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Mini-Review

Anthrax in Pakistan

Shahzad Ali[1*] **and Mohammad Ejaz**[2]

[1] Wildlife Epidemiology and Molecular Microbiology Laboratory (One Health Research Group), Discipline of Zoology, Department of Wildlife & Ecology, University of Veterinary and Animal Sciences, Lahore, Ravi Campus, Pattoki, Pakistan
[2] Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan

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***Corresponding author:**
Shahzad Ali
shahzad.ali@uvas.edu.pk

Abstract

Anthrax is a highly fatal zoonotic disease caused by a Gram-positive and spore-forming bacterium, *Bacillus anthracis*. The epidemiological situation of anthrax is unstable worldwide, and outbreaks have been reported in all the continents and commonly cause high mortality in domestic and wild herbivores as well as several mammals and bird species. The disease is endemic in Pakistan, and outbreaks were reported in domestic animals and wildlife species in limited zones. Sporadic cases also reemerged infrequently in some areas, and anthrax became a public health concern in Pakistan. *Bacillus anthracis* has been isolated from soil samples in different localities of Pakistan, and the risk of the emergence of anthrax is increased after flooding that has occurred recently. Therefore, this review aims to provide an update on the current trends and incidence of anthrax in Pakistan to increase awareness and will be useful for stakeholders and healthcare providers to suspect and manage anthrax.

Keywords: Anthrax, Epidemiology, Zoonosis, *Bacillus anthracis*, Livestock

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Introduction

Anthrax is a bacterial disease primarily infecting herbivores but is also clinically significant in some omnivores, carnivores, and other vertebrates. Anthrax is caused by the Gram-positive, non-motile, aerobic, rod-shaped, spore-forming bacteria called *Bacillus anthracis* (*B. anthracis*). Spores produced by *B. anthracis* are highly resistant to any extreme chemical, temperature, gamma, or ultraviolet radiation and can survive over a longer period in the environment. The spores remain dormant until suitable conditions are met and can spark an outbreak in the region (Raymond et al., 2010). Anthrax is one of the oldest known diseases, and evidence from paleopathology and ancient literature of Egyptians, Greeks, and Hindus described the presence of the disease in that era (Ngetich, 2019). The term "anthrax" is derived from the Greek word "anthracites" which means coal, referred to as a typical symptom of black eschar in cutaneous anthrax (Sternbach, 2003).

In 1850, Casimir Joseph Davaine and Pierre Raver discovered the causative agent of anthrax, followed by the discovery of the complete life cycle of *B. anthracis* in 1876 by Robert Koch (Kamal et al., 2011). Anthrax is also notorious for the "Black Bane" that brushed through Europe in the 16th century, causing >60,000 deaths in cattle and humans (Schwartz, 2009). Similar events occurred in 1978-1980 in which 10,000 Zimbabweans population suffered from anthrax, considered one of the largest zoonotic events in recorded history (Bower et al., 2022). In humans, anthrax is characterized by three main clinical pictures based on the route of infection (Simonsen and Chatterjee, 2023). Cutaneous form occurs when *B. anthracis* spores enter through injured skin, the inhalational form occurs when *B. anthracis* spores are inhaled and ingestion form when humans consumed contaminated food or water (Chambers et al., 2023; Simonsen and Chatterjee, 2023). Cutaneous anthrax accounts for >95% of human cases and is characterized by skin lesions that eventually form black necrotic eschars (Thompson et al., 2022). Inhalation and gastrointestinal anthrax account for <5% of the cases with a mortality rate ranging from 25% to 60% of untreated cases (Sweeney et al., 2011).

B. anthracis mostly affects ruminant animals, in-

cluding goats, sheep, and cattle infected by ingesting water, vegetation, or soil contaminated with spores. Humans are accidental hosts in which transmission of bacteria occurs due to handling infected animals, their carcasses, or animal products (Li et al., 2020). Consumption of dead animals infected with *B. anthracis* can transmit pathogens to wildlife animals and humans (Lehman et al., 2017). Similarly, animals grazing in contaminated soils can potentially ingest or inhale spores of bacteria and consequently may develop the disease (Abrahams, 2002). Anthrax is not highly contagious, and human-to-human transmissions have rarely been documented (Kamal et al., 2011). The incubation period in animals ranges from 1 to 6 days and animals usually suffer from fever, sudden excitement followed by depression, lack of rumination, difficulty in breathing, staggering, convolution, trembling, and death (Beyer and Turnbull, 2009; Kamal et al., 2011), accompanied by the oozing of unclotted dark blood from all orifices (WHO, 2023; Simonsen and Chatterjee, 2023), resulting in contamination of soil and the environment by spores.

