

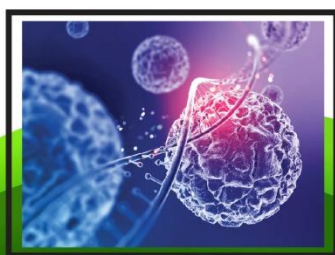
IJAB



INDUS JOURNAL OF AGRICULTURE AND BIOLOGY

VOLUME 1, ISSUE 1

JAN-JUNE 2022



ALI INSTITUTE OF RESEARCH AND SKILLS DEVELOPMENT (AIRSD)

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Indus Journal of Agriculture and Biology(IJAB) is an International peer reviewed open access journal that publishes articles in the field of agro-sciences, biological, ecological and toxicological Studies, Cell biology, developmental biology, genetics, biology, Toxicology, Ecology and Environmental biology, Entomology, Biotechnology.

IJAB is a scientific journal that provides academicians and researchers a unique platform to collect and disseminate latest research on agriculture and biological sciences. The journal focuses on improving agricultural production systems, enhancing agricultural sustainability and addressing issues of toxicology and food security whilst protecting the environment.

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Indus Journal of Agriculture and Biology(IJAB)

Publisher: Ali Institute of Research and Skill Development (AIRSD)

Office No1, Moiz Clinic Building, Khan Village Road, New Gulgust Multan, Pakistan.

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Publisher: Ali Institute of Research and Skill Development (AIRSD)

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Indus Journal of Agriculture and Biology(IJAB)

Publisher: Ali Institute of Research and Skill Development (AIRSD)

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Botulism a Major Risk in Animals After Flood in Pakistan; A Review

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ARTICLE INFO

Article History:

Received:	January	1, 2022
Revised:	January	20, 2022
Accepted:	February	5, 2022
Available Online:	March	10, 2022

Keywords:

Botulism; *Clostridium botulinum*; Livestock;
Economic impact; Epidemiology; Flood; Pakistan

ABSTRACT

Background: Botulism, a potentially fatal disease, poses an elevated risk to livestock in flood-prone regions like Pakistan. Flooding facilitates the spread of the causative bacteria, leading to substantial losses in the livestock sector. This review delves into the multifaceted aspects of botulism in animals post-flooding. **Objectives:** To summarize the impacts of botulism on animals post-flooding, covering its epidemiology, transmission routes, symptomatology, and management, based on an exhaustive literature review. **Methods:** A comprehensive review of literature was conducted, focusing on studies and articles that discussed botulism in animals following flood events. The collected data was analyzed and categorized based on different aspects of the disease, from its transmission to its management. **Results:** Flooding events, intensified by rapid urbanization, global warming, and other factors, foster the proliferation of spore-forming anaerobic bacteria prevalent in decaying organic matter. Animals, notably cattle, contract botulism either through consumption of toxin-laden rotting substances or endogenous toxin formation. Identified forms of botulism include carrion-associated, toxico-infection, wound, and forage botulism. Symptoms predominantly involve the neuromuscular system, characterized by progressive paralysis, with a high likelihood of resultant death. Treatments are limited in efficacy, making prevention the frontline defense against the disease. **Conclusion:** Botulism, though not widely acknowledged among dairy farmers, can cause grave losses in livestock populations after floods. The absence of a definitive cure emphasizes the importance of awareness and proactive preventive measures. Dairy farmers should be educated on the risks of botulism and the criticality of early intervention and preventive strategies.



INTRODUCTION

Flooding causes a variety of issues, but the most visible ones, such lameness and mastitis, are obvious, so the quality of reaction is typically pretty good. However, there are other less urgent but potentially more severe issues that could arise. Since parasite, worm, coccidia, and fluke problems are more likely to arise after wet circumstances, farmers must be aware of this and prepare for it by strategically watering their crops.

OBJECTIVES: Botulism is the major risk for livestock and human being. The aim of current review article was to know about the effects of botulism on animals, so current review of literature was done.

Animal diseases

Various illnesses including leptospirosis, anthrax, clostridial infections, foot Rot, oedema, mastitis, bovine ephemeral fever, lumpy wool in Sheep, infectious anaemia in horses, botulism, and other vector-borne diseases cause the animal health to suffer following floods ¹⁻².

Botulism

Among all these mentioned diseases, botulism is the major one which spread in animals after flooding ³⁻⁴. Toxins can be consumed orally and cause intoxication, or they can be created inside the digestive system. The spore-forming anaerobic bacteria thrives in decaying animal tissue, as well as occasionally in plant matter ⁵⁻⁶. In addition to the outbreak of various bacterial and vector-borne diseases, post-natural catastrophes like floods in underdeveloped countries can result in diseases and infections ranging from epizootics to parasitosis (internal and external). Physical and emotional strain, a lack of wholesome food and drink, environmental damage leading to unsanitary surroundings, etc. are all causes of these post-disaster effects ⁷⁻⁹.

Spread of Botulism in livestock after flood

The population of livestock is the first to be impacted by any unstable condition brought on by a natural disaster, such as an earthquake. Livestock in particular has remained extremely sensitive to practically all types of natural disasters, but flooding is the most common and dangerous of all types ¹⁰⁻¹¹. The primary cause of floods is the natural ecological system, which includes the monsoons, heavily contaminated river systems, and steep, easily eroded mountains, especially those found in the arctic regions ¹². Other factors cited as contributing to floods include population growth, fast urbanization, severe strain on rural lands, expansion of development in flood plains, and global warming. Decomposing plants and animal carcasses can contain the botulinum toxin after floods. Animals may accidentally eat rotting matter, intentionally if they are hungry or low in phosphorous ¹³.

Mode of transmission

Toxins can be swallowed accidentally or, in the case of bone chewing, in an effort to gain phosphorus in times of deficiency. Bones, corpses, rotting plants, and silage can all be dangerous. Progressive paralysis brought on by the poison usually results in death. Cattle that have been infected initially may drool and exhibit tongue paralysis. This rapidly worsens into being unable to stand, and in 1 to 2 days, paralysis of the breathing muscles results in death. Mode of action is given in table 1.

Table 1. Production, mode of action and effects of *C. botulinum* neurotoxins

Feature of neurotoxin	Effects of <i>Clostridium botulinum</i>
Genes which regulate production	Usually in genome
Site of production	In carcasses, Occasionally in wounds or in intestine, decaying vegetation
Mode of action	Inhibition of neuromuscular transmission
Antigen type	Eight antigenically distinct toxins A -G
Clinical effects	Flaccid paralysis

EPIDEMIOLOGY

Occurrence

Clostridium botulinum pathogens are found in soil, rotting and decaying plant matter, and dead bodies all over the world. Geographically, botulism cases are more common in temperate nations than *C. botulinum* does in the soil, and the disease-causing strains are compatible with different types of soil ¹⁴.

Etiology

The majority of the time, botulism is an intoxication brought on by ingesting a toxin found in food rather than an illness. A, B, C1, D, E, F, and G are the seven different varieties of *C. botulinum*, which are distinguished by the antigenic specificity of the toxins. The most significant types are A, B, and E in humans; C1 in the majority of animal species, particularly in cattle, horses, wild ducks, pheasants, chickens, mink, and D in cattle ¹⁵⁻¹⁶.

Source of infection and type of botulism

There are various source of infection and types of botulism. Some are mentioned here in this review of literature.

Carrion Associated Botulism

The best sources of poison are rotting cattle carrion, fly maggots, and bone. There is a significant risk of ingesting BoNT in cattle with pica because they often gnaw on bones and corpses to make up for their mineral deficiencies. When calves consume carrion directly after developing osteophagia from a diet low in phosphorus, this is known as direct carrion consumption. The condition is likely to manifest as an outbreak ¹⁶.

Toxico-infection Botulism

Toxico infectious botulism is the term used to describe the illness that C. A living animal's tissue is where botulinum develops and creates toxins. Animals can contract toxico infectious botulism, another type of botulism, by ingesting bacteria from the digestive tract of cattle that are actively developing ¹⁶.

Wound Botulism

A illness caused by the expansion of C is wound botulism. In-vivo toxin generation by botulinum spores in a polluted wound. It is a rare acquired neuromuscular junction disorder characterized by descending flaccid paralysis brought on by botulinum neurotoxins (BoNTs) created after *Clostridium botulinum* infection of wounds¹⁵⁻¹⁶.

Forage botulinum

Consumption of food contaminated with which has grown there and produced a toxin, is what causes the sickness. Consuming water or feed that has been polluted by animal corpses and rotting feed has also been documented to cause poisoning¹⁶.

Toxins and sources of *C. botulinum*

The toxins produced by *C. botulinum* which insert bad impacts on livestock health are describes below. Progressive paralysis brought on by the poison usually results in death. Cattle that have been infected initially may drool and exhibit tongue paralysis.

Table 2. Toxins and the source of *C. botulinum*

Serial number	Toxins	Sources
1	Type A	carcasses, meat and toxico infection
2	Type B	carcasses, meat and toxico infection
3	Type C	maggots, dead invertebrates, rotting vegetation carcasses of poultry, hay or silage contaminated with rodent carcasses and ensiled poultry litter
4	Type D	Bones and carcass
5	Type E	sludge in earth bottomed ponds, dead invertebrates and fish
6	Type F	Fish and meat
7	Type G	soil contaminated food

Quinn et al. (2003)

MECHANISM

An anaerobic, Gram positive, spore-forming rod, *C. botulinum* is serious threat for livestock industry after flood. One microgram of the most potent known poisons, botulin, may kill an animal. It causes respiratory and muscular paralysis by obstructing nerve function. In every instance, the toxin produced by *C. botulinum*, not the bacterium itself, is what causes sickness (Arnon *et al.*, 2001).

Casual agents

In cattle, a bacterium called *Clostridium botulinum* causes botulism. The organism, a spore-forming, anaerobic, gram-positive rod, is frequently found in soil samples and aquatic sediments and thrives mostly in decomposing animal and plant materials¹⁷. The antigenic specificity of the toxins distinguishes the seven different varieties of *C. botulinum*: A, B, C, D, E, F, and G. Types A, B, and E harm people the most; C is the most prevalent in animal species, while D is seen in cattle as given in table 2.

Symptoms or signs of botulinum

There are different symptoms of botulinum that have reported or showed in livestock by many researchers. The major signs are given here.

1. Constipation.
2. Drooling of saliva.
3. Decreased tongue tone
4. Issues with prehension of food and deglutition
5. Restlessness
6. Incoordination
7. Stumbling knuckling
8. Ataxia
9. Unable to eat or drink

Botulism symptoms include progressive motor paralysis, altered eyesight, trouble swallowing and chewing, disturbed vision, and progressive paresis. Typically, cardiac or respiratory paralysis results in death¹⁸.

