Distribution and Implications of Mycobacterium *tuberculosis* Genotypes in Animals: A Comprehensive Review

Abstract

This narrative review discusses the spread of *Mycobacterium tuberculosis* among animal populations, focusing on *M. tuberculosis* genotype distribution and frequency across different species. To develop comprehensive tuberculosis control strategies under the One Health approach, it is important to have an understanding of the genotypes of *M. tuberculosis* in animals. Animal infections are discussed, as well as the pathogenesis and epidemiology of *M. tuberculosis*. Various genotyping methods, including spoligotyping, MIRU-VNTR, and whole genome sequencing, are discussed in the review, emphasizing their role in understanding strain diversity and transmission dynamics. There are many factors influencing the distribution of *M. tuberculosis* genotypes in animals, including environmental conditions, host factors, human-animal interactions, and animal trade practices. It is clear from the review that the Euro-American lineage is widely distributed across animal species, with the Beijing genotype becoming increasingly prevalent in regions where it is prevalent in humans. The study highlights the need for more comprehensive genotyping studies as well as the development of better diagnostic tools. This will enhance our understanding of *M. tuberculosis* in animal populations. As a conclusion to the review, it emphasizes that standardizing genotyping protocols, utilizing whole genome sequencing, and fostering international collaboration are essential for improving our understanding of *M. tuberculosis* diversity in animals and developing targeted tuberculosis control strategies.

Keywords: *Mycobacteriu tuberculosis*; Animal tuberculosis; Genotyping methods; Zoonotic transmission; One Health approach

Commented [u1]: The abstract content miss standard formats such as introduction, methods you used to review articles, major findings conclusions, and recommendations based on the review result

Commented [u2]: Please consider appropriate standard of key word selection

Introduction

In animal populations, *Mycobacterium tuberculosis* (*M. tuberculosis*) has emerged as a significant threat, challenging our understanding of its host range and transmission dynamics (1). *Mycobacterium bovis*(*M.bovis*) has historically been associated with animal tuberculosis, but growing evidence suggests that *M. tuberculosis* can also infect and persist in other animals. The impact of this phenomenon is profound, both in terms of animal health as well as the potential for zoonotic transmission. (2).

Genetic diversity, manifested in the numerous genotypes of *M. tuberculosis* identified globally, is intricately linked to its ability to infect animals. In addition to virulence, host specificity, and drug resistance, these genotypes can influence critical factors. To understand the complicated epidemiology of tuberculosis across species barriers, it is crucial to understand how these genotypes are distributed and how often they occur in animal populations (3).

There are several reasons why studying *M. tuberculosis* genotypes in animals is important. The concept of One Health recognizes the interconnectedness of human, animal, and environmental health. Developing comprehensive strategies to control tuberculosis requires this approach. Furthermore, *M. tuberculosis*-infected animals may serve as reservoirs for human infection, complicating eradication efforts. As well as contributing to our understanding of the bacterium's evolution, studying *M. tuberculosis* in animals provides insights into its adaptability and host range (4).

Traditional methods for detecting *M. bovis* may be confounded by the presence of *M. tuberculosis* in animals, necessitating refined testing strategies. In addition, understanding the dynamics of *M. tuberculosis* in animals is crucial for assessing and mitigating potential public health risks, particularly in areas with close human-animal interactions (5).

An overview of the frequency and distribution of *M. tuberculosis* genotypes in different animal species is presented in this narrative review. In addition to analyzing genotype prevalence across geographical regions and animal populations, this paper will discuss the implications of these findings for zoonotic potential, animal health, and tuberculosis control. The review will also identify knowledge gaps and areas for future research in animal-associated *M. tuberculosis* genotypes. Finally, it will examine the potential impact of animal-associated

Formatted: Font: Italic

Commented [u3]: Please review the langiage

tuberculosis on diagnostics, treatment, and prevention strategies in both veterinary and human medicine.

