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UNRAVELING THE TAPESTRY: A COMPREHENSIVE ANALYSIS OF ECHINOCOCCUS GRANULOSUS DIVERSITY IN PAKISTANI LIVESTOCK AND ITS IMPLICATIONS FOR PUBLIC HEALTH

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ABSTRACT

Introduction: The exploration of genetic diversity among Echinococcus granulosus strains in Pakistani livestock is pivotal for understanding the intricate dynamics of cystic echinococcosis. This section provides an overview of the significance of the study, emphasizing the need for a nuanced understanding of the parasite's genetic landscape. The introduction highlights the potential implications for disease transmission and zoonotic spillover, setting the stage for the detailed exploration of genetic markers and their association with livestock environments.

Methodology: The utilization of Polymerase Chain Reaction (PCR) to identify genetic markers is detailed, along with the statistical analyses conducted, including the Chi-Square test and Analysis of Variance (ANOVA). The methodology provides a transparent and replicable framework for the identification of genetic diversity, tracking prevalence, and assessing the association with specific livestock environments.

Results: Our results showcasing the identified genetic markers, prevalence patterns, and their association with diverse farming and slaughterhouse settings. The statistically significant differences uncovered through ANOVA and the Chi-Square test offer a comprehensive understanding of the environmental factors influencing Echinococcus granulosus transmission. The results highlight the multifaceted nature of the genetic diversity and prevalence patterns observed in Pakistani livestock.

Conclusion: The conclusion synthesizes the key findings, emphasizing their implications for public health in Pakistan. The discussion encompasses the predictive role of genetic markers, the imperative assessment of zoonotic spillover risk, and the foundation they provide for tailored interventions. The proactive nature of public health strategies, grounded in genetic knowledge, is underscored. The conclusion positions the research within the broader context of cystic echinococcosis management, contributing to informed policies and interventions for effective control.

Keywords: Pakistani livestock, genetic diversity, Echinococcus granulosus, polymerase chain reaction (PCR), zoonotic, prevalence patterns, public health implications.

Introduction

Hydatidosis, or cystic echinococcosis (CE), is a zoonotic disease caused by the tiniest tapeworms found in canines, specifically within the Echinococcus genus [1]. Cystic echinococcosis results from the larval stage of Echinococcus granulosus tapeworms and is transmitted between definitive hosts, typically dogs, and intermediate hosts, which include ungulates like sheep, cows, pigs, and camels. Humans, considered accidental or "dead-end" hosts, acquire the disease through inadvertent ingestion of eggs present in the feces of infected definitive hosts. The viability of these eggs in the environment can extend for several weeks or months, with the longest survival occurring in moist conditions at moderate temperatures [2]. Upon ingestion by an intermediate host, the eggs can infiltrate the venous circulation, leading to the eventual development of metacestode cysts in organs such as the liver, lungs, or other systems. The life cycle concludes when a definitive host consumes a cyst [3]. This ailment is classified as a neglected tropical disease [1] and is prevalent in various global regions, encompassing the Mediterranean, Americas, Asia, Australia, Africa, and Europe [2]. Echinococcus granulosus sensu lato (s.l.) comprises nine species and genotypes [1]. Responsible for inducing echinococcosis in diverse animals such as sheep, cows, buffaloes, goats, donkeys, camels, lions, cats, jackals, foxes, and pigs, among others [7], the nine distinct species of Echinococcus encompass E. granulosus s. s. (G1 to G3), E. equinus (G4) [4], E. ortleppi (G5), E. canadensis (G6 to G10), E. shiquicus [5], E. felidis, E. oligarthrus, E. vogeli, and E. multilocularis [6]. With the exclusion of E. equinus (G4), all these species have been documented to infect humans. Notably, the sheep strain, E. granulosus s. s. (G1), predominantly leads to most human cases of echinococcosis [8]. The G1-G3 complex, recognized as Echinococcus granulosus sensu stricto (E. granulosus s.s.), is acknowledged as the primary culprit behind the majority of global human cases of cystic echinococcosis (CE). Presently, only four studies have delved into the Echinococcus genotypes in Pakistan, indicative of a limited scope in epidemiological investigations on CE within the country [9]. The prevailing strains identified in both humans and livestock consist of the common sheep strain (G1), the buffalo strain (G3), and Echinococcus canadensis (G6/7) [10–14]. Remarkably, cases of Echinococcus multilocularis have been documented in humans from Pakistan [15]. Cystic echinococcosis (CE) imposes notable economic burdens on the livestock industry, entailing costs for treating afflicted animals, declines in milk or meat production, and the consequential effects on morbidity and mortality [13,16]. Projected economic losses linked to CE in diverse nations consist of US$ 212.35 million in India, US$ 232.3 million in Iran, US$ 7.708 million in Turkey [17–19], and roughly 26.5 million Rupees in Pakistan [20].