Management or treatment

The antitoxic serum should be either selective or polyvalent since it only works on homologous strains. To eliminate or inactivate toxins in the digestive tract, purgatives, lactic acid administration, and ruminal lavage have all been recommended. Therapy is primarily symptomatic, and you should provide normal nursing care along with hydration and nutritional assistance. Some of the paralysis brought on by the toxin has been successfully treated with guanidine hydrochloride. The best method for preventing botulism is to keep your vaccination schedule current. Supplemental phosphorus may also be helpful¹⁹⁻²².

CONCLUSION

The aftermath of flooding in Pakistan amplifies the vulnerability of livestock to botulism, a potentially lethal disease often overshadowed by other flood-induced challenges. The disease's ability to rapidly compromise the neuromuscular system in animals, coupled with the absence of a reliable curative measure, underscores the urgency of shifting the focus from reactive responses to proactive preparedness. Addressing the threat of botulism necessitates a multi-faceted approach, centering on heightened awareness among dairy farmers and the wider agricultural community. This includes recognizing early disease indicators, understanding transmission routes, and most crucially, implementing evidence-based preventive measures. As the threat of climate change-induced floods persists, integrated strategies that encompass education, early detection, and preventive action become paramount to safeguard Pakistan's livestock sector against the crippling impacts of botulism.

ACKNOWLEDGMENT

Authors are highly thankful to their concern institute.

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Rhizobacterial Inoculation to Improve Wheat Growth and Soil Quality in a Saline Environment

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ARTICLE INFO

Article History:

Received: January 10, 2022
Revised: February 10, 2022
Accepted: February 28, 2022
Available Online: March 23, 2022

Keywords:

Wheat, Rhizobacteria, Salinity, Soil physico-chemical Properties

ABSTRACT

Objective: To elucidate the effects of PGPR inoculation on the growth and productivity of wheat (cultivar AZRC-84) cultivated under saline conditions. **Methods:** Four salt-resistant PGPR strains were isolated from the wheat rhizosphere at the Arid Zone Research Center (AZRC) DI Khan. A pot experiment, involving these PGPR strains, was conducted on wheat grown in saline soil with an electrical conductivity (ECe) of 7.3 dS m⁻¹. Plant biomass and various soil parameters were analyzed 25 days post-germination. **Results:** Compared to controls, inoculation with PGPR strains significantly improved the wheat's dry biomass, especially with MB3 *Pseudomonas aeruginosa*, MB2 *Enterobacter mori*, and MB4 *Enterobacter asburiae* treatments. In terms of microbial population under saline conditions, the maximum bacterial populations were observed in MB3 *Pseudomonas aeruginosa* and MB4 *Enterobacter asburiae* treatments. Soil analyses post-PGPR inoculation revealed an increase in organic carbon and water-holding capacity. Concurrently, there was a reduction in ECe, pH, and sodium content, whereas the soil's bulk density remained unaltered. **Conclusion:** PGPR inoculation demonstrates potential in enhancing wheat growth in saline environments by not only promoting plant growth but also ameliorating certain detrimental soil parameters. The findings hold promise for improving crop yields in salt-affected areas through bio-inoculation strategies.



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INTRODUCTION

About 20% of irrigated agricultural land suffers unfavorable effects from salinity¹. Salt management issues can cause sodicity in clay soils. When negatively charged clay encounters sodium (Na), the clay expands and disperses. Saline soils have poor plant nutrition and high levels of osmotic stress, have a major detrimental effect on plant growth. Salt stress affects all a plant's key metabolic processes, including development, photosynthesis, protein synthesis, and lipid metabolism². Proline may aid in osmotic regulation, guard macromolecules from desiccation, and serve as a vital part of the body's antioxidative defense mechanism in the pentose phosphate pathway³. When exposed to salinity, plants of the chickpea (*Cicer arietinum* L.) species exhibited heightened Na/K fraction and lower P assimilation in shoot⁴. But inoculating plants with PGPR can benefit plants to grow better under osmotic stress⁵. Utilizing PGPR bio-inoculants, i.e., *Agrobacterium*, *Pseudomonas*, *Azospirillum*, and numerous other bacterial species, is an ecologically responsive, and economically feasible method of recovering salinity-stricken land and improving biomass output⁶. As PGPR colonize plant roots, its use can be beneficial in the creation of strategies to elevate the growth of wheat in salty conditions⁷. Inducing PGPR chemotaxis on root surfaces by root exudates i.e., carbs and amino acids enhances the possibility that bacteria will reach the plant roots⁸. There have been reports of higher agronomic yields because of PGPR due to the generation of growth-stimulating plant hormones i.e., gibberellic acid (GA), indole-3-acetic acid (IAA), ethylene, zeatin, abscisic acid and phosphorus solubilization⁹. Salinity significantly reduces the yield of wheat, which on moderately salinized soils results in a loss of about 65 percent of the crop¹⁰. Although there is very little information available regarding the role of PGPR in wheat under salinity, it has been observed that using PGPR inoculum for cereal growth can decrease salt stress¹¹. This research investigated the effects of PGPR application on the development and productivity of wheat in a saline environment.

MATERIALS AND METHODS

PGPR Collection

The current research utilized four salt resistant PGPR, which were isolated from the wheat rhizosphere at Arid Zone Research Center (AZRC) DI Khan. These PGPR were found to have a w/v concentration of 6% sodium chloride. These strains have growth-promoting properties i.e., IAA, P solubilization, GA, proline, siderophores, reducing sugars (RS), and total solvable sugar synthesis at 6% sodium chloride for plants⁵.

Testing and analyzing cannabis

A pot experiment with the salt-tolerant wheat cultivar AZRC-84 that was inoculated with PGPRs (MB₁ *Pseudomonas putida*, MB₂ *Enterobacter mori*, MB₃ *Pseudomonas aeruginosa* and MB₄ *Enterobacter asburiae*) was carried out at AZRC DI Khan. The salinity of the soil was measured at EC_e = 7.3 dS m⁻¹. 25 days after the seedlings emerged, the plants were dug up and observations were conducted on the overall as well as the shoot and the roots (dry biomass). According to the procedures developed by Kalra and Maynard, the bulk density, organic carbon, electrical conductivity (ECe), and water-holding capacity of the pre-treated (saline soil prior to bacterial inoculum) and post-treated (rhizosphere soil following bacterial inoculum 25 days after sowing) soil from wheat grown in containers were analyzed¹². Bacterial populations in the soil around the rhizosphere were assessed for the number of colony-forming units (CFU) that they produced on a NA medium (Upadhyay et al.⁵ and Upadhyay et al.⁷ methods were used to

calculate the salt content of the soil.

Statistical analysis

Statistix version 8.1 was utilized throughout all of the analyses of variation. When conducting multiple range studies, the LSD test was utilized to identify significant differences between the several sets of data. When $p < 0.05$, the findings were deemed to be significant.

RESULTS

Wheat plants' response to PGPRs in salty environments

Controls consisted of non-inoculated treatments that were either subjected to or not subjected to NaCl stress. Extreme biomass was attained with inoculation of isolates MB₃ *Pseudomonas aeruginosa*, MB₂ *Enterobacter mori* and MB₄ *Enterobacter asburiae*. Highest root biomass was attained following inoculation with MB₃ *Pseudomonas aeruginosa*. Inoculation with isolates MB₃ *Pseudomonas aeruginosa*, MB₂ *Enterobacter mori* and MB₄ *Enterobacter asburiae* significantly improved total dry biomass (Table 1).

Effect of PGPRs on microbial population under saline conditions

The rhizosphere possessed the most bacterial populations, and all of the PGPR strains showed rhizo-adaptation in wheat (i.e., the optimal CfU population; see Table 2). The greatest was observed in MB₃ *Pseudomonas aeruginosa* and MB₄ *Enterobacter asburiae*.

Effect of PGPRs on soil parameters under saline conditions

After PGPR was inoculated into the soil, there was a greater increase in organic carbon and water-holding capacity in comparison to the control. On the other hand, the EC_e, pH, and sodium content of the soil all decreased, but the bulk density of the soil remained the same (Table 3).

Table 1: The influence of PGPR on the growth characteristics of wheat (AZRC-84) grown in salty environments

Treatments	Shoot		Root		Total	
	Fresh biomass (g)	Dry biomass (g)	Fresh biomass (g)	Dry biomass (g)	Fresh biomass (g)	Dry biomass (g)
Uninoculated (Normal Soil)	0.69 c	0.17 b	0.30 b	0.08 b	0.99 c	0.25 c
Uninoculated (Saline Soil)	0.42 e	0.11 d	0.18 d	0.05 c	0.60 e	0.16 e
MB ₁ <i>Pseudomonas putida</i>	0.57 d	0.14 c	0.25 c	0.07 bc	0.82 d	0.21 d
MB ₂ <i>Enterobacter mori</i>	0.73 b	0.18 b	0.32 b	0.09 ab	1.05 b	0.27 b
MB ₃ <i>Pseudomonas aeruginosa</i>	0.87 a	0.22 a	0.38 a	0.10 a	1.25 a	0.32 a
MB ₄ <i>Enterobacter asburiae</i>	0.71 b	0.18 b	0.31 b	0.08 b	1.02 bc	0.26 bc

Table 2: PGPR's impact on the bacterial population under salty environments

Treatments	BP (cfu g ⁻¹ soil)
Uninoculated (Normal Soil)	6 x 10 ⁴
Uninoculated (Saline Soil)	3 x 10 ²
MB ₁ <i>Pseudomonas putida</i>	7 x 10 ⁵
MB ₂ <i>Enterobacter mori</i>	4 x 10 ⁶
MB ₃ <i>Pseudomonas aeruginosa</i>	7 x 10 ⁷
MB ₄ <i>Enterobacter asburiae</i>	6 x 10 ⁷

Table 3: The influence of PGPR on soil characteristics in salty environments

Treatments	EC _e (dS m ⁻¹)	pH	Na (ppm)	BD (Mg m ⁻³)	Organic Carbon (%)	WHC (%)
Uninoculated (Normal Soil)	2.69 d	8.1 ab	13 c	1.18 d	0.96 c	25.2 b
Uninoculated (Saline Soil)	6.8 a	8.3 a	38 a	1.38 a	0.65 e	17.8 d
MB ₁ <i>Pseudomonas putida</i>	3.94 b	8.2 ab	18 b	1.33 b	0.81 d	21.3 c