By addressing these objectives, this review provides a comprehensive overview of the current state of knowledge regarding *M. tuberculosis* genotypes in animals, highlighting its importance in the broader context of tuberculosis research. To develop effective strategies for managing tuberculosis at the human-animal interface and to advance our understanding of this complex pathogen, we need these data.

Commented [u4]: Language edition

Commented [u5]: Please mention the methodology you used to review the articles. Now adays systematic review is recommended

Overview of *M. tuberculosis*

Characteristics of M. tuberculosis

In the Mycobacteriaceae family, *M. tuberculosis* is a rod-shaped, non-motile bacterium. A rich source of mycolic acids in its cell wall provides it with exceptional resilience. This contributes to the pathogenicity of the bacterium. Due to its cell wall composition, *M. tuberculosis* is resistant to many antibiotics and can survive for long periods in a dormant state (6).

M. tuberculosis is an obligate aerobic and facultative intracellular pathogen, primarily infecting macrophages. Its slow growth rate, with a generation time of 15-20 hours, poses significant challenges to diagnosis and treatment. When stained with acidic solutions, the bacterium is usually identified using acid-fast staining techniques, such as Ziehl-Neelsen staining (7).

Genetically, *M. tuberculosis* shows less diversity than many other bacterial pathogens, suggesting a relatively recent evolutionary bottleneck. Despite this, genetic variations do exist, and they play an important role in the bacterium's virulence, adaptability, and drug resistance. As a result of these genetic differences, different genotypes are observed in both human and animal populations (8).

Pathogenesis in Humans and Animals

There is a delicate interplay between the bacterium and the immune system in the pathogenesis of *M. tuberculosis* infection. In humans and animals, aerosolized bacteria are the primary route of infection. Inhaled bacteria are phagocytosed by alveolar macrophages, triggering an immunological cascade (9).

A sophisticated mechanism has evolved in *M. tuberculosis* for surviving and replicating inside macrophages, including inhibiting phagosome-lysosome fusion and modulating host cell death pathways. Infection typically results in the formation of granulomas, which are organized structures of immune cells that try to contain bacteria. In many cases, granulomas can effectively control infection, but they can also provide a home for bacterial persistence, leading to latent tuberculosis (9).

Commented [u6]: The source seems taken mainly from one section

Pathogenesis of animal tuberculosis is similar to that of human tuberculosis, but there are notable differences depending on the species. A degree of host adaptation may be evident in cattle infected with *M. tuberculosis* as compared to *M. bovis* infections. There is a strong similarity between the pathogenesis of tuberculosis in non-human primates and humans, which makes them a useful model for the study of tuberculosis (10).

The virulence and tissue tropism of *M. tuberculosis* were shown to change according to the hosts, according to recent studies. As a result of this adaptability, animal populations display diverse genotypes, which has implications for cross-species transmission and pathogen evolution (11).

Both humans and animals respond to *M. tuberculosis* by activating CD4+ T cells, CD8+ T cells, and macrophages. Controlling the infection relies on the production of cytokines such as interferon-gamma (IFN-) and tumor necrosis factor-alpha (TNF-). *M. tuberculosis*, however, has evolved mechanisms to modulate and evade the host's immune response, increasing its effectiveness as a pathogen (12).

There are several reasons why understanding the pathogenesis of *M. tuberculosis* in different animal species is crucial. Animal models for tuberculosis research can be developed using this information, and animal-adapted strains can be assessed for their zoonotic potential based on their host range and adaptability. The study of pathogenesis across species may also reveal novel aspects of host-pathogen interactions, leading to the development of new strategies for tuberculosis control and treatment (13).

Commented [u7]: Idea/concept is taken from one section which is not recommended, please rephrase and or include different sources

Epidemiology of *M. tuberculosis* in Animals

Global Distribution of M. tuberculosis in Animals

There is a complex and evolving landscape in which *M. tuberculosis* is distributed in animals. There is increasing evidence that *M. tuberculosis* is more prevalent in animal populations than previously believed, although *M. bovis* remains the primary source of tuberculosis in animals. Animal species and geographical regions have different distributions (14).