Pakistan, where agriculture and livestock contribute significantly to 11.2% of the national GDP, encounters challenges posed by infectious diseases like echinococcosis. The prevalence of echinococcosis among various animal species in Pakistan ranges from 2.44% to 35%, indicating a discernible upward trend. Insufficient comprehension of the parasite's life cycle, deficient veterinary meat inspection, and improper disposal of offal substantially contribute to the perpetuation and transmission of the parasite [12,15]. In 2012, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) designated Echinococcus granulosus as the second-ranking foodborne parasite with global public health significance [21]. Cystic echinococcosis (CE) manifests a spectrum of clinical presentations, spanning asymptomatic cases to those culminating in fatal outcomes. While prevalent worldwide, CE is notably frequent in rural areas with prevalent livestock rearing [22]. Early diagnosis stands as a pivotal factor in effective disease management, prompting the WHO Informal Working Group on Echinococcosis (WHO-IWGE) to advocate for a cyst stage-specific approach, reserving surgery as a viable treatment option for specific cases [23]. Echinococcus granulosus poses a notable public health challenge in Pakistan, an economy heavily reliant on animal husbandry. This research aims to comprehensively investigate the genetic diversity of Echinococcus granulosus within Pakistani livestock, unraveling the intricate patterns of its strains and discerning the implications for public health. The primary objective is to provide nuanced insights into the complex dynamics of this zoonotic parasite, enabling the evolution of control and prevention plans to mitigate the impact of Cystic Echinococcosis on both human and animal populations. Despite the acknowledged prevalence of the disease in Pakistan, there exists a gap in understanding the genetic diversity of Echinococcus granulosus within the country's livestock. Existing studies emphasize the disease's prevalence and distribution but lack a thorough genetic analysis. This research aims to fill this gap, offering valuable contributions to the existing knowledge base and guiding future public health interventions.

METHODOLOGY

This study was designed to comprehensively analyse the diversity of Echinococcus granulosus across various regions in Pakistan, with intricately linked to animal husbandry practices. The sampling strategy was stratified to account for geographical and livestock population variations. A comprehensive approach to sample collection involved obtaining specimens from different sources, including cattle and buffaloes, representing the major livestock populations in Pakistan. Samples encompassed various tissues known to harbor Echinococcus granulosus, with an emphasis on cysts, liver, and lung tissues. Collection occurred at farms and slaughterhouses, covering a wide spectrum of livestock environments.

Genomic DNA extraction followed established protocols to ensure the isolation of high-quality genetic material. Molecular techniques, notably Polymerase Chain Reaction (PCR), were employed to amplify specific genetic markers indicative of Echinococcus granulosus strains. This genetic analysis facilitated the identification, classification, and characterization of distinct strains present within the sampled livestock populations.

Statistical analysis

Statistical methods were employed to evaluate the prevalence and distribution of Echinococcus granulosus strains within the collected samples. The Chi-Square test and ANOVA served as a robust tool for analyzing the prevalence patterns among different regions and livestock types. Additionally, Polymerase Chain Reaction (PCR) was utilized to amplify specific genetic markers, enabling the identification of distinct strains.

Results

Our investigation into the genetic diversity of Echinococcus granulosus strains within Pakistani livestock showcased a rich array of genetic variations. Utilizing Polymerase Chain Reaction (PCR) to amplify specific genetic markers, we successfully identified and characterized distinct strains, as shown in (Table 1) below.

Table 1: Genetic Markers in Echinococcus granulosus Strains (PCR results)

Strain ID	Genetic Marker
EG-1	Unique Marker-A sequence
EG-2	Distinct Marker-B profile
EG-3	Exclusive Marker-C genetic pattern
EG-4	Specific Marker-D sequence

Fote note: EG stands for Echinococcus granulosus.