MB₂ <i>Enterobacter mori</i>	3.05 c	7.9 c	14 c	1.31 bc	0.97 bc	27.5 a
MB₃ <i>Pseudomonas aeruginosa</i>	2.98 cd	7.9 c	13 c	1.28 c	1.15 a	27.9 a
MB₄ <i>Enterobacter asburiae</i>	3.17 c	8.0 b	13 c	1.30 bc	1.03 b	26.8 a

DISCUSSION

Wheat has a tolerance level that falls in between moderate and high for salinity, and there have been reports of species differences in salinity tolerance ¹³. Free-living bacteria that either directly or indirectly promote plant growth are referred to as plant growth-promoting bacteria ¹⁴. In trials conducted in greenhouses, all four PGPR utilized had a substantial impact on the development of wheat and the health of the soil in pot experiments conducted inside a saline environment; in other words, all four PGPR stimulated growth ⁵. After PGPR inoculation, there may be an improvement in nutrition in the saline environment, which may account for the rise in biomass. The detrimental effects of salt on the growth of tomato, pepper, canola, cotton, and wheat have been demonstrated to be partially mitigated by the use of certain PGPR in prior studies ¹⁵. Rhizo-adaptation or a rhizosphere effect can be demonstrated by the fact that the population of PGPR grew over time after sowing in the rhizosphere soil of PGPR-treated plants (Table 2). Hiltner, was the first person to describe the rhizosphere effect, which can be defined as the attraction of microorganisms to nutrients that are released from plant roots, leading to an increase in the number and activity of microorganisms in the area surrounding plant roots ¹⁶. However, in addition to providing an environment rich in carbon, plant roots also initiate crosstalk with soil microbes. This causes plant roots to produce signals that are recognized by soil microbes, which then cause soil microbes to send signals that initiate colonization ¹⁷. These bacterial populations will colonize the rhizosphere and interact with one another by a variety of mechanisms, including root exudates and chemotaxis, symbiosis, quorum- sensing, and others ¹⁸. In addition to having an effect on plant growth, PGPR also improves the health of salty soil. In PGPR-treated soil, organic C and water-holding capacity increased, whereas ECE, sodium content, and pH fell. This was in comparison to the controls, which were grown in soil that had not been treated. The production of organic acids with low molecular weight by bacteria capable of phosphate solubilization may contribute to a decrease in pH.¹⁹. The solubilization and mineralization processes can co-exist in certain bacterial strains, according to Tao et al. ²⁰. This contributes to an improvement in soil health by keeping the pH close to neutral. The formation of exopolysaccharides by PGPR strains also assists in binding cations, including sodium, and as a result, it may reduce the amount of sodium that is available for plant absorption and contribute to the alleviation of salt stress ⁷.

CONCLUSION

Based on the findings from this investigation, we can ascertain several key insights. Despite the evident benefits of PGPR inoculants, there remains a significant gap in our comprehensive understanding of their exact mechanisms and modes of action. In environments burdened by salinity, the introduction of PGPR appears to be a promising strategy to enhance soil health and vitality. Particularly noteworthy among the studied rhizobacteria is *Pseudomonas aeruginosa*, which stands out for its robust salt-resistance and potential to substantially augment wheat growth under saline conditions. Moreover, PGPR serve as not only eco-friendly alternatives but also effective

countermeasures against the detrimental impacts of excessive fertilizer usage in agricultural practices. Consequently, they play a pivotal role in ensuring a harmonious balance between optimal crop yield and soil preservation, furthering the cause of sustainable agricultural endeavors.

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Importance of Livestock and Blackleg Disease Spread in Livestock After Flood

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ARTICLE INFO

Article History:

Received: February 25, 2022
Revised: March 20, 2022
Accepted: April 10, 2022
Available Online: May 30, 2022

Keywords:

Flood, impacts, Livestock, infectious disease, Blackleg

ABSTRACT

Background: Livestock plays a pivotal role in the global agricultural system, with farm animals significantly contributing to the socio-economic and nutritional needs of humans. Floods, being one of the predominant natural disasters, impact livestock health, productivity, and grazing, thereby affecting the human population dependent on them. **Objective:** This review was undertaken to determine the effects of blackleg disease on animals in the aftermath of the 2022 flood. **Methods:** A comprehensive examination of the literature was conducted, focusing on the significance of livestock, the types and effects of floods on the livestock industry, and a deep dive into the etiology, symptoms, and impact of blackleg disease. **Results:** Floodwaters can disturb soil habitats, releasing the dormant spores of the bacterium *Clostridium chauvoei*, responsible for blackleg disease. Although the flood may have receded, the aftermath can still be conducive to the spread of blackleg, primarily among cattle, with symptoms ranging from gassy swellings under the skin to sudden death. The disease has potential ramifications for the livestock industry, as it affects young, fast-growing cattle under two years old. **Conclusion:** The 2022 flood has exposed livestock, especially cattle, to heightened risks of blackleg disease. Such disruptions emphasize the need for continuous monitoring, vaccination, and interventions to safeguard livestock health and by extension, human food security and economic stability in livestock-dependent regions.



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INTRODUCTION

There are different causes of animal mortality in the globe, but among them, infectious diseases are the top one. Among the infectious diseases which causes animals mortality is the blackleg disease which spread in animals after flood. The flood of 2022 has caused severity death of animals in Pakistan due to this disease.

OBJECTIVES

The aim of current review of literature was to determine the effects of blackleg disease in animals after the flood of 2022.

Significance of animals

Allah made both living things and non-living things in the globe. Animals are utilized for a variety of things, including food production, companionship, and scientific research. Different products such as wool, hides, skin, and hoofs used to make different products for the benefit of human. Animals can used since the creation of creatures for transport and agriculture purposes by human. According to estimates, livestock provide 30% of the food and agricultural needs of humans in the form of meat, milk, carbs, and proteins. There are numerous varieties of milk. Some are based on the quantity of milk fat in the final product, while others are based on the kind of processing done¹⁻². Whole milk or full-fat milk are other names for fresh milk. Fresh milk is still a low-fat food in spite of this. It is a good calcium source as well. For a number of reasons, even though they are sometimes disregarded, meat, milk, eggs, and other animal products, such as fish and other seafoods are very crucial in establishing food security³.

Importance of farm animals

Farm animals are essential to a sustainable agricultural system, particularly for smallholder farmers, who make up the majority of farmers worldwide⁴⁻⁵. Farm animals also contribute other resources including manure for fertilizers, on-farm electricity, and other byproducts, as well as economic diversity and risk distribution⁶. A significant number of resources, including water and land, have been saved as a result of improved management techniques and genetic selection during the past few decades, and the carbon footprint of animal production has decreased significantly⁷.

Importance of ruminant animals

If managed properly, grazing can also improve the health and biodiversity of grasslands. Ruminants like buffalo, cattle, goats, and sheep efficiently transform the forages from grasslands into high-quality animal products. This is significant because grassland pastures, which house over a billion people and span more than 25% of the Earth's land surface, predominantly consist of marginal or non-arable land⁸⁻¹⁰.

Role of Animals products

Animal products provide a crucial source of high-quality, balanced, and highly bioavailable protein as well as a number of essential micronutrients, such as zinc, iron, vitamins B-12 and A, all of which a huge section of the world's population is deficient¹¹. So, maintaining a nutritionally balanced diet is mostly dependent on the moderate consumption of items derived from animals, particularly in developing nations¹².

Flood

One of the most significant natural disasters that can completely damage the physical and economic environment has nearly always been a flood. Floods may occasionally occur suddenly throughout the world due to physical causes and human activities, or they may occur seasonally in the same region. Flooding is a prevalent disaster in the globe ¹³.

Types of flood

There are different types of flood as reported by many researchers in the globe. Flash flood, coastal flood and river flood are the major one. The livestock losses caused by each type of flood. An unwarranted disaster is a flash flood. A cloud burst may strike one location while leaving another many kilometers away unaffected. Because of this, it is impossible to determine when a river will overflow its banks ¹⁴.

Effect of flood on livestock industry

The most frequent and likely type of natural disaster to strike both wealthy and developing nations is flooding. The populations affected by flooding face serious direct and indirect health hazards ¹⁵. While the flood results in significant losses of human life or suffering, the losses to animals are much greater. Lack of drinking water, fodder, and feedstuffs are only a few of the detrimental repercussions of the flood on the livestock industry that have a significant negative impact on cattle health and output. Flood-related nutritional deficiencies prevent animals from growing, reproducing, and working as efficiently as possible ¹⁶⁻¹⁷. Animal feed security becomes of the utmost importance to livestock farmers at that point. Livestock must have enough of food and clean water during the floods. Crop livestock systems in emerging nations contribute significantly to the livelihoods of millions of people while producing sizable amounts of both crop and livestock food products. These include dealers, market intermediaries, and processors in addition to livestock farmers. Currently, mixed systems generate over 50% of the world's cereals, 75% of the milk, 55% of the lamb, and 65% of the beef in developing countries ¹⁸. Agriculture is typically linked to the rearing of livestock. Animals losses occur in two areas of Pakistan are shown in figure 1 ¹⁹.

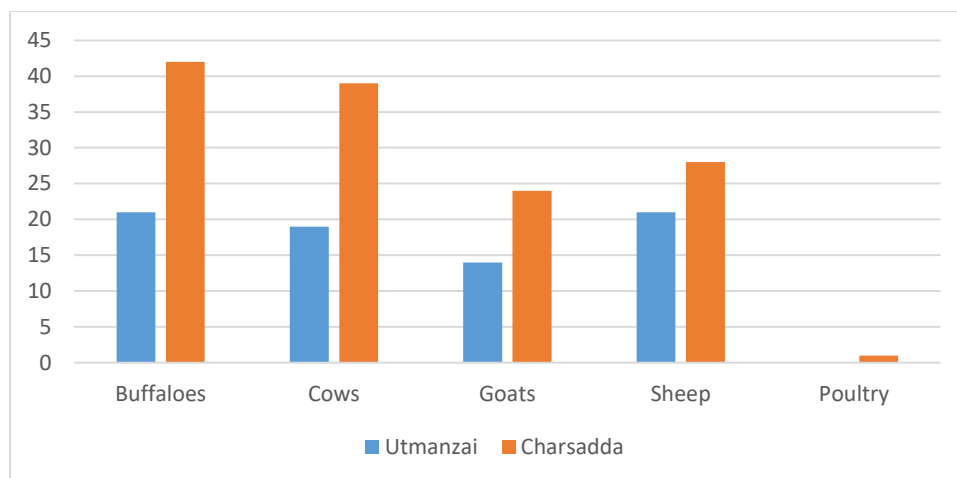


Figure 1. : Livestock losses in Utmanzai and Charsadda Khas, Pakistan

Effects on livestock performances

Flood changes the physiology of livestock, which lowers productivity and production. According to a study published to analyze the effects of climate change on milk production. It has been reported that high-yielding Holstein-Friesian cattle decline the milk production during unfavorable environmental conditions²⁰. The quantity and quality of pasture decreased to such an extent during the dry seasons that livestock might not get enough energy to maintain their body weight. Loss of body weight and a decrease in milk production are the results of this. Therefore, the decreased and likely insufficient nutritional supply restricts the growth rate of broilers and layers, as well as egg output and egg mass²¹⁻²².

