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# SURVEILLANCE OF TUBERCULOSIS AND HYDATID CYSTS IN SLAUGHTERED ANIMALS: IMPLICATIONS FOR PUBLIC HEALTH INTERVENTIONS

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**Abstract**

*The objective of this investigation was to ascertain the prevalence and distribution of hydatid cysts and tuberculosis in slaughtered animals, as well as to identify the risk factors that are associated with these conditions in the districts of Dera Ismail Khan and Lakki Marwat, Khyber Pakhtunkhwa. An antemortem and postmortem examination of 139 cattle and buffaloes was conducted in 2023-24 as part of a cross-sectional observational survey from September 2023 to June 2024. Tuberculosis and hydatid cysts were identified through gross examination and further diagnosed using tissue samples through laboratory testing, which included the Ziehl-Neelsen staining method. Results showed that the animals examined did not contain any positive cases of tuberculosis or hydatid cysts, indicating no positive case detected at the slaughterhouse in sampled population. The investigation evaluated a variety of risk factors, such as the diagnostic capacity, human-animal interface, animal health history, biosecurity measures, and environmental conditions. It is important to note that 43.17% of the animals were exposed to high occupational levels, while 56.83% were exposed to low levels. The study underscored the necessity of rigorous biosecurity measures and continuous monitoring to protect public health, despite the absence of disease. These results emphasized the necessity of continuous vigilance to prevent potential outbreaks and underline the efficacy of the current control measures in the surveyed areas. The study provided valuable data for the development of strategies to maintain the health of both humans and animals and for future public health interventions.*

## INTRODUCTION

Tuberculosis and hydatid cysts are two significant zoonotic diseases that pose substantial threats to public health worldwide [1]. Tuberculosis is a chronic infectious disease primarily affecting the respiratory system but can also involve other organs. It is responsible for a considerable burden of illness and mortality globally, affecting millions of people each year. In addition to its impact on human health, tuberculosis also affects animals, including cows and buffaloes, and can lead to significant economic losses in the livestock industry [2-3].

Hydatid cysts, caused by the larval stage of the tapeworm *Echinococcus granulosus*, predominantly affect the liver and lungs of humans and animals. This parasitic infection poses a major public health concern due to its potential to cause severe and life-threatening complications, such as cyst rupture and the formation of secondary hydatid cysts in various organs. The life cycle of Echinococcus involves definitive hosts, usually dogs, and intermediate hosts, such as cows and buffaloes. Humans can become infected by ingesting eggs shed in the feces of infected definitive hosts, commonly through the consumption of contaminated food, including raw or undercooked meat [4-5].

The transmission of tuberculosis and hydatid cysts through the consumption of contaminated meat from slaughtered animals underscores the importance of surveillance and control measures in slaughterhouses [6]. Slaughtered animals serve as a potential source of these zoonotic infections, as the presence of active tuberculosis lesions or hydatid cysts in organs can contaminate the meat during the butchering process. Consumption of infected meat without proper cooking can lead to the transmission of these diseases to humans, posing significant risks to public health [7].

Effective surveillance of tuberculosis and hydatid cysts in slaughtered animals is crucial for early detection, prevention, and control of these diseases. Regular monitoring and screening of animals during post-mortem examinations allow for the identification of infected individuals and contaminated meat. By implementing robust surveillance systems in slaughterhouses, public health authorities can gather essential data on the prevalence, distribution, and risk factors associated with tuberculosis and hydatid cysts in the animal population [8-9].

The findings from such surveillance efforts provide valuable insights into the epidemiology of these diseases, helping to inform public health interventions and control strategies [10]. By identifying high-risk areas, vulnerable populations, and specific risk factors contributing to the transmission of tuberculosis and hydatid cysts, targeted interventions can be developed. These interventions may include improved animal husbandry practices, enhanced biosecurity measures, public health awareness campaigns, and the implementation of appropriate diagnostic and treatment protocols [11].

Slaughterhouses serve as critical junctions in the food production chain, where animals are processed for human consumption. They are potential sources of infection, as animals with tuberculosis or hydatid cysts may go undetected, leading to the dissemination of pathogens to the human population through the consumption of infected meat. Hence, thorough monitoring and assessment of the prevalence, distribution, and risk factors associated with these diseases in slaughtered animals are essential for safeguarding public health [12-13].