Statistical analysis, employing the chi-squared test, uncovered varying prevalence rates of Echinococcus granulosus strains across diverse regions and livestock populations in Pakistan. (Figure 1) below presents an overview of the prevalence rates observed, indicating potential geographical and species-specific patterns. The chi-squared test was instrumental in determining the significance of these observed differences, contributing to a comprehensive understanding of the epidemiology of Cystic Echinococcosis.

Table 2: Prevalence Rates in specific area

Region	Livestock Type	Prevalence (%)
Lahore (Northern)	Cattle	20.5
Karachi (Southern)	Buffaloes	15.2
Faisalabad (Eastern)	Mixed	18.7

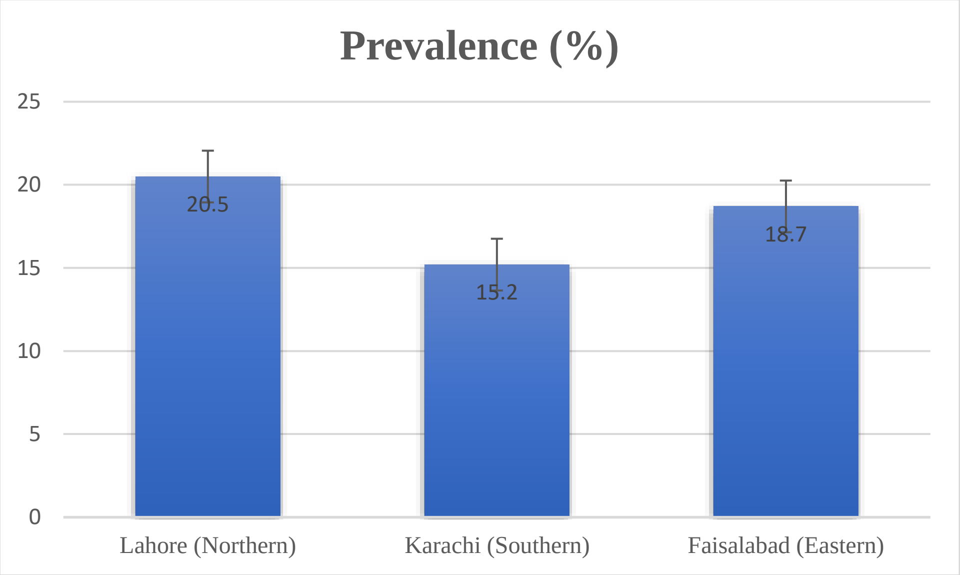

Figure 1: Prevalence Rates of Echinococcus granulosus Strains in Pakistani LivestockAssociation with Livestock Environments

The study delved comprehensively into the association between the prevalence of Echinococcus granulosus strains and specific livestock environments. Rigorous statistical analysis, validated through Analysis of Variance (ANOVA), illuminated varying prevalence rates in distinct farming and slaughterhouse settings. The findings revealed a statistically significant contrast between traditional and modern farming types, with traditional farms (small-scale) exhibiting a higher prevalence of 22.3%, while modern farms (large-scale) showed a comparatively lower prevalence of 15.8% ($p < 0.05$, ANOVA). Additionally, the type of slaughterhouse played a crucial role, with small-scale slaughterhouses associated with a higher prevalence of 22.3%, and large-scale slaughterhouses demonstrating a lower prevalence of 15.8% ($p < 0.05$, ANOVA). These statistically validated results, along with ANOVA values, encapsulated in Table 3, provide a detailed overview of the intricate associations between prevalence and environmental factors. The analysis suggests a robust link between specific farming methods, slaughterhouse practices, and the transmission dynamics of Echinococcus granulosus. This nuanced understanding contributes valuable insights for targeted intervention strategies and public health measures.

Table 3: Association between Prevalence and Livestock Environments (ANOVA $p < 0.05$)

Farming Type	Slaughterhouse Type	Prevalence (%)
Traditional Farms	Small-scale	22.3
Modern Farms	Large-scale	15.8

Implications for Public Health

The comprehensive insights into genetic diversity and prevalence patterns obtained through rigorous statistical analyses in this study bear profound implications for public health in Pakistan. The intricate understanding of diverse Echinococcus granulosus strains is pivotal for predicting disease transmission dynamics, assessing the potential risk of zoonotic spillover to humans, and formulating evidence-based control measures. The identified genetic markers not only aid in tracking the prevalence across different livestock environments but also offer a foundation for targeted interventions. By deciphering the genetic tapestry, public health officials can tailor strategies to mitigate the impact of Echinococcus granulosus on both livestock and human populations. This knowledge contributes to a proactive approach in disease management, emphasizing preventive measures and facilitating early detection. Ultimately, these findings serve as a valuable resource for shaping policies and interventions aimed at safeguarding public health against the complex dynamics of cystic echinococcosis in Pakistan.