Effect on Feed Availability

Hidosa and Guyo, reported both quality and quantity of feed reduce due to flooding. It is anticipated that the flooding will increase the sensitivity to livestock feed²³. When cultivating different varieties of grass in low land areas, the hot and dry seasons have the biggest impact on biomass yield²⁴. Had reported that the lack of food and shelter poses a hazard to the animals that have survived these floods. These animals are stressed and immune-suppressed due to lack of food and shelter, making them more vulnerable to infectious infections.

Effects on Livestock Health

Flooding has had an effect on the animals' performance and health both directly and indirectly. The results demonstrated that the introduction of vector-borne, soil-related, flood-related, and humidity-related factors had a direct impact on animal health. The researchers found that the flood also altered microbial ecosystems, which in turn had an indirect effect on animal health performance²⁵⁻²⁶.

Effect on Pastoral Livelihood

Livestock output and pastoral livelihoods are more closely tied to rangeland productivity. In addition, this rangeland has been negatively impacted by floods in the lowlands, which causes droughts to reoccur frequently and cause animal mortality. It is obvious that livestock mortality has an impact on pastoralists' livelihoods²⁷.

Effects of flood on drinking water of animals

Over the past few decades, increasing urbanisation has largely been to blame for a rise in the frequency of floods and the damage caused by urban flood events. Since faeces are likely to be present in floodwater when urban flooding occurs in regions with combined sewer systems, those who are exposed to pathogens in these waters may be at risk for health problems²⁸. Floods have a significant negative effect on the ecosystem. The present devastating flood and severe rains have had a significant negative impact on the Nowshera district (80%)²⁹. The research area's drinking water sources were impacted by flooding water as shown in figure 2¹⁹.

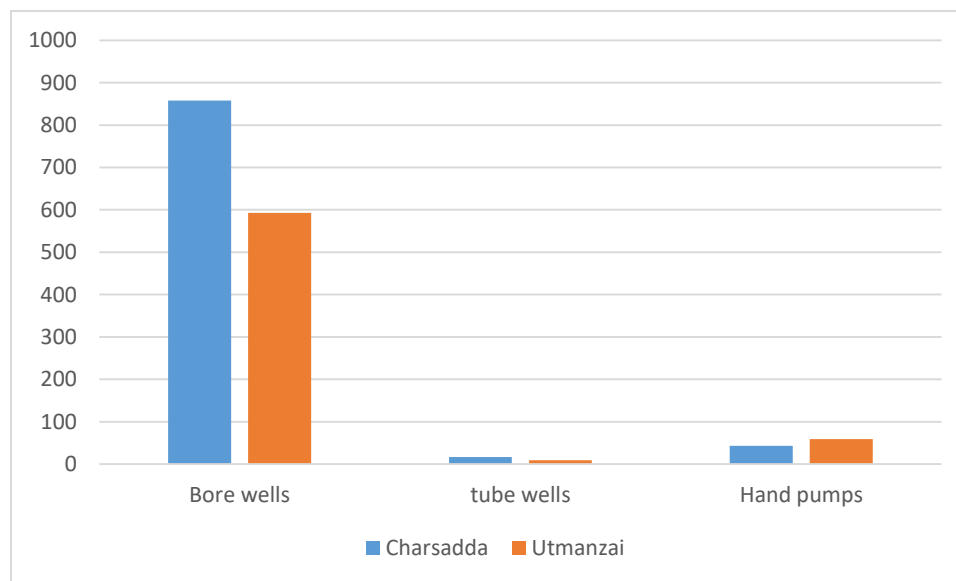


Figure 2. Contaminated Water sources in Utmanzai and Charsadda Khas villages of Pakistan

Effect of flood on livestock grazing

Livestock grazing is a long-standing agricultural practice on terrestrial grasslands all over the world. In addition to having a direct impact on plant productivity, livestock grazing also indirectly affects the soil biota through a number of mechanisms³⁰.

Drivers of disease threats

There are two types of drivers which caused diseases in animals. In terms of their radical and extensive effects on the development of infectious illnesses in animals and plants, two modern mechanisms stand out. The first is climate change, which has a significant impact on how disease organisms are distributed while also making agriculture in some areas more susceptible to drought, salinity, flooding, and other extreme weather occurrences. This phenomenon presents difficulties for border controls, food supply chains, and trade patterns, but it is also a driving force behind the creation of both national and international regulatory systems³¹.

Infectious diseases

In Pakistan, the amount of rainfall fluctuates greatly from year to year. This impacts the upper catchments of the major rivers and depressions coming from the Bay of Bengal, causing unusually high flood peaks and widespread flooding.

Numerous illnesses can have detrimental effects on livestock as they grow during and after damp weather. Flood-affected farmers should examine their livestock for any odd signs of sickness. Some important diseases emerge in animals are given below³².

Blackleg

Blackleg, also known as quarter ill or black quarter, is an acute infectious disease that affects cattle, occasionally affecting sheep and pigs. Young, fast-growing cattle under the age of two are most frequently afflicted by blackleg. Bacterial spores from polluted settings enter the animal through tiny wounds or are swallowed, which is what causes the disease. The signs of this condition might not appear right away and they might take some time to develop. Floods' erosion and movement create ideal circumstances for blackleg spore survival. Fever, severe depression, gassy swelling under the skin or in the muscles even before death, or sudden death frequently accompanied by fast bloating of the corpse are examples of common symptoms³³⁻³⁴.

Etiology

The bacterium *Clostridium chauvoei*, which causes blackleg, is anaerobic, extremely pathogenic, endospore-forming, and gram-positive. It generates endospores with a lemon-like shape and needs enriched conditions to develop.

Table 1. Toxins produced by bacteria, Clostridium chauvoei

Toxins	Cellular effects	Mechanism of action
Flagella	increased pathogen spread	Motility of bacteria
Hyaluronidase	reduction of extracellular matrix	hyaluron cleavage
Neuraminidase	Reduction in rigidity of cell membrane and loss of cellular attachments	Cleavage of sialic acids
DNase	Degradation of DNA	Cleave DNA phosphodiester bonds
Hemolysins	Cytolysis	Pores formation in cell membrane

Abreu *et al.* (2017)

Epidemiology

Occurrence

Between the ages of six months to two years, non-vaccinated cattle are most commonly affected by blackleg, while cases occasionally affect animals older than this. Most animals in good nutritional health and frequently on pasture are affected by the disease. The bacteria can be recycled by faeces contaminating the soil and growing easily in the intestinal tracts of cattle. *C. chauvoei* quickly produces spores when exposed to the environment, and these spores may persist in the soil for a very long time (several years)³⁵.

Source of infection and mode of entry

A soil-borne infection is blackleg. There is considerable debate around the entry point for the organisms into the body. It is assumed that the entrance point occurs through the alimentary mucosa following consumption of tainted feed or in connection with the eruption of teeth. The bacteria can be present in the spleen, liver, and digestive system of healthy animals, and

contaminated feces or the decomposition of animal carcasses can contaminate pastures and soil. When factors like trauma or malnutrition lead spores that are not normally stuck in normal tissues to grow, true blackleg might result ³⁶.

Mode of transmission

In cattle, the disease typically develops without a history of trauma, whereas in sheep, a wound infection almost invariably occurs. Local lesions may develop as a result of infections in skin wounds sustained during docking, shearing, and birth, as well as lesions on the navel ³⁷.

Clinical signs and histopathological findings

Blackleg is often an acute or subacute illness, and many animals pass away rapidly or hardly ever survive for more than 36 hours from the onset of clinical sickness. There may occasionally be chronic conditions. Lameness, anorexia, lethargy, recumbence, and inability to move are the major symptoms of blackleg in the livestock as reported by many researchers in the globe ³⁸. Clinical symptoms may include one or more of the following as shown in figure 1 while histopathological findings are shown in figure 2.