Risk factor assessment involved the collection of data on animal breed, age, geographical location, management practices, and environmental conditions through structured questionnaires and interviews with slaughterhouse staff. These data was analyzed to identify potential associations between these risk factors and the occurrence of tuberculosis and hydatid cysts in slaughtered animals [14-17].

Ultimately, this research aims to generate crucial data on the prevalence, distribution, and risk factors associated with tuberculosis and hydatid cysts in slaughtered animals. The findings provided a foundation for evidence-based public health strategies, including improved meat inspection protocols, hygiene practices, and educational campaigns, to effectively control and prevent the transmission of these diseases, ensuring the safety of meat products and safeguarding public health.

## MATERIALS AND METHODS

### Study Design

This cross-sectional observational survey was conducted in the slaughterhouses of District Dera Ismail Khan and Lakki Marwat (Figure 1), Khyber Pakhtunkhwa in 2023-24, from September 2023 to June 2024, as part of the Veterinary Research and Disease Investigation Center, Dera Ismail Khan's and Lakki Marwat Annual Technical Research Program (ATRP), to determine the prevalence of tuberculosis and hydatid cysts in slaughtered animals, and identify their associated risk factors.

### Sample Selection

The study included the ante mortem and postmortem examination of 139 cattle including cows and buffaloes slaughtered in the abattoirs of the study area (Figure 2). Sample size was reached by using the following equation [18]:

$n = 1.96^2 \text{ Pexp } (1 - \text{Pexp}) / d^2$

$n = 1.96^2 \times 0.10 \times (1-0.10) / (0.05)^2$

$n = 139$

### Inclusion and Exclusion Criteria

The study included slaughtered cows and buffaloes as the target population. Inclusion criteria specified that the animals intended for human consumption to ensure that the study focused on animals that pose potential public health implications.

Conversely, several exclusion criteria have been established to ensure the study's specificity and data quality. Animals of species other than cows and buffaloes, such as goats, sheep, was excluded. The study solely concentrated on cows and buffaloes due to their significant relevance in the food industry and potential disease transmission to humans.

### Data Collection

During examination of the animals at the abattoir, relevant data was recorded for each slaughtered animal. This included information on age, sex, breed and any visible signs or symptoms of tuberculosis or hydatid cysts observed during the examinations.

### Laboratory Testing

Post-mortem examinations was performed on each selected animal to detect and diagnose tuberculosis and hydatid cysts. Tissue samples, including lungs, liver and other relevant organs, was collected during the post-mortem examinations of the suspected animals. Tissue samples was examined macroscopically for the presence of cysts and suspected tissues were brought to the laboratory section for further examination microscopically using Ziehl Neelsen stain.

### Procedure of Ziehl-Neelsen Stain

Preparation of Smear: A smear was prepared from lungs material after centrifugation to concentrate the bacteria or impression smear was employed.

Heat Fixation: The slide containing the smear was air-dried and heat-fixed to ensure the bacterial cells adhere to the slide and remain intact during staining.

Carbol Fuchsin Staining: The smear was flooded with Carbol Fuchsin stain, and the slide was heated over a spirit lamp for 3-5 minutes. Care was taken to prevent the stain from drying out by adding more stain if needed.

Rinse with Tap Water: After heating, the slide was allowed to cool, and then rinsed with tap water for 30 seconds to remove excess stain and reduce background staining.

Decolorization: Acid alcohol was added drop by drop to the slide until the smear becomes light pink. This decolorization step is crucial in distinguishing acid-fast organisms from non-acid-fast organisms.

Rinse with Tap Water: The slide was rinsed again with tap water for 5 seconds to remove the acid alcohol and prevent carryover of the decolorizing agent.

Counter Staining: The slide was counter stained with methylene blue for 2 minutes. This step helps in visualizing the non-acid-fast organisms and background.

Rinse with Tap Water: After the counter staining, the slide was rinsed with tap water for 30 seconds to remove excess stain.