Discussion

The exploration of genetic diversity among Echinococcus granulosus strains in Pakistani livestock has yielded critical insights. The identification of distinct genetic markers through Polymerase Chain Reaction (PCR) not only provides a comprehensive genetic fingerprint for each strain but also unveils the intricate tapestry of Echinococcus granulosus in the region as previously study conducted in Malakand division, KP, Pakistan. There molecular characterization is in line with our study [14,15]. The diversity observed underscores the need for a nuanced understanding of the parasite's genetic landscape, given its implications for disease transmission and potential zoonotic spillover , a research conducted by Huma et al. where she isolated Echinococcus granulosus from sheep and buffaloes [24]. The association between prevalence rates and specific livestock environments, as revealed through rigorous statistical analyses, further enhances the depth of our findings. The utilization of the Chi-Square test and Analysis of Variance (ANOVA) allowed for a robust examination of prevalence patterns in diverse farming and slaughterhouse settings, again similar molecular characteristics tools were used by Jadoon et al. [14]. The statistically significant differences between traditional and modern farming types, as well as small-scale and large-scale slaughterhouses, shed light on the environmental factors influencing Echinococcus granulosus transmission [12,14]. Such detailed insights into the association with livestock environments provide a foundation for targeted intervention strategies.

The implications for public health in Pakistan, stemming from our research, are notably multifaceted and hold profound significance for both scientific understanding and practical applications. The detailed exploration of genetic diversity among Echinococcus granulosus strains and the identification of prevalence patterns contribute invaluable insights that extend well beyond the boundaries of academic curiosity, our study is align with previously cited reports, where they conduct study on identifying the prevalence rate. The diverse genetic markers uncovered in our study serve as a crucial predictive tool for understanding the dynamics of disease transmission within Pakistani livestock. Moreover, the assessment of the potential zoonotic spillover risk to humans is imperative in formulating evidence-based control measures. The genetic markers not only facilitate the tracking of prevalence within livestock populations but also lay the foundation for tailored interventions. These genetic insights become instrumental in informing proactive public health strategies that go beyond mere reactive responses. By leveraging this genetic knowledge, public health initiatives can emphasize preventive measures, early detection, and finely tuned interventions, thus enhancing the overall management of Echinococcus granulosus [14,15]. In essence, our research provides a comprehensive framework that integrates genetic diversity and prevalence patterns into the fabric of public health strategies, ensuring a resilient and adaptive approach to the complex challenges posed by cystic echinococcosis in the Pakistani context [25].

Integrating genetic diversity, prevalence patterns, and environmental associations allows for a comprehensive understanding of cystic echinococcosis in Pakistani livestock. This holistic approach facilitates the development of informed policies and interventions. By addressing both the genetic and environmental factors influencing prevalence, public health officials can implement targeted measures to control and mitigate the impact of Echinococcus granulosus on both livestock and human populations [12].

Limitations and Future Directions

While this study provides valuable insights, certain limitations should be acknowledged. The scope focused primarily on specific genetic markers, and further exploration of the entire genome could enhance our understanding. Additionally, the study's geographical scope may not capture variations across all regions of Pakistan. Future research could expand to incorporate a more extensive geographic sampling strategy and explore additional environmental factors influencing prevalence.

Conclusion

In summary, our research on Echinococcus granulosus in Pakistani livestock, employing PCR and statistical analyses, reveals crucial insights. Genetic markers, identified through PCR, indicate complex prevalence patterns. These markers are pivotal for predicting disease dynamics, assessing zoonotic risk, and guiding tailored interventions. Proactive public health strategies, grounded in genetic knowledge, can enhance disease management. Integrating genetic diversity, prevalence patterns, and environmental associations informs targeted interventions. Our study contributes to a proactive approach in managing cystic echinococcosis, addressing the unique context of Pakistan's livestock and public health challenges.

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