Animals, such as cattle, elephants, and various zoo animals, are frequently infected with *M. tuberculosis* in Asia, particularly in countries with high human TB burdens such as India and China (15). According to a comprehensive study in India, up to 15% of tuberculous cattle were infected with *M. tuberculosis* rather than *M. bovis* (15). In Southeast Asia, there have been several reported cases of *M. tuberculosis* infection in elephants (16).

There is a high prevalence of both human and animal tuberculosis in Africa, complicating the situation. Ethiopia and Tanzania have reported cases of *M. tuberculosis* in cattle, although at a lower rate than *M. bovis*. It is interesting to note that a study in South Africa found *M. tuberculosis* in wild animals, such as lions and baboons, highlighting the potential for the pathogen to establish itself in wild populations (17, 18).

There is a different picture in the Americas. Most cases of *M. tuberculosis* in animals in North America are associated with close contact with human TB patients. Nevertheless, *M. tuberculosis* has been isolated from cattle and domestic animals in South America, particularly in areas with high human TB prevalence (19, 20).

A low rate of *M. tuberculosis* in animals is generally reported in Europe, with most cases occurring in zoo animals and pets with close human contact. It is important to note that there is a possibility for underreporting because routine animal TB testing is often limited to *M. bovis* (5).

Transmission Dynamics Between Animals and Humans

Although human-to-animal transmission is more common, *M. tuberculosis* is transmitted bidirectionally between animals and humans. Tuberculosis ~~B~~ control efforts are further complicated by *M. tuberculosis*' zoonotic and reverse zoonotic potential (21). Usually, animals acquire *M. tuberculosis* from close, prolonged contact with infected humans. It is especially evident in cases involving pets, zoo

Commented [u8]: At least there should be indicators of M. tuberculosis presence in animals with evidences tested againist this bacteria.

Commented [u9]: Please provide tables that should indicate the presence of this bactria in different animals across different geographical locations

Field Code Changed

Commented [u10]: Indicat emeasurable figures rather than using low

Commented [u11]: Where is the evidence ?

animals, and livestock close to human settlements. In elephants, *M. tuberculosis* has been associated with their handlers in several cases (15, 22).

There is a risk of transmission from animals to humans, even though it is less common. It has been reported that veterinarians, animal handlers, and farmers have contracted *M. tuberculosis* from infected animals. Further research is needed to determine the extent of this risk and its contribution to the overall TB burden in humans (23).

Numerous factors influence transmission dynamics, including (24, 25):

- Human-animal proximity and duration of contact
- Host immunity, both human and animal
- Number of bacteria and stage of infection in the source (human or animal)
- Environments that favor the survival of the bacilli outside of the host

It is crucial to understand these dynamics to develop effective strategies for preventing cross-species transmission and for implementing appropriate biosecurity measures.

Host Species Affected by *M. tuberculosis*

Despite being the primary host for *M. tuberculosis*, the bacterium is capable of infecting a wide variety of animals. There are considerable differences in the susceptibility and manifestation of disease among different animal hosts (26).

Commented [u12]: This is not clear please mention the host here

- Cattle: Although less susceptible to *M. tuberculosis* than *M. bovis*, cattle can become infected, particularly in regions where TB is prevalent in humans (27).
- Elephants: Asian elephants are particularly susceptible to *M. tuberculosis*, with numerous cases reported in captive and wild populations (15).
- Non-human Primates: Different species of monkeys and apes are susceptible to *M. tuberculosis*, often showing similar disease progression as humans (28).
- Domestic pets: In dogs and cats, *M. tuberculosis* can be transmitted, though the clinical disease is rare (29).
- Zoo animals: Many zoo animals have developed *M. tuberculosis*, often from human sources, including big cats, bears, and ungulates (30).

- Wild animals: *M. tuberculosis* infections have been reported in meerkats, meerkats, and wild boars (5).
- Birds: In addition to humans, birds are susceptible to *M. tuberculosis*, particularly psittacines (31).