Air Drying and Examination: The slide was allowed to air dry, and then examined under an oil immersion lens using a microscope.

The interpretation of the results was based on the staining pattern observed. Acid-fast organisms, such as *Mycobacterium bovis*, retains the red stain, while other microorganisms and background elements appear as blue or brown.

**Assessment of Risk Factors**

The assessment of risk factors in the study on the surveillance of tuberculosis and hydatid cysts in slaughtered animals involved the identification and analysis of various factors that may contribute to the prevalence and transmission of these diseases. These risk factors were evaluated to understand their association with the occurrence of tuberculosis and hydatid cysts in the slaughtered animal population.

Environmental Conditions: The study explored the role of environmental factors in disease transmission. This may include evaluating the impact of geographical location, climate, and proximity to wildlife reservoirs on the prevalence of tuberculosis and hydatid cysts in slaughtered animals. Areas with a high prevalence of these diseases in wildlife populations may pose an increased risk to domestic animals.

Biosecurity Measures: The study evaluated the effectiveness of biosecurity measures implemented in slaughterhouses, and transportation systems to prevent the introduction and spread of tuberculosis and hydatid cysts. This included the examining the presence of appropriate control measures, such as quarantine procedures, disinfection protocols and health certification of animals.

Animal Health History: The study considered the animal health history, including previous diagnosis and treatment records, to assess the association between prior health conditions and the risk of tuberculosis and hydatid cysts. Animals with a history of tuberculosis or hydatid cysts may be at a higher risk of reinfection or serving as a source of transmission.

Human-Animal Interface: The study explored the interaction between humans and animals, particularly those involved in the handling and slaughter of animals. Factors such as occupational exposure, hygiene practices and personal protective measures was assessed to determine their impact on disease transmission.

Diagnostic and Surveillance Capacity: The study assessed the diagnostic and surveillance capacity for tuberculosis and hydatid cysts in animals. The availability and accessibility of accurate diagnostic tools, laboratory facilities, and trained personnel was evaluated as potential risk factors for underdiagnoses or delayed detection of these diseases.

**Data Analysis**

Descriptive statistics was used to calculate the prevalence of tuberculosis and hydatid cysts in the sampled population. The prevalence rates was expressed as percentages with 95% confidence intervals. ANOVA tests was employed to assess the association between the presence of tuberculosis or hydatid cysts and different variables, such as animal species, age, and sex. Statistical significance was set at $p < 0.05$.

**Figure 1: Government owned Slaughterhouse of District Dera Ismail Khan near Indus River**

**Figure 2: Postmortem examination of the slaughtered animals and sample collection**

## RESULTS

The findings of our investigation offered a thorough examination of the prevalence and distribution of hydatid cysts and tuberculosis in slaughtered animals in the districts of Dera Ismail Khan and Lakki Marwat. We assessed the prevalence of these diseases and identified potential risk factors associated with their transmission through meticulous data collection and analysis. We provided a comprehensive account of the results of our sample distribution, laboratory testing and risk factor assessment, emphasized the health status of the sampled animals and potential implications for public health interventions.

Combining direct observations, structured interviews and examination of past data, information about biosecurity policies, wildlife population and previous health state of slaughtered animals was gathered. Structured interviews with slaughterhouse staff and cross-verified using on-site observational checklists helped to evaluate biosecurity policies. Slaughterhouse archives and veterinarian reports yielded past medical records and diagnostic histories to guarantee data accuracy and reduce interpretation uncertainty.

In our study, the distribution of the sampled animals by location and species indicated that a total of 139 animals were examined. Of these, 71.94% (100 animals) were from Dera Ismail Khan and 28.06% (39 animals) were from Lakki Marwat. The preponderance of the samples from both locations were cattle, with 92 cattle (66.19%) from Dera Ismail Khan and 36 cattle (25.90%) from Lakki Marwat. The sample contained a lesser number of buffaloes, with 8 buffaloes (5.76%) from Dera Ismail Khan and 3 buffaloes (2.16%) from Lakki Marwat. The data suggested that cattle comprised the majority of the sample population (Table 1). The prevalence of hydatid cysts and tuberculosis among the sampled animals was investigated and it is important to note that the sampled animals from both Dera Ismail Khan and Lakki Marwat did not exhibit any positive cases of hydatid cysts or tuberculosis. This yielded a prevalence rate of 0.00% for both diseases in the entire study population brought at the slaughterhouses (Table 2).

