC, Total leukocyte count.

Mycotoxin detection

Analysis of feed samples showed a higher amount of Aflatoxin B1 in spoiled gram and Berseem which were fed to camels whereas a low quantity of Aflatoxin B1 was recorded in grams stored properly ([Table 2](#)).

Table 2: Aflatoxin B1 in feed samples collected at the study area.

| Feed | Status | Aflatoxin B1 (Mean \pm SE) |
|---------|---------|------------------------------|
| Gram | Normal | 10 ± 0.26 |
| | Spoiled | 50 ± 0.81 |
| Berseem | Normal | 12 ± 0.25 |
| | Spoiled | 45 ± 0.54 |

Camel has gained much less attention and generally is ignored specie comparatively among the research institutes in the world more specifically in Pakistan. Though farmers use their camels for routine activities like transportation and drought purposes due to a lack of awareness and facilities, the farming community is unable to treat their camels in time and their production and working potential have drastically decreased.

Postmortem examination of the dead camels at the time of the outbreak revealed liver enlargement, congestion, and necrotic patches. Almost all of the carcasses had severe congestion in the colon and rectum, with a few cases having hemorrhagic enteritis. Similar lesions were reported in camels died of *Bacillus cereus* ([Khan et al., 2017a](#)) and aflatoxicosis ([Al-Hizab et al., 2015](#); [Osman et al., 2004](#)). There was

severe hepatotoxicity in dogs died after consumption of camel meat. Normally, camels prefer to feed on Indigoferous species of plant but this plant contains indospicine toxin. This toxin is absorbed in camel meat and when dogs consume camel meat, causes severe hepatotoxicity in dogs and death because dogs are highly sensitive to indospicine toxin as compared to other species. Recent mortality in dogs may be due to the severe hepatotoxicosis due to indospicine as reported by [FitzGerald et al. \(2011\)](#).

The total erythrocytic count of the infected camels was slightly decreased, though non-significant, while hemoglobin and packed cell volume were decreased significantly and these results were indicative of anemia. During the summer season, camels consume more water due to which blood is diluted and PCV is decreased. There is a negative correlation between hematocrit values and atmospheric temperature. This statement is in agreement with the findings of [Badawy et al. \(2008\)](#) that in the summer season, there is a reduction of circulating erythrocytes and an increased rate of destruction of RBCs which results in decreased PCV. Anemia was classified as microcytic normochromic based on erythrocytic indices because the mean corpuscular volume was significantly decreased while MCH and MCHC were in the normal range.

Microbiological examination revealed sporulated bacilli on gram staining and the results of all the confirmatory tests were closely related to *Bacillus cereus* while no evidence of *Bacillus anthracis* was recorded ([Quinn et al., 2002](#)). *Bacillus cereus* is a gram-positive organism, capable of producing endospores. It is widely distributed in the environment due to which it can easily contaminate animal feed ([Czernomys-Furowicz et al., 2000](#)). *Bacillus cereus* is an important cause of food poisoning and produces different types of toxins (endotoxins) ([Logan, 2012](#)). Endotoxins cause severe endothelial damage which activates clotting factors in the blood and causes disseminated intravascular coagulation (DIC) and bleeding manifestations. There might be involvement of *Bacillus cereus* in the current camel mortality because there is a history of floods in the study area, due to which gram fed to camels were spoiled. *Bacillus cereus* mainly causes two forms of illness i.e., diarrheal illness and emetic illness, in which the latter form causes infection in less than 5-6 hrs after consuming contaminated feed ([Kotiranta et al., 2000](#)). *Bacillus*

cereus intoxication was also reported as a suspected cause of camel mortality in the Thal districts of Punjab, Pakistan where more than 100 camels died ([Malik et al., 2016-17](#)).

Floods and high temperature in the study area favor the growth of the fungus (which produce Aflatoxin B1) and the level of aflatoxin in Gram and Berseem was recorded as higher than the permissible level. [Osman et al. \(2004\)](#) reported a similar pattern of camel mortality fed with a feed having an increased level of Aflatoxin B1. The fungus can grow well in raw feed components under suitable conditions because of its high level of nutrients. Temperature and moisture content are important indicators for the growth and proliferation of fungus and hence the production of mycotoxin ([Yaling et al., 2008](#)).

Conclusions and Recommendations

From the findings of the current study, it can be concluded that *Bacillus cereus* intoxication (*Haemorrhagic diathesis*) may be the prime cause of camel mortality, whereas aflatoxin may not be directly involved in camel mortality but may reduce the body's resistance to diseases.

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Novelty Statement

To my knowledge, this is the first study of its kind, as *Bacillus cereus* poisoning in camels has never been reported, especially in Khyber Pakhtunkhwa.

Author's Contribution

Rafiullah, Anwar Ali, and Imtiaz Ali Shah: Collected various samples.

Rafiullah, Imtiaz Ali Shah, and Anwar Ali: Processed hematological samples.

Said Sajjad Ali Shah and Muhammad Ilyas Khan: Conducted parasitological examinations.