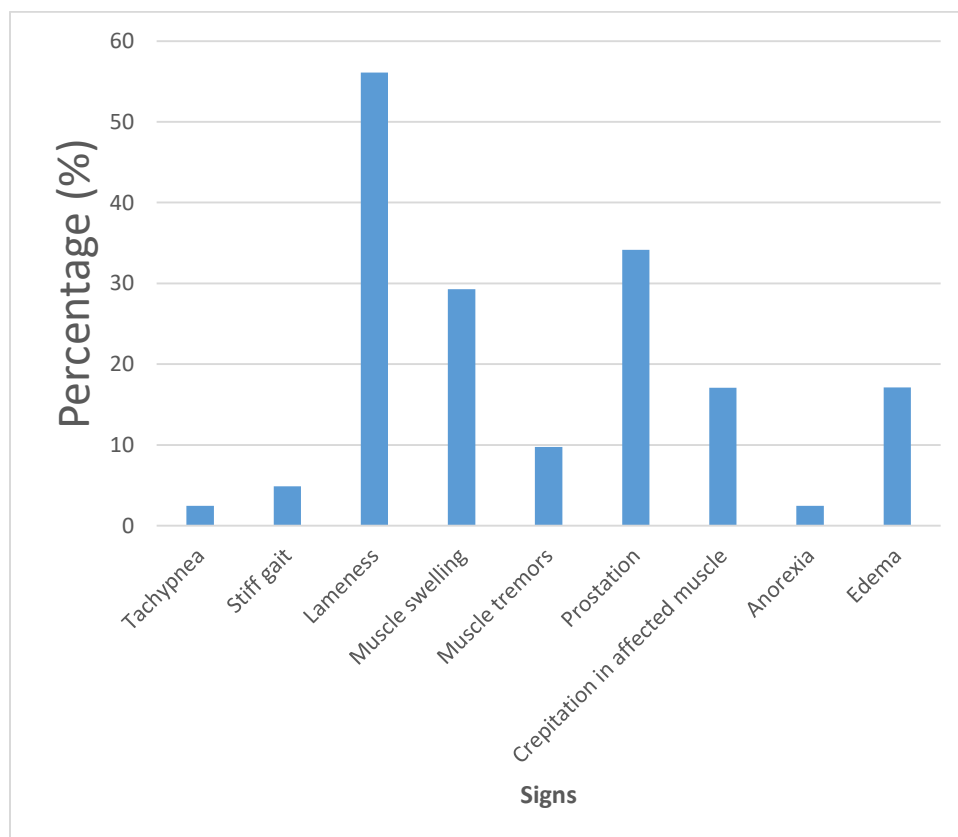


Figure 1. Major clinical signs of blackleg in livestock

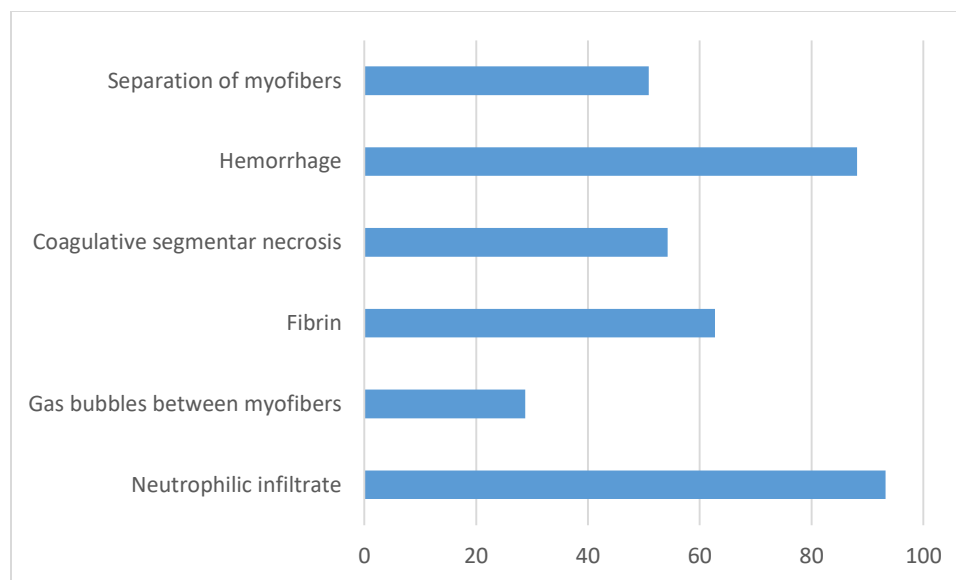


Figure 2. Histopathological findings in the muscle of blackleg infected animals

CONCLUSION

After the flood waters have subsided, cattle can develop blackleg in dry conditions. Floodwaters can reveal disease-causing spores by upsetting the soil. The spores may subsequently be spread into places where cattle graze by the floodwaters. Although blackleg is typically linked to cattle, other ruminants can also contract the disease.

ACKNOWLEDGMENTS

Authors are highly thankful to the concern institute.

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Frequency of Theileriosis in Cows in Tehsil Paharpur, Dera Ismail Khan

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ARTICLE INFO

Article History:

Received: February 25, 2022
Revised: March 20, 2022
Accepted: April 10, 2022
Available Online: May 30, 2022

Keywords:

Epidemiology; Hematology; Pyrexia; Theileria; Ticks

ABSTRACT

Objective: This study aimed to investigate the prevalence of theileriosis in cows within Tehsil Paharpur, District Dera Ismail Khan, Pakistan.

Materials and Methods: A total of 384 blood samples were collected randomly from cows in Tehsil Paharpur over a year (2021-22) and examined microscopically for Theileria parasite detection. The findings were statistically analyzed using chi-square and one-way ANOVA through SPSS version 24. **Results:** The overall prevalence of theileriosis in cattle was 12.23%. Cows exhibited a higher rate (5.62%) compared to goats (3.020%), sheep (0.420%), and buffaloes (0.590%). Female cows revealed a prevalence rate of 12.35% against 10.71% in male counterparts. Seasonally, theileriosis was more prevalent in the summer (19.79%), followed by rainy (14.58%), spring (8.33%), and winter (6.25%) seasons. Age-wise, animals under a year old were most susceptible (16.09%), compared to those aged 1-3 years (11.79%) and above 3 years (9.41%). **Conclusion:** Theileriosis shows a significant prevalence in Tehsil Paharpur, with a higher incidence in young and female cows, especially during the summer season. Molecular research is suggested for a deeper understanding of infection carriers, and proactive measures are needed for its mitigation.



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INTRODUCTION

Pakistan has an estimated population of 207 million people and ranks fifth among the world's most populous nations. Agriculture and livestock are vital to the nation's food, economic, and job sectors. Annually, livestock accounts for 60.07 percent of the agricultural sector and 11.53 percent of the national GDP (Gross Domestic Product). It relates to a 1.505-billion-rupee

increase in the national GDP with a 3.06% annual growth rate throughout the 2020-21 fiscal year. It is crucial to highlight here that 35% of the livelihood of the 10 Million households in Pakistan is closely associated with livestock and their products ¹.

Bovine *Theileria* species are intracellular parasites that infect their hosts with both severe and mild illnesses. Infected buffalo exhibit fever (40.5–41.5 °C), dyspnea, anorexia, lymphadenitis (parotid, prefemoral, prescapular), diarrhea, minor nasal, ocular discharges, pale mucous membrane, congested conjunctiva and hyper-salivation. In acute instances, theileriosis is diagnosed upon clinical manifestations and microscopy of Giemsa stained smears. However, in long-term carriers, blood smears are negative under the microscope and disease can only be diagnosed using most advanced molecular techniques ².

Therefore, this research was done for determining the prevalence of theileriosis in cows in Tehsil Paharpur, District Dera Ismail Khan.

MATERIALS AND METHODS

The current research was executed in Livestock Research and Development Station Paharpur, District Dera Ismail Khan of Khyber Pakhtunkhwa, Pakistan, for determining the frequency of theileriosis in cows. The investigation was conducted over one year (2021-22). For this purpose, 384 random blood samples were taken from cows of varying ages and sexes from various farms in Tehsil Paharpur ³. The specimens were examined microscopically under an oil immersion lens to diagnose *Theileria* parasite ⁴. Also the clinical-epidemiological details of the animals were collected.

The acquired findings were statistically examined using chi-square and one-way ANOVA through SPSS version 24.

RESULTS & DISCUSSION

Overall prevalence of theileriosis in cattle was found to be 12.23%, in Tehsil Paharpur of Dera Ismail Khan, whereby 47 cases were detected with *Theileria* through Giemsa stained thin blood smear techniques (Figure 1). A study reported that cows had the highest prevalence of theileriosis (5.62%), than goats (3.020%), sheep (0.420%) and buffaloes (0.590%). It was significantly higher in goats ($2 = 4.68$, $p < 0.05$) and cows ($2 = 11.76$, $p < 0.05$) ⁵. Our findings were also supported by a study conducted in cow herds, whereby the relative prevalence of *T. annulata* was 12.8% and 23.3% by microscopy and PCR, respectively ⁶.

Most of the affected population comprised females revealing 12.35% (44/356) positive cases with *Theileria*, while a 10.71% prevalence rate was recorded in males (3/28) (Figure 2). Our findings were highly supported by a study in which it was reported that theileriosis was more common among female animals (3.56%) than among males (1.92%). There was a non-significant correlation between theileriosis prevalence and animal gender ($2 = 2.13$, $p > 0.05$) was observed. Female animals had a 1.89-fold greater prevalence (0.79–4.4) than male animals ^{5,7}.

Season-wise frequency of *Theileria* in cows was also recorded and a significantly high population of cows was affected with bovine theileriosis ($p < 0.05$) in the summer season (19.79%), followed by rainy (14.58%), spring (8.33%) and the lowest incidence was recorded in winters (6.25%) (Figure 3).

Age-wise prevalence of theileriosis indicated that animals of age less than a year (16.09%) were more prone to the infection ($p < 0.05$) than 1-3 years cows (11.79%) and above 3 years (9.41%) (Figure 4). Our findings were in agreement with the study stating that Theileriosis was more prevalent in cattle that were young and female animals ⁶. Another study probed the prevalence of Bovine Tropical Theileriosis in cows. In the examined 300 cows, theileriosis was detected in 73 (24.33%) animals. The prevalence was higher among females (27.37%) than males (19.07%). The prevalence was also higher in adults than three years 28.33, followed by 21.33 in one to three years age group and 13.33% in 0-1 years. The Holstein Friesian cross showed the highest prevalence (30.53%) of tropical theileriosis, followed by the Jersey cross (25.33%), the Sahiwal (23.33%), and the Red Sindhi (15.71%) ⁸.