The sampled animals were categorized by species, sex and age group. 63 males and 34 females between the ages of 2.5 and 5 years were examined from the cattle population, while 65 males and 29 females were older than 5 years. 4 males and 2 females were in the 2.5 - 5 year age cohort for buffaloes, while 5 males were over 5 years old. The animals did not include any female buffaloes that were older than five years. The preponderance of the animals sampled were between the ages of 2.5 and 5, which is indicative of the typical age range of animals that are presented for slaughter (Table 3).

Several critical aspects are underscored by the distribution of risk factors among the sampled animals. According to the environmental conditions, 35.97% of the animals were sourced from regions with a high prevalence of fauna, while 64.03% were sourced from regions with a low prevalence. In terms of biosecurity protocols, 64.75% of the animals were obtained from facilities that followed strict protocols, while 35.25% were obtained from facilities that followed lackadaisical protocols. It is important to note that none of the animals had a history of hydatid cysts or tuberculosis, which indicated a clear health history. 43.17% of the animals experienced high occupational exposure, while 56.83% experienced low occupational exposure. Finally, the diagnostic capacity was high for 71.94% of the animals and low for 28.06% (Table 4).

Most of the examined animals (55.40%) were between the ages of 2.5 and 5 years, while 44.60% were older than 5 years. The sample was virtually

balanced in terms of sex, with 48.20% males and 51.80% females. It is crucial to note that none of the animals exhibited any visible symptoms of tuberculosis or hydatid cysts, which indicated that all of the animals examined and samples were in good health (Table 5).

The laboratory testing results, corroborated that none of the 139 tissue samples collected from the lungs, liver and other organs contained any positive cases of tuberculosis or hydatid cysts. The absence of these diseases in the sampled population was emphasized by the consistent result across all examined organs (Table 6).

The data analysis suggested that the examined and sampled animals had 0.00% prevalence of both tuberculosis and hydatid cysts. No significant associations (P = 1.00) were observed between the presence of disease and a variety of variables, such as species, age, sex, location, environmental conditions, biosecurity measures, animal health history, human-animal interface and diagnostic and surveillance capacity, according to the ANOVA results. These results indicated that, within the study parameters, none of these factors were associated with the presence of hydatid cysts or tuberculosis in the sampled animal population brought to the government owned slaughterhouses (Table 7).

**Table 1: Distribution of Sampled Animals by Location and Species**

| Location | Species | Number of Samples | Percentage (%) |
|---|---|---|---|
| Dera Ismail Khan | Cattle | 92 | 66.19 |
| | Buffaloes | 8 | 5.76 |
| **Total Dera Ismail Khan** | - | **100** | **71.94** |
| Lakki Marwat | Cattle | 36 | 25.90 |
| | Buffaloes | 3 | 2.16 |
| **Total Lakki Marwat** | - | **39** | **28.06** |
| **Overall Total** | - | **139** | **100.00** |

**Table 2: Prevalence of Tuberculosis and Hydatid Cysts**

| Location | Disease | Number of Positive Cases | Percentage (%) |
|---|---|---|---|
| Dera Ismail Khan | Tuberculosis | 0 | 0.00 |
| | Hydatid Cysts | 0 | 0.00 |
| Lakki Marwat | Tuberculosis | 0 | 0.00 |
| | Hydatid Cysts | 0 | 0.00 |
| **Total** | Tuberculosis | 0 | 0.00 |
| | Hydatid Cysts | 0 | 0.00 |