Both veterinary medicine and public health are affected by the varying susceptibility of different animal species to *M. tuberculosis*. The interplay between human, animal, and environmental health highlights the need for a One Health approach in TB control (32).

Genotyping of *M. tuberculosis* in Animals

Genotyping of *M. tuberculosis* isolates from animals is crucial for understanding the epidemiology, transmission dynamics, and evolution of the pathogen across different host species. Several methods have been developed and applied for this purpose, each with its own advantages and limitations (33). Table 1 provides the information about different genotyping methods in *M. tuberculosis*.

Commented [u13]: Weak review, please provide strong evidences

Methods for Genotyping *M. tuberculosis*

Spoligotyping (Spacer Oligonucleotide Typing)

In the *M. tuberculosis* genome, spoligotyping is based on the Direct Repeat (DR) locus polymorphism. It involves PCR amplification of the DR region followed by hybridization of 43 immobilized spacer oligonucleotides to a membrane. In the absence or presence of these spacers, a binary pattern is created that can be easily compared between isolates. As a first-line method for strain typing, spoligotyping can differentiate *M. tuberculosis* complex species (34).

Commented [u14]:

Mycobacterial Interspersed Repetitive Units-Variable Number Tandem Repeat (MIRU-VNTR) Typing

Using MIRU-VNTR analysis, we can determine how many repetitive sequences occur in the genome at different locations. MIRU-VNTR typically uses 24 loci, although 15 or 12 loci sets are also common. This method involves PCR amplification of these loci, followed by size determination of the amplicons by capillary electrophoresis. Repeats at each locus create a numerical code that can be used to identify strains (35).

Whole Genome Sequencing (WGS)

As the most comprehensive genetic analysis of *M. tuberculosis* isolates available, WGS provides the most information. To determine the complete genome sequence, DNA is extracted from cultured bacteria, sequencing libraries are prepared, and next-generation sequencing platforms are used. Single nucleotide polymorphisms (SNPs), insertions, deletions, and other genomic variations can be detected in the resulting data. By using WGS, phylogenetic analyses can be performed at high resolution, transmission chains can be identified, and drug resistance mutations can be detected (36).

IS6110-Restriction Fragment Length Polymorphism (RFLP)

In the past, this method was the gold standard for *M. tuberculosis* genotyping, though it is less commonly used today. A Southern blot hybridization with a probe for the IS6110 insertion sequence is performed after genomic DNA is digested with restriction enzymes, separated by gel electrophoresis, and then hybridized with a probe for the IS6110 insertion sequence. Strain identification is based on the resulting banding pattern. In particular, strains with a high copy number of IS6110 can benefit from this method (37).

Pulsed-Field Gel Electrophoresis (PFGE)

PFGE involves embedding bacteria in agarose plugs, lysing the cells, and digesting the chromosomal DNA with restriction enzymes. A system of electrophoresis is used to separate the large DNA fragments by periodically changing the direction of the electric field. In certain situations, PFGE can provide valuable information about *M. tuberculosis*, particularly for strains with low copy numbers of IS6110 (37).

Table 1. Comparison of genotyping methods for *M. tuberculosis*.

Method	Duration	Efficiency	Advantages	Disadvantages
Spoligotyping	1-2 days	Medium	• Rapid and simple • Standardized	• Limited discriminatory

			nomenclature • Can differentiate *M. tuberculosis* complex species	power • Cannot detect mixed infections
MIRU-VNTR	2-3 days	High	• High discriminatory power • Easily digitized results • Can detect mixed infections	• More time-consuming than spoligotyping • Requires specialized equipment
Whole Genome Sequencing	2-7 days	Very High	• Highest resolution • Provides comprehensive genetic information • Can detect drug resistance mutations	• Expensive • Requires sophisticated equipment and expertise • Data analysis can be complex
IS6110-RFLP	3-4 weeks	Medium	• High discriminatory power for strains with many IS6110 copies	• Time-consuming • Requires large amounts of DNA • Labor-intensive • Difficult to compare results between labs
PFGE	3-5 days	Medium	• Useful for strains with low IS6110 copy numbers • Can analyze large DNA fragments	• Time-consuming • Labor-intensive • Limited discriminatory power for *M. tuberculosis*