Figure 1: Overall prevalence of Theileria in cows

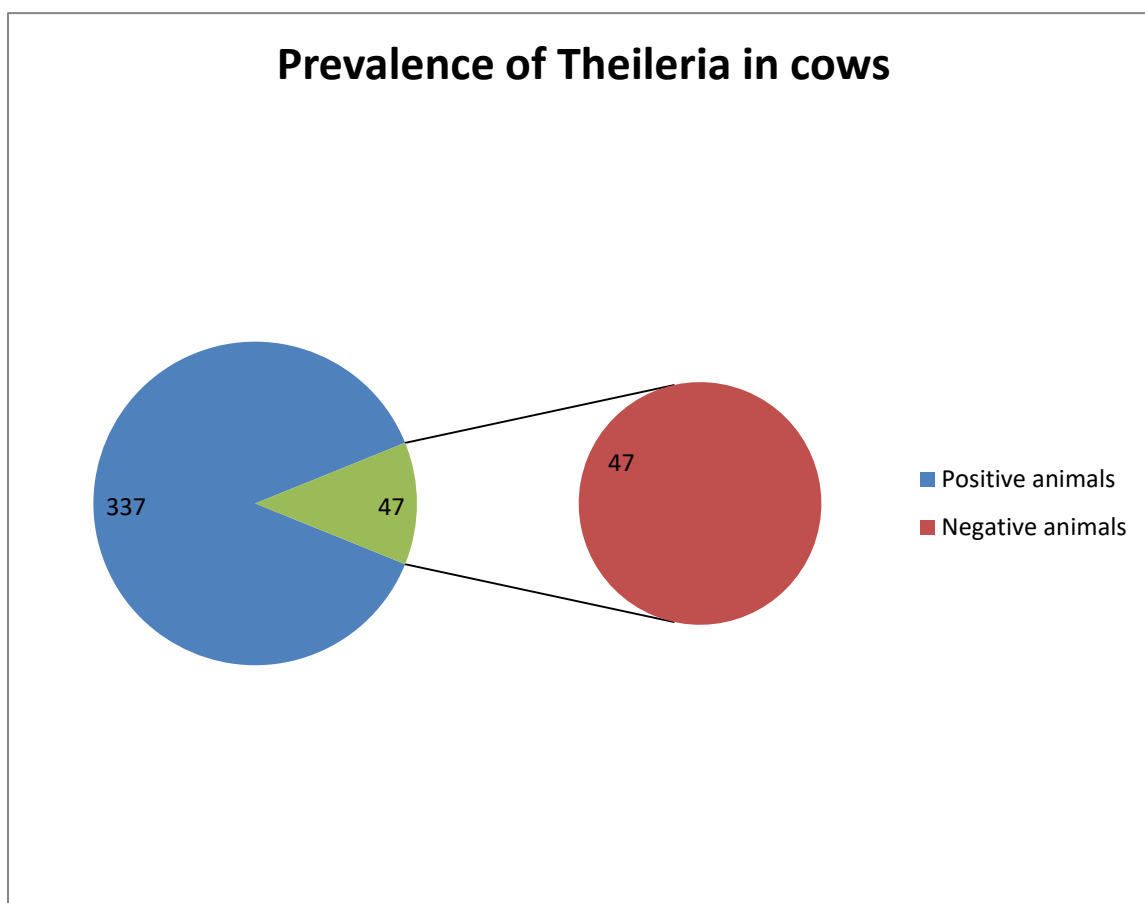


Figure 2: Sex-wise prevalence of Theileria in cows

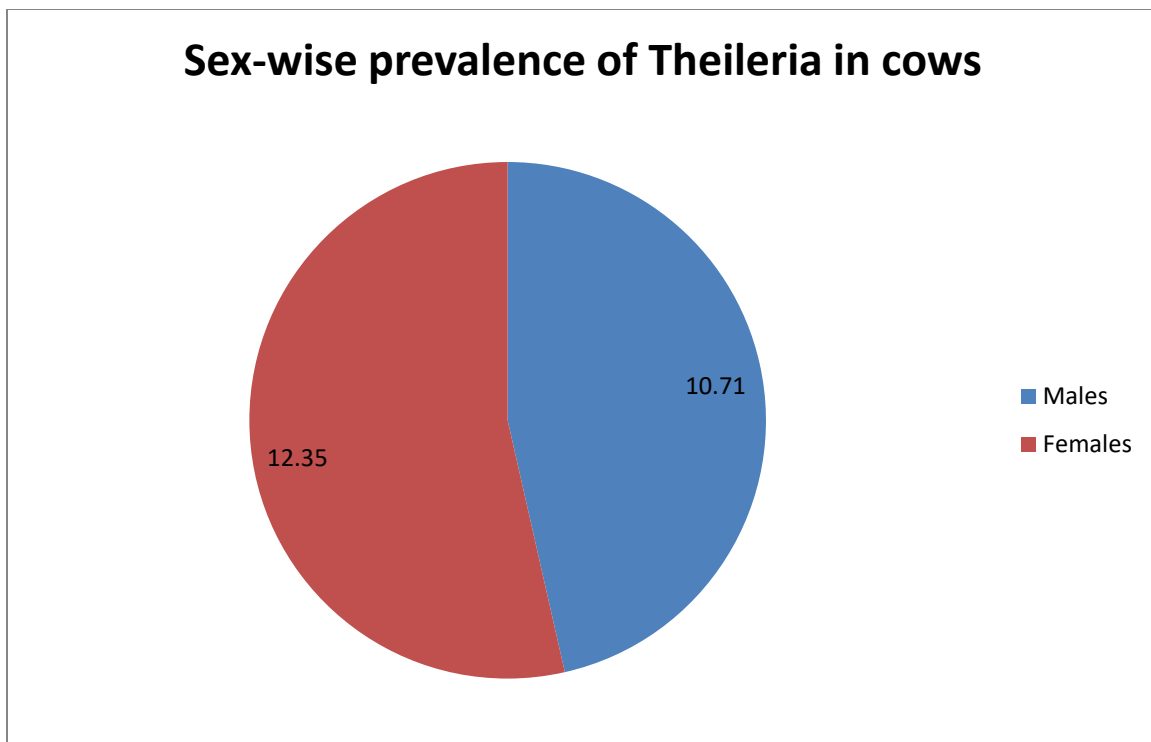


Figure 3: Season-wise prevalence of Theileria in cows

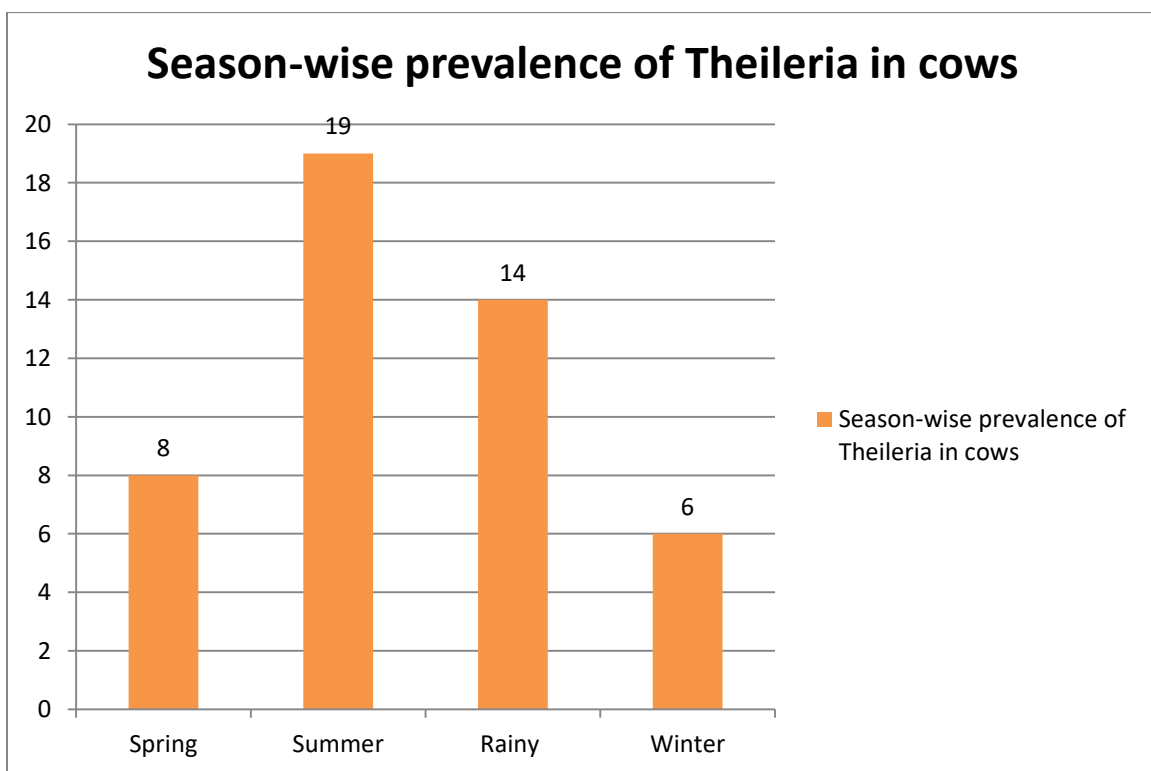
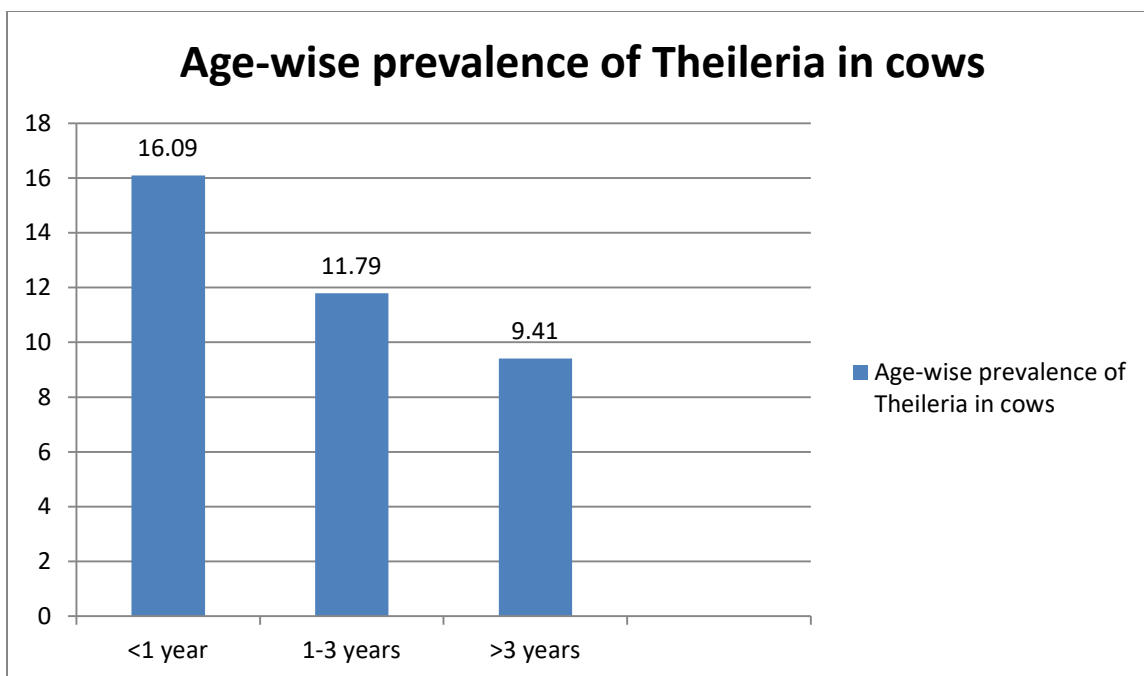


Figure 4: Age-wise prevalence of Theileria in cows



CONCLUSION

In Tehsil Paharpur, Dera Ismail Khan, Pakistan, theileriosis was determined to be quite widespread in cattle. There is a need for additional research using molecular techniques to identify the infection's carriers, and preventative measures must be implemented to eradicate the infection.

CONFLICT OF INTEREST

None.