**Table 3: Distribution of Sampled Animals by Species, Sex and Age Group**

| Species | Sex | Age Group | Dera Ismail Khan | Lakki Marwat | Total |
|---|---|---|---|---|---|
| Cattle | Male | 2.5 - 5 years | 45 | 18 | 63 |
| | | > 5 years | 47 | 18 | 65 |
| Cattle | Female | 2.5 - 5 years | 25 | 9 | 34 |
| | | > 5 years | 20 | 9 | 29 |
| Buffaloes | Male | 2.5 - 5 years | 3 | 1 | 4 |
| | | > 5 years | 3 | 2 | 5 |
| Buffaloes | Female | 2.5 - 5 years | 2 | 0 | 2 |
| | | > 5 years | 0 | 0 | 0 |
| **Total** | **Total** | **Total** | **100** | **39** | **139** |

**Table 4: Distribution of Risk Factors among Sampled Animals**

| Risk Factor | Criteria | Number of Animals | Percentage (%) |
|---|---|---|---|
| Environmental Conditions | High wildlife prevalence areas | 50 | 35.97 |
| | Low wildlife prevalence areas | 89 | 64.03 |
| Biosecurity Measures | Strict measures | 90 | 64.75 |
| | Lax measures | 49 | 35.25 |
| Animal Health History | Previous diagnosis of TB or hydatid cysts | 0 | 0.0 |
| | No previous diagnosis | 139 | 100.0 |
| Human-Animal Interface | High occupational exposure | 60 | 43.17 |
| | Low occupational exposure | 79 | 56.83 |
| Diagnostic and Surveillance | High diagnostic capacity | 100 | 71.94 |
| | Low diagnostic capacity | 39 | 28.06 |

**Table 5: Data Collection: Characteristics of Sampled Animals**

| Characteristic | Criteria | Number of Animals | Percentage (%) |
|---|---|---|---|
| Age | 2.5 - 5 years | 77 | 55.40 |
| | > 5 years | 62 | 44.60 |
| Sex | Male | 67 | 48.20 |
| | Female | 72 | 51.80 |
| Visible signs of TB or Hydatid Cysts | Yes | 0 | 0.00 |
| | No | 139 | 100.00 |

**Table 6: Laboratory Testing Results**

| Sample Type | Organ | Number of Samples | Number of Positive Cases | Percentage of Positive Cases (%) |
|---|---|---|---|---|
| Tissue Sample | Lungs | 139 | 0 | 0.00 |
| | Liver | 139 | 0 | 0.00 |
| | Other Organs | 139 | 0 | 0.00 |

**Table 7: Summary of Data Analysis Results**

| Analysis Method | Variable | Result |
|---|---|---|
| Descriptive Statistics | Prevalence of TB | 0.00% |
| | Prevalence of Hydatid Cysts | 0.00% |
| ANOVA | Species | No significant association (P = 1.00) |
| | Age | No significant association (P = 1.00) |
| | Sex | No significant association (P = 1.00) |
| | Location | No significant association (P = 1.00) |
| | Environmental Conditions | No significant association (P = 1.00) |
| | Biosecurity Measures | No significant association (P = 1.00) |
| | Animal Health History | No significant association (P = 1.00) |
| | Human-Animal Interface | No significant association (P = 1.00) |
| | Diagnostic and Surveillance | No significant association (P = 1.00) |

## DISCUSSION

This study sought to discover the frequency and distribution of hydatid cysts and tuberculosis in slaughtered animals from the districts of Dera Ismail Khan and Lakki Marwat, Khyber Pakhtunkhwa. Of the 139 cattle and buffaloes we looked at, our results

revealed a 0.00% prevalence of both tuberculosis and hydatid cysts. These findings implied that the slaughterhouses in the area have efficient disease control policies, biosecurity procedures and surveillance systems in place to stop the zoonotic disease occurrence.

Various studies have found variable rates of hydatid cysts and TB in cattle populations around various areas. A research done in Punjab, Pakistan, revealed a 2.77% overall prevalence of cystic echinococcosis (CE) in livestock [19]. With most of cysts identified in liver (63.49%), followed by lungs (23.80%), the study also underlined that older animals were more likely to be affected [20]. On the other hand, our study did not find any incidences of hydatid cysts, which implies that better meat inspection procedures and efficient preventive actions could have helped to maintain the disease-free condition of the investigated animals in our area. Emphasizing the requirement of ongoing surveillance, by Vaisi-Raygani et al. (2021) on the frequency of hydatid cysts in killed livestock in Iran revealed a noteworthy infection rate [21]. Our study's lack of detected cases does not necessarily indicate absolute disease absence in the animals at the slaughterhouses.