The methods included are Spoligotyping (Spacer Oligonucleotide Typing), MIRU-VNTR (Mycobacterial Interspersed Repetitive Units-Variable Number Tandem Repeat), WGS (Whole Genome Sequencing), IS6110-RFLP (Insertion Sequence 6110-Restriction Fragment Length Polymorphism), and PFGE (Pulsed-Field Gel Electrophoresis).

Frequency of *M. tuberculosis* Genotypes in Different Animal Species

Veterinary epidemiology has been interested in the distribution and prevalence of *M. tuberculosis* genotypes across various animal species. It provides an overview of the current understanding of *M. tuberculosis* genotype frequencies in domestic animals, wildlife, and exotic animals in captivity, emphasizing the interaction between pathogen genetics and host genetics (38).

Domestic Animals

Cattle

Although *M. bovis* is the primary cause of bovine tuberculosis, *M. tuberculosis* infection in cattle has been documented, although less frequently. The Beijing genotype is predominant in cattle, especially in regions with a high prevalence of human TB. The Euro-American lineage has also been isolated from bovine hosts, suggesting human-to-cattle transmission. However, *M. tuberculosis* genotypes remain relatively rare in cattle compared to *M. bovis* strains (39).

Commented [u15]: Mentioned above

Dogs

The incidence of canine tuberculosis caused by *M. tuberculosis* is uncommon, but not unprecedented. In most cases, the genotypes observed match those of the local human population. The most frequently reported genotypes in dogs are those of the Euro-American lineage, particularly the Haarlem and LAM (Latin American-Mediterranean) families. Dogs with the Beijing genotype have been detected sporadically in regions where the strain is endemic in humans (29).

Cats

There are very few cases of feline tuberculosis caused by *M. tuberculosis*, with most cases associated with close contact with infected humans. Cat genotypes in the geographical area are largely a reflection of human genotypes. Reports of Euro-American isolates have been reported, with the T1 sublineage being more prevalent. Occasionally, the Beijing genotype has been detected in feline hosts, particularly in Asian countries where this strain is common (29).

Commented [u16]:

Wildlife

Deer

Deer populations are less likely to contract *M. tuberculosis* since these animals are more susceptible to *M. bovis*. However, when *M. tuberculosis* is detected in cervids, the genotypes are often similar to those circulating in human settlements. Some deer populations have been reported to have the Euro-American lineage, particularly the Uganda and X-type families. Geographic location and human-wildlife interface dynamics greatly influence the frequency of specific genotypes (40).

Elephants

There is evidence that elephants, particularly those in captivity, are susceptible to tuberculosis infection. Genetic studies have revealed a wide variety of strains, with certain genotypes predominating in various regions. The East African-Indian lineage and the Beijing genotype have been reported more frequently in Asian elephants. Euro-American lineages, including the Haarlem family, have been detected in Asian and African elephants in captivity, perhaps as a result of human handling (41).

Other Wildlife Species

In addition to non-human primates, meerkats, and badgers, *M. tuberculosis* has been detected sporadically in other wildlife species. The genotype frequencies in these species are often similar to those in local human populations, suggesting anthroponotic transmission. It has been reported that the Euro-American lineage is more prevalent in non-human primates, particularly the LAM and Haarlem families. It has been discovered that some primate populations, particularly in Asian countries, carry the Beijing genotype (5, 42).

Exotic Animals in Captivity

Zoo Animals

Due to the proximity of diverse species and human interaction, zoological collections present a unique environment for *M. tuberculosis* transmission. The genotyping of *M. tuberculosis* strains in zoo animals has revealed a heterogeneous distribution. Many species have been identified with the Euro-American lineage, including the T, Haarlem, and LAM families. In some cases, the Beijing genotype has been reported, particularly in zoos located in regions where this strain has become endemic (5).