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Unlocking the Potential of Zinc Efficiency for Enhanced Crop Production in Low Zinc Environments

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ARTICLE INFO

Article History:

Received:	March	25, 2022
Revised:	April	30, 2022
Accepted:	May	25, 2022
Available Online:	June	30, 2022

Keywords:

Zinc (Zn), Nutrient, Zn efficiency, Crop production, Suboptimal Zn

ABSTRACT

Background: An estimated three billion people worldwide suffer from deficiencies in zinc (Zn) and iron (Fe), resulting in the death of 500,000 children every year. As the global shift towards healthier diets continues, the importance of essential mineral nutrients, especially Zn, becomes more pronounced. **Objective:** To provide a comprehensive insight into the critical aspects of Zn efficiency in plants, especially in terms of uptake, transport, and utilization, to address global nutritional needs. **Methods:** Zn plays a vital role in multiple plant processes, including enzyme activation, chlorophyll synthesis, gene expression, signal transduction, and defense. **Results:** Zn deficiency, especially in alkaline soils, is a widespread issue, impacting crop yield and growth. Increasing Zn efficiency in plants involves optimizing Zn uptake, transport, and utilization. Focusing on the cultivation of crops like rice, beans, wheat, soybeans, and maize, which inherently have a robust ability to absorb and utilize Zn, can be instrumental in ensuring sustainable food production. The review delves into the root system's capability in Zn absorption, the contribution of Zn transporters, and the importance of Zn utilization in the shoot system. **Conclusion:** Enhancing Zn efficiency in crops is paramount in addressing global nutritional deficiencies, promoting sustainable food production, and ensuring a brighter future with food security for all.



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INTRODUCTION

Zinc, as a critical micronutrient, plays a crucial role in various vital physiological and biochemical processes in plants, such as those associated with photosynthesis, respiration, and hormone metabolism¹. Despite the significance of zinc, soil deficiency of this micronutrient is a prevalent issue that hampers crop productivity in numerous regions worldwide. Zinc, being an essential micronutrient, plays a critical role in the growth and development of plants, as well as in various physiological and biochemical processes². Despite its crucial role, widespread soil Zn deficiency has a detrimental impact on crop productivity. This meta-analysis review paper seeks to bring attention to the significance of zinc efficiency for the future of crop production in regions characterized by suboptimal zinc conditions³. In this paper, we aim to give a

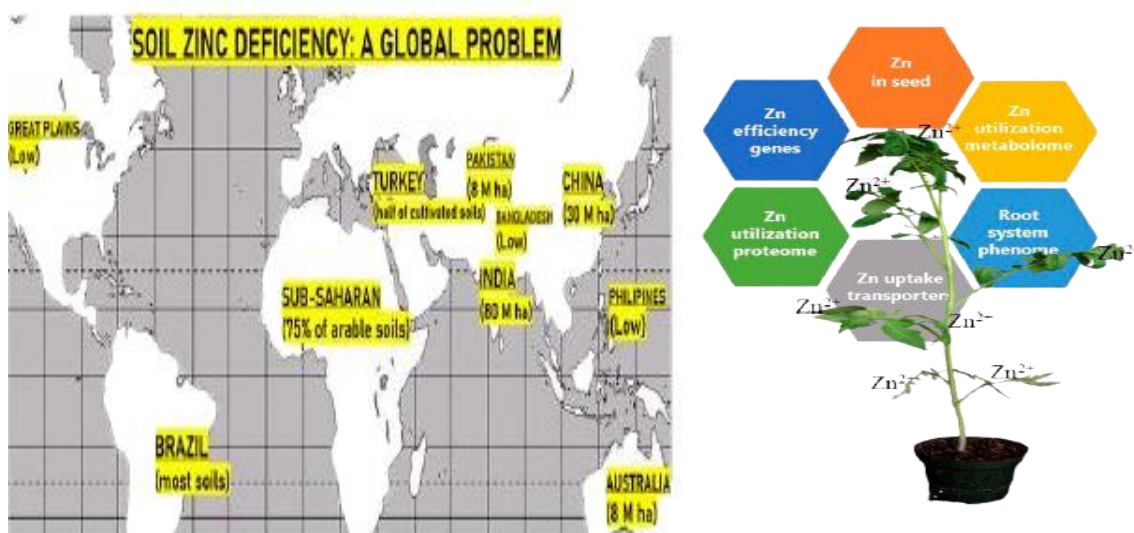


Figure 1. A brief summary of Zinc Deficiency and its impact on plants, including: (a) a map of the world highlighting major regions and countries with soil lacking in Zinc, (b) potential strategies for increasing plant efficiency in obtaining Zinc[4].

overview of the issue of soil Zn deficiency, showcasing the evidence of naturally occurring genetic variation in terms of zinc efficiency in plants⁴. Additionally, we delve into various zinc efficiency strategies implemented in crop plants and examine the underlying mechanisms of zinc efficiency, including the zinc uptake systems and transporters, utilization of zinc within the shoot, and other relevant mechanisms. We conclude by highlighting future challenges and perspectives in the field. The objective of this review paper is to highlight and summarize the latest advancements and research findings related to zinc efficiency and its potential impact on future crop production under suboptimal zinc conditions. Projections estimate that by the year 2050, the world's population will reach a staggering 10 billion, which means that in order to meet the food demands of this growing population, global food crop production must significantly increase and double in quantity⁵. Zinc deficiency is a widespread nutritional disorder that poses a significant threat, particularly in developing countries, with an estimated 17.3% of the world's population at risk of being affected by this condition⁶. Zinc, being an essential mineral, plays a critical role in various plant processes that are vital to the growth, development, and overall

functioning of the plant. These processes include enzyme formation, photosynthesis, protein synthesis, and signal transduction⁷. However, soil Zn deficiency affects over 49% of arable lands globally, negatively impacting plant growth and crop yield⁸. In Zn-deficient countries like Turkey, Australia, Brazil, India, and China, there is a significant focus on conducting research and developing crops that are more efficient in terms of zinc utilization. This is being done with the aim of reducing yield losses and maximizing productivity in these regions⁹⁻¹⁰. Another important aspect of increasing zinc efficiency is improving the zinc content in staple food crops like rice, wheat, maize, and beans. This can be done through techniques such as biofortification, which is crucial for ensuring adequate human nutrition and promoting healthy development. This review focuses on the strategies plants use to cope with low Zn availability and the advancements in Zn efficiency research, including future directions¹¹.

Zinc may be the last element in the list of essential nutrients, but it is by no means any less important. This mineral is crucial for the growth and proper functioning of plants, as well as human nutrition through the consumption of plant-based foods¹². Ongoing research is constantly improving our understanding of the significance of zinc and its impact. This includes exploring the characteristics of crop varieties that are able to grow and prosper in soil conditions that are deficient in zinc¹³.

The exploration of Zn efficiency strategies, cellular mechanisms, and genes holds the potential to advance agricultural sustainability, enhance human nutrition, and reduce the reliance on synthetic fertilizers. A thorough understanding of these aspects can lead to significant advancements and improvements. Improved Zn efficiency in crops can lead to enhanced crop production and better nutritional quality, which will be essential in meeting the needs of an increasing global population¹⁴.

In order to further expand our understanding of Zinc (Zn) efficiency, a number of areas require further research. Future studies should aim to pinpoint the specific genes and processes involved in zinc efficiency in plants, make use of advanced genome editing tools such as CRISPR-Cas9, enhance the measurement of zinc efficiency in food crops, analyze the metabolic changes that occur in response to low zinc conditions through metabolomic profiling, and investigate the genetic factors contributing to zinc efficiency and the accumulation of seed zinc under low zinc conditions through genome-wide association studies. By exploring these avenues of research, we can contribute to the improvement of agricultural sustainability, human nutrition, and the reduction of the need for synthetic fertilizers.

Zinc Deficiency in Soil:

To ensure that crops are able to grow and flourish, they require a range of nutrients, including zinc (Zn). Since 1926, zinc, a type of divalent cation, has been widely recognized as a crucial micronutrient for higher green plants. Nonetheless, various soil types can suffer from zinc deficiency, hindering the growth and output of crops. Such soil types include those with limited zinc availability, soils with high pH and high levels of calcium, those that have undergone extensive farming, sandy soils, and soils with high levels of phosphorus¹⁵. It's estimated that a significant portion of the world's soils, about 50%, have low levels of zinc naturally. This lack of zinc in the soil can lead to deficiencies in crops and impact their growth and productivity. As zinc is an essential micronutrient for plant growth, it's important to study ways to improve zinc efficiency in crops to ensure sustainable food production for a growing population¹⁶. It is well-

known that a large percentage of soils worldwide are affected by Zn deficiency, leading to reduced crop production. However, the exact extent of this issue varies from region to region, with some areas being particularly hard hit. Despite this, a comprehensive understanding of the issue and ongoing research efforts aim to address this challenge and improve agricultural sustainability for farmers and communities around the world ¹⁷. Zinc (Zn) is an indispensable micronutrient that holds a key place in ensuring the well-being and development of crops. Zinc deficiency can arise in several soil types, including those with low zinc availability such as soils with high pH and high levels of calcium, soils that have undergone extensive farming, sandy soils, and soils with high levels of phosphorus. While zinc deficiency is a widespread issue in many developing countries, it is also a concern in developed nations, such as the United States. For instance, zinc deficiency is prevalent in the Great Plains and western regions of the US as well as in sandy soils in Florida. However, the exact extent of Zn-deficiency in these regions is still being studied ¹⁸. To ensure that crops are able to grow and thrive in soil conditions with low levels of zinc (Zn), identifying and cultivating varieties that are efficient in utilizing the mineral is a more favorable approach than solely relying on synthetic fertilizers. This approach helps to minimize any adverse effects on yield and quality that can occur as a result of Zn deficiency in the soil ¹⁹. Screening of crops like wheat, beans, chickpeas, and rice to identify Zn-efficient genotypes is a critical step in managing low-Zn soil stress and reducing yield and quality losses. For this purpose, numerous genotypes of these crops have undergone assessment to determine their Zn efficiency. The process of evaluating crops for zinc efficiency involves a comprehensive analysis that includes analyzing their visual symptoms and assessing the impact on their biomass and yield under conditions of both low and sufficient zinc levels ⁴. The technology of phenotyping, which is the process of evaluating physical and biological traits of an organism, has come a long way in recent times. This advancement has the potential to bring about improvements in the way zinc efficiency is evaluated and predicted ²⁰. The cultivation of Zn-efficient cereal and vegetable cultivars is a crucial step towards a more sustainable agriculture and ensuring sufficient food supply for growing populations. By minimizing the need for synthetic fertilizers and increasing crop yields, Zn-efficient crops play a significant role in meeting the global demand for food ²¹.

Soil Zn insufficiency is a global challenge that has a significant impact on agricultural yields across various regions. It happens when there is not enough Zn in the soil to support healthy growth and development of plants ²². There are a number of factors that can cause soil zinc deficiency, including low soil pH, excessive amounts of phosphorus, and overuse of other micronutrients. These factors can result in insufficient zinc availability for optimal plant growth and development, a problem that is prevalent in many regions around the world ²³. The severity of soil Zn deficiency varies depending on the soil type and management practices ²⁴.