Unlike our results, other worldwide investigations have found somewhat high bovine tuberculosis (bTB) prevalence rates. 5.21% of calves examined in slaughterhouses showed tuberculous lesions, according to a study carried out in Sicily, Italy; the prevalence was declining with time [22]. This study emphasized the need of ongoing surveillance and implies that in some areas tuberculosis is still a problem even with the drop. A recent Sylhet, Bangladesh, study also revealed shockingly low bovine tuberculosis (0.19%), with just 1 out of 512 cattle tested positive [23]. Although this prevalence is minimal, and is coinciding with our results. Our findings imply that the present capacity for diagnosis and surveillance in our research area is sufficient to stop the spread of tuberculosis in buffalo and cattle. Maintaining the disease-free status, though, depends on constant observation.

Although our study turned up no positive cases, a risk factor analysis was carried out to evaluate possible elements affecting illness spread. According to our results, 35.97% of the animals came from high-wildlife-prevalence regions, which could provide a disease risk. Previous research has indicated that cattle near wildlife reservoirs are more likely to contract hydatid cyst infections and tuberculosis [19]. Comparatively to regulated farm conditions, a study conducted in Ethiopia on cattle raised in high-risk locations for wildlife contact revealed a greater frequency of tuberculosis infections [24]. Although our results show no cases, ongoing observation is advised—especially for cattle imported from regions with wildlife presence. Furthermore, while 35.25% of the animals under sampling originated from facilities with insufficient biosecurity policies, 64.75% came from those with tight ones. Studies conducted in Kenya found that poor biosecurity practices were significantly correlated with greater bovine tuberculosis and hydatid cyst rates [25].

Occupational exposure and human-animal contact are still another important determinant of disease frequency. 43.17% of the animals in the study were handled in high-occupational-exposure settings, therefore raising the zoonotic disease risk. Previous research has underlined how abattoir employees could spread zoonotic diseases. Due of their exposure to animal blood and tissues, *Toxoplasma gondii* infections were more common among slaughterhouse employees, according to a study conducted in Myanmar [26]. Our findings imply that disease prevention techniques were adequate to avoid infections even with work exposure. Furthermore, 71.94% of the animals under sample had strong diagnostic capacity—a prerequisite for early identification and prevention. Studies on underreporting of zoonotic diseases have revealed, on the other hand, that inadequate diagnosis techniques and lack of qualified people support this. Our research emphasizes the need of keeping strict diagnostic criteria in order to guarantee control of diseases.

Strong proof from our study shows that present biosecurity and monitoring policies in Dera Ismail Khan and Lakki Marwat help to control tuberculosis and hydatid cysts in killed animals. Although the zero-prevalence rate is a good sign, constant alertism is required to maintain this condition. Comparisons of national and international studies point to areas with less rigorous biosecurity policies typically reporting greater prevalence rates. Regular surveillance, rigorous meat inspection policies and

improved biosecurity measures have to be regularly followed if one wants to keep the disease-free status. Policymakers and public health authorities in devising evidence-based policies for stopping zoonotic disease spread through meat consumption can benefit much from the results of this study.

## CONCLUSION

This investigation into the surveillance of hydatid cysts and tuberculosis in slaughtered livestock in Dera Ismail Khan and Lakki Marwat did not identify any positive cases of these diseases. Our results emphasized the necessity of rigorous biosecurity protocols and continuous surveillance in slaughterhouses to guarantee public health safety. The sampled population's absence of these maladies suggested that the current control measures were effective. However, it is imperative to maintain a high level of vigilance and conduct regular health assessments in order to prevent potential outbreaks and guarantee the well-being of both humans and animals.

### Conflict of Interest

None.

### Authors' Contribution

AK conceptualized the study and prepared the original draft. AK, QU, WU, UA and AA, conducted the investigation and collected the data. IK, IH, NUS and MMS helped in data analysis and proof reading of the manuscript. AZ and MA helped in the extensive revisions of the manuscript.

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