Circus Animals

As circus animals travel frequently and are in close contact with humans, they present a unique epidemiological scenario for *M. tuberculosis* transmission. Despite the lack of data on genotype frequencies in circus animals, available studies indicate a predominance of strains of the Euro-American lineage. It has been reported that the Haarlem and S-type families exist in some cases. In circus animals, the Beijing genotype appears to be less common than in zoo animals, possibly due to differences in geographic exposure (43).

Therefore, the frequency of strains of *M. tuberculosis* in animals in particular geographical regions is closely related to that of the strains circulating among humans in those areas. It appears that the Euro-American lineage is the most

widely distributed across a variety of animal species, with the Beijing genotype becoming increasingly prevalent, particularly in regions where it is endemic in humans. In contrast with species-adapted mycobacterial pathogens, the overall prevalence of *M. tuberculosis* in animals is relatively low. To clarify the complex dynamics of *M. tuberculosis* genotype distribution in different animal populations and the implications for cross-species transmission and control, further research is required (44, 45).

Factors Influencing the Distribution of *M. tuberculosis* Genotypes in Animals

A multitude of interconnected factors influence the distribution of *M. tuberculosis* genotypes in animal populations. For elucidating the epidemiology of tuberculosis in animals and developing effective control strategies, it is crucial to understand these factors. Various animal species are examined to understand the factors shaping *M. tuberculosis*' genotypic landscape (46).

Environmental Factors

As a result of environmental factors, *M. tuberculosis* is susceptible to survival and transmission, thereby affecting the distribution of specific genotypes within animal populations. Environmental factors such as temperature, humidity, and UV radiation influence bacterial persistence. Certain ecological niches may benefit from genotypes that exhibit enhanced environmental stability, such as those belonging to the Beijing lineage (47).

There is also an impact of soil composition and pH levels on *M. tuberculosis* in the environment. Several genotypes, particularly in the Euro-American lineage, have adapted to specific soil conditions, potentially influencing their prevalence in geographically distinct populations. Additionally, environmental mycobacteria may exert selective pressure on *M. tuberculosis* genotypes, favoring strains with unique survival mechanisms or competitive advantages. As a result of climate change and habitat changes, animal species may be exposed to novel genotypes of *M. tuberculosis*. Environmental change and host distribution may interact dynamically, resulting in the emergence of previously uncommon genotypes in certain animal populations (48).

Host Factors

There is a significant influence of the genetic makeup, immune status, and physiological characteristics of animal hosts on the success of specific genotypes of *M. tuberculosis*. Certain bacterial strains may be selectively favored because of differences in innate and adaptive immune responses between host species. Among some animal species, the Beijing genotype has been found to modulate immune responses effectively (48).

Additionally, intra-species genetic variation is crucial in determining susceptibility to specific genotypes of *M. tuberculosis*. Toll-like receptor or cytokine polymorphisms, for example, can influence infection outcomes and, by extension, bacterial genotype distribution in a population (49).

The nutritional status and overall health of animal hosts can affect their susceptibility to *M. tuberculosis* infection. Animals with malnutrition or immunosuppression may be more susceptible to a broader range of genotypes, potentially altering the genotypic landscape. The genotype distribution can also be affected by age-related differences in immune function. Some genotypes are more prevalent in young and elderly animals, resulting in age-structured patterns of genotype prevalence (49).

Human-Animal Interactions

In animal populations, *M. tuberculosis* genotype distribution is heavily influenced by human-animal interactions. Human-animal contact, particularly in settings with high human TB prevalence, can cause anthroponotic transmission of *M. tuberculosis*. As a result of this phenomenon, animal infections are often caused by genotypes that are prevalent in the local human population (50).