Natural Genetic Variation in Plants for Zinc Efficiency: Evidence

To achieve successful crop production and avoid economic loss, researchers are exploring ways to address soil Zn deficiency which can negatively impact yields. This includes the study of beneficial alleles and the investigation of natural variation in Zn efficiency through association studies ²⁴. The natural variation in Zn efficiency in crops is of great importance for crop breeding and selection, as this variation can impact crop yields. Some crops are known to be Zn efficient, such as alfalfa, carrots, and sunflowers, while others, such as beans, citrus, and lettuce, are considered Zn inefficient. Researchers are utilizing this variation to understand Zn efficiency and

make improvements to crop yields. Crops like barley, potato, and sugar beet display medium Zn efficiency²⁵. The ability to efficiently utilize Zn in soil can result in increased crop yields, even in soils with low Zn availability. Research has identified variations in Zn efficiency among different genotypes of crops, including rice, wheat, beans, maize, and others²⁶⁻²⁷. In recent years, the study of Zn efficiency in various crops such as wheat, beans, and rice has gained significant attention and research efforts have been invested in this field²⁸. Extensive field studies have identified Zn-efficient wheat genotypes in low-Zn soil in several countries²⁹. The common bean and rice are two crops that are particularly important in many regions across the world. While the common bean is known to be sensitive to soil Zn deficiency, research has shown that it is possible to identify Zn-efficient genotypes through screening experiments. Rice, on the other hand, is a staple food crop for over half of the world population and holds a yearly value of USD 3 billion in the United States, making it an economically significant crop as well³⁰. Recent advancements in high-throughput phenotyping systems will provide opportunities for enhanced evaluation and forecast of the Zn efficiency of rice. Despite being grown in soil with low Zn content, rice displays a broad genetic diversity in terms of Zn efficiency³¹. The genotypic variation in Zn efficiency among maize cultivars has been observed in various regions around the world, including Brazil, where maize is a staple food crop and considered to be one of the most important cereal crops globally. The significant variation provides opportunities for crop breeding and selection efforts aimed at improving yields in low-Zn soil environments³⁰.

Studies have increasingly revealed the presence of natural genetic variations in plant's ability to absorb and utilize zinc (Zn). This opens up new avenues for exploiting these differences to enhance crop resilience to low Zn conditions³⁰. Investigations in recent times have brought to light the specific genetic locations responsible for the efficient utilization of Zn in crops like rice, wheat, maize, and soybean. This discovery presents a positive opportunity to enhance crop Zn



Figure 2. A Venn diagram displays the plant species categorized as (a) zinc-deficient and (b) zinc-sufficient, with the overlapping area representing plant species that are only mildly efficient in obtaining zinc [32].

efficiency through innovative breeding techniques and the utilization of genetic engineering.

Strategies for Improving Zinc Efficiency in Crop Plants:

Zinc is a vital element for the growth and development of plants, and different species and varieties have evolved their own methods for acquiring the necessary Zn or making the most efficient use of it³³⁻³⁵. Crops and varieties that are proficient in utilizing Zinc (Zn) are capable of achieving sustainable growth and producing high yields, particularly in alkaline soil conditions. These Zn-efficient crops can play a significant role in resolving Zn deficiency challenges faced by farmers³⁶. Despite the numerous studies conducted on Zn efficiency in food crops like wheat, beans, rice, and chickpeas, a comprehensive comprehension of the underlying mechanisms and natural genetic variations of Zn utilization still eludes us. In order to advance our understanding of zinc efficiency in crops, it is essential to gain a more in-depth understanding of the physiological and genetic factors that determine zinc efficiency³⁷. The efficiency of Zn is a multifaceted characteristic that can be attributed to a combination of various mechanisms operating at different levels. This complexity makes it difficult to determine the precise reasons behind its efficiency³². There are several strategies for improving Zn efficiency in crop plants, including genetic improvement, nutrient management, and agronomic practices. In this section, we discuss the mechanisms of Zn efficiency in crops³⁸.

Candidate Mechanism 1 for Improving Plant Zinc Efficiency: Zinc Uptake Systems and Zinc Transporters:

Plants absorb Zinc (Zn) through various structures like the root epidermis, cortex, endodermis, pericycle, xylem, stem, leaves, phloem, and seeds. Over the last three decades, researchers have been striving to understand the science behind Zn efficiency in plants and to formulate efficient breeding approaches for crops that would optimize Zn utilization³⁹. Although several Zn efficiency mechanisms have been proposed in literature, root uptake studies have provided the most evidence⁴⁰. However, recent studies have shown that root Zn²⁺ influx is not strongly correlated with Zn efficiency, especially in wheat, suggesting that Zn efficiency may be more of a shoot-focused trait than a root-focused one⁴¹. Soil type and pH play a crucial role in Zn availability to crops. Alkaline soils and sandy soils with low total Zn levels are prone to Zn deficiency⁴². Plants produce organic compounds known as Phyto siderophores, which can impact the accessibility of Zinc (Zn) to the plant. The process of Zn uptake by the roots is a complex phenomenon and occurs in two stages, which are characterized by high-affinity and low-affinity transport systems. The absorption of Zn into the roots is facilitated by several types of transporter proteins, including ZIP family, HMA family, MTP family, VIT family, and PCR proteins⁴³. These transporter genes are regulated by transcription factors and may also be influenced by Phyto siderophores. "Conducting additional studies on the transporter proteins responsible for Zinc (Zn) uptake will greatly enhance our understanding of how crops can withstand low Zn soil conditions, which often hinder their growth and productivity⁴⁴. The systems and transporters responsible for Zinc (Zn) uptake in plants play a pivotal role in determining the efficiency of Zn utilization in crops. Current research has pinpointed a number of genes and transporters involved in Zn uptake and transportation in different crop species⁴⁵. These findings provide a promising avenue for improving Zn efficiency through genetic engineering and breeding approaches.

Candidate Mechanism 2 for Improving Plant Zinc Efficiency: Internal Utilization of Zinc in Shoots:

"Zinc (Zn) is a singular metal in that it contributes to the functioning of all classes of enzymes, including lyases, transferases, isomerases, oxidoreductases, and hydrolases. This wide-ranging involvement in enzymatic activities can have a significant effect on the overall efficiency of Zn utilization⁴⁶. It has been reported that Zn deficiency can inhibit carbonic anhydrase in crops, and as such, it is needed for the proper functioning of over 300 enzymes⁴⁷. To maintain crop yields, it is crucial to cultivate crops that are more efficient in their use of Zn⁴⁸. Research suggests that Zn efficiency is related to a shoot-coordinated pathway, and that Zn-efficient crops use more effective internal utilization mechanisms than Zn-inefficient crops⁴⁹. This is because Zn is an essential component of several key enzymes. Studies suggest that the utilization of Zinc (Zn) in the shoots of plants is dependent on Zn-requiring enzymes. Elevated activity of enzymes such as carbonic anhydrase and Cu/Zn superoxide dismutase in Zn-efficient wheat varieties has been linked to improved Zn utilization in these crops⁵⁰. Research conducted on wheat has shown that the physiological utilization of Zinc (Zn) is a key determinant of Zn efficiency. The concentration of Zn in wheat grain has been linked to the activities of two important enzymes, superoxide dismutase and carbonic anhydrase⁵¹. To gain a more comprehensive understanding of Zinc (Zn) efficiency, it is crucial to continue research efforts aimed at discovering new genes associated with internal Zn utilization within the shoots of crop plants and exploring the connections between Zn efficiency and Zn-dependent enzymes in crops.

Internal Zn utilization in the shoot is also an important mechanism for improving Zn efficiency in crops. Studies have identified genetic loci and biochemical pathways involved in Zn utilization in the shoot⁵². These findings provide a promising avenue for improving Zn efficiency through genetic engineering and breeding approaches⁵³.

Additional Mechanisms:

Future research is needed to uncover additional mechanisms contributing to Zn efficiency in crops, such as root architecture or seed Zn levels⁵⁴. The soil conditions and environmental factors of a location can affect the micronutrient levels, like Zn, in seeds⁵⁵. Seed Zn content is important for human nutrition, and QTLs for seed Zn have been identified in crops like wheat, rice, maize, and beans, offering opportunities for breeding Zn-biofortified varieties. Studies have also found QTLs for Zn, Cu, and Cd concentrations in brown rice, as well as major QTLs for Zn efficiency and seed Zn accumulation in wheat and grain Zn and Fe QTLs in rice⁵⁶. In order to enhance global crop tolerance to low-Zn soils, it is important to expand our knowledge of the physiological and molecular genetics aspects of Zn efficiency in plants. A deeper understanding of this will aid in developing better strategies for improving crop growth and yields in low-Zn soil environments⁵⁷.

In addition to Zn uptake and utilization, there are other mechanisms that contribute to Zn efficiency in crops, such as root morphology, metal tolerance, and phytohormones⁵⁸. Further research is needed to fully understand the mechanisms involved in Zn efficiency in crops.

Final Thoughts, Upcoming Challenges, and Prospective Outlook:

The significance of zinc in human nutrition cannot be overstated, as it is a crucial element in ensuring a well-rounded and nutritious diet. Its role is essential for maintaining a healthy balance in our diet and overall well-being. A considerable amount of the zinc intake of humans comes from plants, which are a predominant source of nourishment. Understanding the role that zinc plays in the growth and function of plants is therefore vital to ensuring the continued health and well-being of the global population. The study of Zn-efficient crops that can withstand low Zn soil stress is contributing to our growing understanding of Zn's impact on living organisms. A thorough understanding of the strategies for maximizing zinc efficiency, the inner workings of plant cells, and the genes involved can lead to a more sustainable agriculture, enhanced human nutrition, and a decrease in the reliance on synthetic fertilizers. "Improving the productivity and nutritional value of crops through enhanced zinc efficiency can assist in meeting the food requirements of the rapidly expanding global population. In order to delve deeper into the topic of zinc efficiency, future studies should concentrate on the following aspects: (1) Establishing the genes and processes associated with zinc efficiency in plants; (2) Examining the capabilities of cutting-edge genome editing technology (CRISPR-Cas9); (3) Improving techniques for measuring zinc efficiency in food crops; (4) Examining the metabolic changes that crops undergo in response to low zinc conditions; and (5) Undertaking genome-wide association studies to gain insight into the genetic foundations of zinc efficiency and the buildup of seed zinc in low zinc conditions.

Funding: This study was not funded by any outside sources.

Acknowledgments: Faran Muhammad expresses gratitude towards colleagues studying plant zinc efficiency, as well as Manzoor Ul Haq and members of the Cereal Crops Section lab at the Agricultural Research Institute in Dera Ismail Khan, Pakistan. The author sincerely apologizes to those whose contributions were not included due to space constraints.

Conflicts of Interest: No conflict of interest is declared by the author.

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