Said Sajjad Ali Shah: Analyzed the data and drafted the manuscript along with other co-authors.

Conflict of interest

The authors have declared no conflict of interest.

References

- Ahmad, S., M. Yaqoob, N. Hashmi, S. Ahmad, M. Zaman and M. Tariq. 2010. Economic importance of camel: Unique alternative under crisis. *Pak. Vet. J.*, 30(4): 191-197.
- Al-Hizab, F., N. Al-Gabri and S. Barakat. 2015. Effect of aflatoxin B1 (AFB1) residues on the pathology of camel liver. *Asian J. Anim. Vet. Adv.*, 10(4): 173-178. <https://doi.org/10.3923/ajava.2015.173.178>
- Anonymous 2006. Pakistan Livestock Census. 2006, Pakistan: Agricultural Census Organization, Ministry of Economic Affairs and Statistics
- Badawy, M., H. Gawish, M.A. Khalifa, F. El-Nouty and G. Hassan. 2008. Seasonal variations in hemato-biochemical parameters in mature one humped she-camels in the north-western coast of Egypt. *Egypt. J. Anim. Prod.*, 45(2): 155-164. <https://doi.org/10.21608/ejap.2008.93879>
- Castro, L.D. and E.A. Vargas. 2001. Determining aflatoxins B1, B2, G1 and G2 in maize using florisil clean up with thin layer chromatography and visual and densitometric quantification. *Food Sci. Technol.*, 21: 115-122. <https://doi.org/10.1590/S0101-20612001000100024>
- Czernomysy-Furowicz, D., A. Furowicz and A. Peruzynska. 2000. Feed intoxication in chinchilla induced by *Bacillus cereus* enterotoxin. Scientifur (Denmark).
- Faraz, A., M.I. Mustafa, M. Lateef, M. Yaqoob and M. Younas. 2013. Production potential of camel and its prospects in Pakistan. *Punjab Univ. J. Zool.*, 28: 89-95.
- Fitzgerald, L., M. Fletcher, A. Paul, C. Mansfield and A. O'hara. 2011. Hepatotoxicosis in dogs consuming a diet of camel meat contaminated with indospicine. *Aust. Vet. J.*, 89(3): 95-100. <https://doi.org/10.1111/j.1751-0813.2010.00684.x>
- Khan, F.M., S. Manzoor, M.N. Malik and S.A. Ali. 2017a. Investigation and Control of Camel Diseases and Folk Anomalies in North and South Punjab of Pakistan. *J. Dairy Vet. Sci.*, 4(1): 555627. <https://doi.org/10.19080/JDVS.2017.04.555627>
- Khan, M.I., S.S.A. Shah, H. Khan, M.I. Khan and U. Aziz. 2017b. Determination of parasitic load in government cattle breeding and dairy farm, Charsadda, Khyber Pakhtunkhwa-Pakistan. *Adv. Anim. Vet. Sci.*, 5(4): 174-178.
- Kotiranta, A., K. Lounatmaa and M. Haapasalo. 2000. Epidemiology and pathogenesis of *Bacillus cereus* infections. *Microb. Infect.*, 2(2): 189-198. [https://doi.org/10.1016/S1286-4579\(00\)00269-0](https://doi.org/10.1016/S1286-4579(00)00269-0)
- Logan, N., 2012. *Bacillus* and relatives in foodborne illness. *J. Appl. Microbiol.*, 112(3): 417-429. <https://doi.org/10.1111/j.1365-2672.2011.05204.x>
- Malik, M.N., F.M. Khan, S. Manzoor and S.A. Ali. 2016-17. Surveillance and prophylactic measures against camel diseases in Punjab. *GoP Livest. Dairy Dev. Dep.*, pp. 40-50.
- Osman, N., F. El-Sabban, A.A. Khawli and E. Mensah-Brown. 2004. Effect of foodstuff contamination by aflatoxin on the one-humped camel (*Camelus dromedarius*) in Al-Ain, United Arab Emirates. *Aust. Vet. J.*, 82(12): 759-761. <https://doi.org/10.1111/j.1751-0813.2004.tb13242.x>
- Quinn, P., B.K. Markey, M. Carter, W. Donnelly and F. Leonard. 2002. *Veterinary microbiology and microbial disease*. Blackwell Science.
- Shah, S., M. Khan, K.M. Rafiullah, H. Khan, A. Ali, M. Ali and R. Jan. 2017. Tick-borne diseases-possible threat to humans-dog interspecie bond. *Adv. Anim. Vet. Sci.*, 5: 115-120. <https://doi.org/10.14737/journal.aavs/2017/5.3.115.119>
- Yaling, W., C. Tongjie, L. Guozhong, Q. Chunsan, D. Huiyong, Y. Meiling, Z. Bert-Andree and S. Gerd. 2008. Simultaneous detection of airborne aflatoxin, ochratoxin and zearlaenone in poultry house by immunoaffinity column and high performance liquid chromatography. *Environ. Res.*, 107: 139-144. <https://doi.org/10.1016/j.envres.2008.01.008>