Human encroachment into natural habitats increases the risk of transmission of *M. tuberculosis* to wildlife populations. These cases frequently have genotypes that are similar to those circulating in nearby human settlements. Animal populations previously untouched by human-associated *M. tuberculosis* can inadvertently become infected by human-associated *M. tuberculosis* genotypes due to conservation efforts and wildlife tourism. New routes for transmission of *M. tuberculosis* have emerged due to the increasing popularity of exotic pets. TB can be transmitted to animals by owners or handlers infected with the disease, introducing human-associated genotypes into novel hosts. *M. tuberculosis* transmission events may be affected by the intensity of human-livestock interactions in agricultural settings. The introduction of human-adapted *M. tuberculosis* genotypes into livestock populations may be made easier by modern farming practices involving close human-animal contact (51).

Impact of Animal Trade and Movement

The distribution of *M. tuberculosis* genotypes across geographical boundaries is profoundly affected by global and local animal trade practices. It is possible to introduce novel genotypes into naive animal populations through the international trade of livestock, exotic pets, and wildlife. As a result of this movement, genotypes can be established in regions where they were previously not found, changing the local tuberculosis landscape (52).

M. tuberculosis genotypes are dispersed across ecological zones through transhumance and nomadic pastoral practices. Using traditional animal husbandry methods can facilitate the mixing of genotypes from diverse geographical origins, resulting in the emergence of novel strain combinations (53).

Natural and human-induced wildlife migrations (e.g., for conservation purposes) may affect the spatial distribution of *M. tuberculosis* genotypes. Specific genotypes may be transported across vast distances and introduced into new ecosystems by migratory species (54).

The illegal wildlife trade is a significant risk factor for the spread of *M. tuberculosis* genotypes. Atypical genotypes may be found in animals transported through this clandestine network, complicating epidemiological investigations and control efforts (55).

M. tuberculosis genotypes are controlled through quarantine practices and health screening protocols in the animal trade. The effectiveness of these measures varies globally, potentially allowing the movement of infected animals and subsequent transmission of specific genotypes (56).

As a result of a complex interplay between environmental factors, host-related factors, and anthropogenic factors, *M. tuberculosis* genotype distribution in animal populations may be influenced by several factors. It is essential to understand these influences to develop comprehensive strategies for monitoring and controlling tuberculosis in animals. Research should focus on elucidating the relative contributions of these factors in different ecological contexts to enable more targeted and effective interventions to manage *M. tuberculosis* across animal species (57).

Need for More Comprehensive Genotyping Studies

There is limited understanding of *M. tuberculosis* genotypes in animals, requiring more comprehensive research. A crucial aspect of these investigations is the use of genotyping methods, which provide valuable information about strain diversity, transmission patterns, and host-pathogen interactions (1).

Current genotyping methods for *M. tuberculosis* include:

Spoligotyping: This PCR-based method identifies the Direct Repeat locus (DR) in the *M. tuberculosis* genome. Simple terminology and standardized nomenclature allow for global comparisons, making it widely used. Its discriminatory power, however, is limited compared to newer methods (34).

Mycobacterial Interspersed Repetitive Units-Variable Number Tandem Repeats (MIRU-VNTR): This method analyzes the number of repeats in multiple loci across the genome. The 24-locus MIRU-VNTR typing provides high discriminatory power and is often used in conjunction with spoligotyping for enhanced resolution (58).

Whole Genome Sequencing (WGS): For *M. tuberculosis* genotyping, this approach provides the highest level of strain differentiation. Using WGS, single nucleotide polymorphisms (SNPs), insertions, deletions, and other genomic variations can be detected, providing a unique insight into strain evolution and transmission dynamics (58).

Future research should focus on:

WGS application expansion: Increasing the use of WGS in animal studies to provide genotypes of *M. tuberculosis* at high resolution. As a result, novel animal-adapted strains could be identified and zoonotic and reverse zoonotic transmissions could be tracked more accurately (59).

Phylogenomic analyses: Conducting comprehensive phylogenomic studies comparing M. tuberculosis isolates from various animal species with human isolates. It could reveal evolutionary adaptations and host-specific mutations that influence host tropism and virulence (60).