The virulence of anthrax lies within two virulence plasmids, pXO1, and pXO2 of *B. anthracis* (Koehler, 2002; Bylaiah et al., 2021). The pXO1 plasmid encodes for protective antigens (PA), edema factor (EF), and lethal factor (LF), which are involved in the formation of anthrax toxin. The pXO2 plasmid encodes for the capsule, which aids in evading bacteria from the host's immune system (Hu et al., 2009). *B. anthracis* is a potential bioweapon agent and is belonging to Category A bioterrorism agents (Gottschalk and Preiser, 2005). Anthrax is infamous for being used in the bioterrorist attack of 2001 in the US in which anthrax spores were sent in letters to US politicians and media personnel, causing 22 infections cases, including five deaths (Jernigan et al., 2001). Anthrax is a global zoonosis with an estimated incidence of 20,000-100,000 human cases in the first half of the 20^{th} century, which decreased to 2,000 cases every year in the second half (WHO, 2023; Yu et al., 2022). A higher incidence of anthrax is reported in tropical countries, including Southeastern and Central Asia, Africa, Central, and South America, the Middle East, and the Caribbean (Parker et al., 2002).

Anthrax is not very common globally, but few incidences are reported regularly from different countries (Dutta et al., 2021; Grace, 2015; Olani et al., 2020). The disease is endemic in developing countries whose economy is mainly agriculture and livestock-dependent (Goel, 2015). Pakistan is an agricultural country with having large livestock population. Animal anthrax is still endemic in Pakistan, and sporadic cases infrequently reemerged in some areas. Therefore, anthrax can be a major public health concern. This review provides an update on current trends in the incidence of anthrax from Pakistan and will be useful for clinicians and healthcare providers to suspect and manage anthrax.

Anthrax in Pakistan

Anthrax has become a neglected disease due to its infrequent sporadic cases in different parts of the world. However, the disease is still endemic in different parts of the world, including Africa, Asia, Europe, and America. Approximately 1.8 billion people live in the anthrax-affected area, and regular reports of sporadic cases and outbreaks were documented from these incubation areas (Carlson et al., 2019). The higher proportion of diseases in humans was of zoonotic origin, and globally 63.8 million livestock keepers live in anthrax-affected regions, primarily in Asia, Africa, and Europe (Sahoo et al., 2020). Globally, the anthrax-risk area has over billion livestock, including 320 million sheep, 268 million cattle, 294 million pigs, 211 million goats, and 0.6 million buffalos (Carlson et al., 2019).

The disease is highly endemic in Southeast Asia, including India, China, and Bangladesh, while sporadic cases were also reported in Pakistan. The official data from the Institute of Epidemiology Disease Control and Research (IEDCR) Bangladesh reported several human and animal anthrax cases in flood-affected areas of Sirajganj, Tangail, Pabna, and Rajshahi (Ferdous et al., 2022). Similarly, Nayak et al. (2019) reported 81 cases of cutaneous anthrax with 3 death in Odisha state of India. According to the 2017 census, the rural population of Pakistan accounts for 62.56% of the total population. Pakistan is an agricultural country, and livestock plays a very important role in its economy. The rural population of Pakistan is highly dependent on agriculture and livestock rearing. Livestock species are always at risk of diverse pathogens. Most of these pathogens are of environmental or wildlife origin and have zoonotic nature. Among these pathogens, *B. anthracis* is of great concern but limited information are available in Pakistan. Anthrax is considered endemic in certain areas of Pakistan and was detected in domestic and wild animals from 2007 to 2021 (WHO, 2023) while the last outbreak was reported in 2017 (APP, 2017).

The endemic areas include FATA, Cholistan, Lahore, Sahiwal, Badin, and Matli, where sporadic cases have been reported in domestic animals and a few studies also reported the contamination of *B. anthracis* spores in soils (Rashid et al., 2020). The spores are highly resistant and can survive for longer periods in soil. The presence of spores in the soil is important in transmitting pathogens to domestic and wildlife animals. *B. anthracis* spores have high floating ability and water can bring the buried spores to the surface, which animals can ingest while grazing (Dragon and Rennie, 1995). The presence of water in the soil can also trigger the vegetative growth of spores. Pakistan suffered from devastating floods recently in different areas including high-risk anthrax areas. The flood water might transport spores to the unaffected area and can potentially result in the emergence of outbreaks (Zasada et al., 2014). The growing soil degradation due to flooding and extreme weather conditions will likely increase animal and human exposure to anthrax in Pakistan.

Table 1: Cases of confirmed *B. anthracis* in humans, animals and soil samples in Pakistan.

Year	Area	Affected species	Suspected cases	Type of samples	Serology	PCR	Isolation of *B. anthracis*	No. of reported death	Reference
2020	Sahiwal Chakwal Sargodha Faisalabad Sheikhupur DG Khan Attock	Cattle Buffaloes Goats Sheep	680	Serum	Cattle [19.2% (n=23)] Buffaloes [36.6% (n=37)] Goats [43.5%(n=124)] Sheep [42.5% (n=74)]	Not performed	Not performed	Not reported	(Rashid et al., 2020)
2018	FATA	Human	13	Blood, pus	Negative	Negative	Negative	0	FATA (2018)
2018	Tharparkar	Goats Sheep	40 40		Not performed	Not performed	30% (12/40) 25% (10/40)	Not Reported	Rajput et al. (2017b)
2018	Tharparkar	-	-	Soil	-	-	40% (32/80)	-	Rajput et al. (2017a)
2018	Punjab	-	-	Soil	-	0.3% (7/1985)	-	-	Rashid et al. (2018)
2017	Badin	-	-	Soil	-	-	20% (20/100)	-	Mari et al. (2017)
2015	Lahore	-	-	Soil	-	9.6% (14/145)	-	-	Shabbir et al. (2015)
2014	Cholistan	Camels	204	Blood	1.8%	Not performed	Not performed	0	Ashraf (2014)
2001	Karachi	Human	1	Blood	Negative	Negative	Negative	0	Ahmad et al. (2004)
1961	Jhang	Human	36	Not reported	Not reported	Not reported	Not reported	3	Khan and Ahmad (1962)

The engagement of the rural population of Pakistan with agriculture and animal-rearing practices and low vaccination coverage increases the risk of anthrax outbreaks.

The situation in humans and animals

Human anthrax has rarely been reported in Pakistan in recent decades, while its last suspected outbreak was reported in 2017 (Figure 1). The first anthrax outbreak in Pakistan was reported in 1961 in the Jhang district of Punjab province, in which cutaneous anthrax was reported in 36 cases with 3 deaths (Khan and Ahmad, 1962). The National Institute of Health, Islamabad, Pakistan received 230 samples collected from packages/letters received by banks in Karachi that were suspected of anthrax spores. Based on the detailed laboratory analysis, these samples were found negative for *B. anthracis* (Ahmad et al., 2004). In the case of humans, federally administered tribal areas and Khyber Pakhtunkhwa reported the most common cutaneous anthrax sporadic cases, while in FATA, thirteen suspected cases of cutaneous anthrax were sent to laboratory testing, which was declared negative(FATA, 2018).

In animals, anthrax is considered one of Pakistan's priority diseases, and results of the National Epidemiological Survey of important livestock diseases showed that anthrax is among the leading cause of death in cattle, goats, and sheep in the desert and hilly areas (Aftab, 2023). In endemic areas of Tharparkar, the incidence of anthrax spores in soil samples was 40%, while in the wool/hair of small ruminants was 28% (Rajput et al., 2017b,a). Whereas, Shabbir et al. (2015) reported a comparatively higher prevalence of *B. anthracis* plasmid gene *Cap*B in 37.9% of samples in the Lahore region (Punjab province) during a study conducted for real-time PCR-based surveillance of soil-borne zoonotic pathogens in Pakistan. Camels of the Cholistan region of Pakistan were also found to be carriers of anthrax based on clinical signs (Ashraf, 2014). In another study, the seroepidemiology of antibodies for the protective antigen of *B. anthracis* in specific districts of Central Punjab, Pakistan, was determined in small and large ruminants using indirect ELISA. Overall, 37.9% of animals were found seropositive, which includes 43.5% goats, 42.5% sheep, 36.6% buffaloes, and 19.2% cattle. The highest prevalence was recorded in Lahore (82.4%), followed by Sahiwal (72.7%), Chakwal (63.6%), Sargodha (54.2%), Faisalabad (15.2%), Sheikhupura (14.1%), and DG Khan (5.5%) (Rashid et al., 2020).

Detected cases in the environment

The ecology of anthrax is highly dependent on climatic and environmental factors (Blackburn et al., 2007). Climatic factors such as average temperature and annual rainfall play a significant role in the incidence of anthrax (Hampson et al., 2011). Similarly, soil factors like high organic content, higher nitrogen level due to the decay of organic matter, alkaline pH, ambient temperature (over 60°F), and moisture serve as an incubator and result in the germination of spores into vegetative bacteria (Parker et al., 2002; Hugh-Jones and Blackburn, 2009). The spores of *B. anthracis* can survive over a longer period in soil when deposited about 15 cm below the upper soil (Goel, 2015). Therefore, the climatic and environmental factors are deciding factors in originating an outbreak in a particular area. Few studies investigated *B. anthracis* spores in soil samples in Pakistan (Table 1). The prevalence of *B. anthracis* was determined in soil samples collected from different locations such as livestock pasturing sites, carcass burial, and disposal sites of district Badin of Sindh province of Pakistan. Moreover, a correlation between soil characteristics and *B. anthracis* incidence was determined. Out of 100 samples, 20% were contaminated with *B. anthracis* in district Badin based on microbiological and biochemical analysis. Soil samples in Tando Bago, Golarchi, and Talhar were more likely contaminated (25%), while 15% and 10% samples of Badin and Matli were positive. Among these, 70%, 18.18%, and 10.86% prevalences were recorded in clay loam type soil, silty clay loam, and silty loam type soil, respectively.

The prevalence of *B. anthracis* was positively correlated with the organic matter and calcium content of soil (Mari et al., 2017). The prevalence of *B. anthracis* DNA plasmid (PA and *Cap*B) was determined using RT-PCR assays in different districts of Punjab province, Pakistan. Out of 1985 soil samples, three were positive based on the *Cap*B gene of plasmid DNA, while four were positive based on the PA gene of plasmid DNA. Soil samples were positive for *B. anthracis* from Chakwal and Attock districts, while samples collected from Gujranwala, Faisalabad, Sargodha, Sahiwal, and Dera Ghazi Khan Districts were negative. The prevalence of *B. anthracis* was higher in regions with high human and animal traffic and slaughterhouses and animal markets (Rashid et al., 2018).

Conclusion and recommendations

Anthrax is an endemic disease in Pakistan, and sporadic cases were infrequently reported from different areas of Pakistan. The distribution of anthrax spores in soils of different areas of Pakistan is alarming and can potentially result in the emergence of outbreaks. The decontamination of soil is impractical, so much of the focus is needed on an enhanced surveillance system to detect the presence of spores or vegetative bacteria in soils of different areas. Prevention of anthrax is largely dependent on the vaccine, so effective vaccination programs in the endemic area need to be employed to eradicate the disease. The accidental spread from animals to humans may be reduced by burying the carcasses to prevent the opening from scavengers which can spread the spores in the soil. Spatial and temporal models of anthrax distribution can ameliorate identifying and predicting high-risk regions for anthrax. The framework for controlling and preventing anthrax-like enhancing surveillance, diagnosis, and outbreak response, is developed by the Centers for Disease Control and Prevention (CDC) using the One-Health approach positively effects to prevent anthrax in both animals and humans in anthrax-endemic countries.

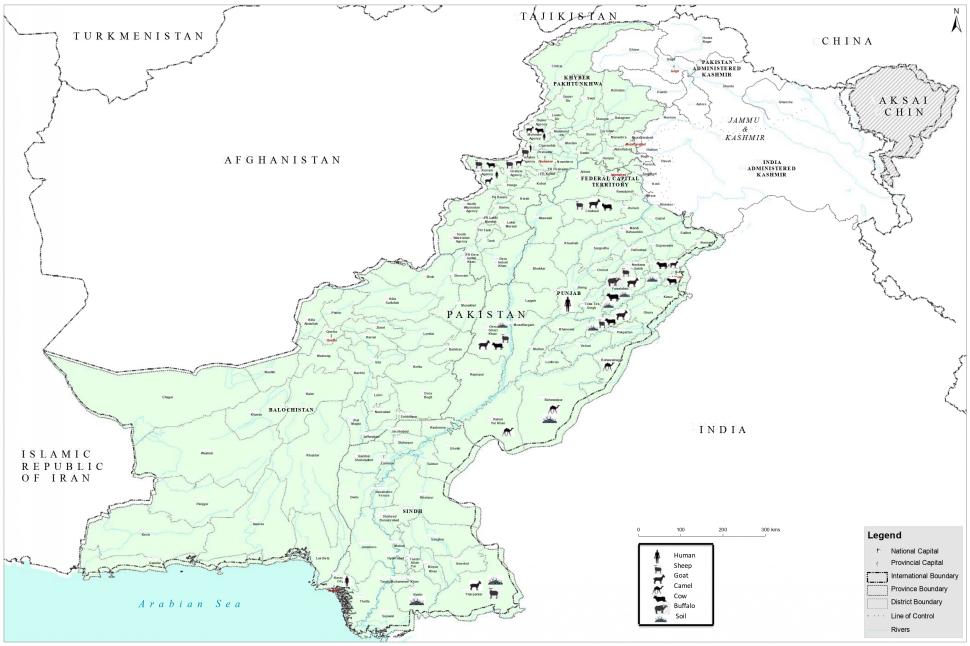

Figure 1: Map of endemic areas of animal and human anthrax cases in different regions of Pakistan

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