Metagenomics approaches: Developing metagenomic methods to detect and genotype *M. tuberculosis* directly from complex environmental or clinical samples without the need for culture. For the study of wildlife populations or environmental reservoirs, this could be especially valuable (61).

Single-cell genomics: Exploring single-cell sequencing technologies to understand the diversity of *M. tuberculosis* populations within an animal host, potentially providing insights into micro-evolution and adaptation (62).

Development of Better Diagnostic Tools

To enhance diagnostic capabilities and epidemiological investigations of *M. tuberculosis* in animals, genotyping methods need to be improved (63). Prioritize the following research in the future:

Methods for rapid genotyping: Development of point-of-care genotyping tools based on loop-mediated isothermal amplification (LAMP) and CRISPR technology. As a result, real-time epidemiological investigations and targeted interventions could be undertaken on *M. tuberculosis* strains identified in animal samples (64).

Multiplexed genotyping assays: Creating diagnostic platforms capable of simultaneously detecting and genotyping *M. tuberculosis* and other mycobacterial species that commonly infect animals. Genetic markers that are specific to each species and genotype can be targeted by multiplex PCR systems or microarray technologies (65).

Technologies for portable sequencing: Adapting portable sequencing devices, like Oxford Nanopore's MinION, for field-based sequencing of *M. tuberculosis* isolates. It would be possible to carry out real-time genomic epidemiology in remote or resource-limited settings (66).

Machine learning integration: Developing machine learning algorithms to analyze complex genotyping data, including WGS data, to predict phenotypic characteristics such as drug resistance or virulence based on genetic markers (67).

Genomics using non-invasive samples: Feces, environmental DNA (eDNA) and other non-invasive samples are being used to genotype *M. tuberculosis*. It is possible to enrich *M. tuberculosis* DNA in complex samples using highly sensitive PCR methods or capture-based sequencing approaches (67, 68).

Biomarker discovery: Combining transcriptomics and proteomics with genotyping to uncover strain-specific biomarkers. Using these techniques, rapid tests could be developed that detect *M. tuberculosis* and genotype simultaneously (69).

Applications of digital PCR: Utilizing digital PCR to detect and genotype *M. tuberculosis* in animal samples with high sensitivity and quantitative accuracy, especially for strains with low abundances or mixed infections (70).

By developing these genotyping methods and integrating them into comprehensive research studies and diagnostic protocols, we can significantly enhance our understanding of *M. tuberculosis* diversity in animal populations. A true One Health approach to TB management will require such knowledge to develop more effective strategies to control tuberculosis at the human-animal interface (65, 69).

Conclusion

Summary of Key Findings

To understand the epidemiology and transmission dynamics of *M. tuberculosis* across species, molecular typing and genotyping have been conducted in animals. This review found the following key findings:

Genotyping Methods: To study *M. tuberculosis* in animals, various molecular typing methods have been used, including spoligotyping, mycobacterial interspersed repetitive units-variable number tandem repeats (MIRU-VNTR), and whole genome sequencing (WGS). Our understanding of strain diversity has been enhanced by different methods with varying levels of discriminatory power.

Strain Diversity: *M. tuberculosis* strain diversity has been revealed through Molecular Typing of animals, often reflecting strains found in local human populations. High-resolution techniques like WGS have provided insights into host adaptations and transmission patterns through the discovery of subtle genetic variations.

Lineage Distribution: The Euro-American lineage is widely distributed across a variety of animal species based on genotyping studies. Animals with the Beijing genotype, identified through specific molecular markers, are increasingly detected in regions where it is prevalent in humans.

Host-Pathogen Interactions: In the field of host-pathogen interactions, several genetic analyses have been performed to identify the genetic basis of host specificity, revealing lineage-specific genome regions and single nucleotide polymorphisms (SNPs) that may influence host tropism and virulence in a variety of animal species.

Transmission Dynamics: As a result of genome sequencing, transmission chains between humans and animals can now be tracked, providing evidence for both anthroponotic and zoonotic transmission events. It has been particularly helpful in investigating outbreaks among captive animals.

Impact on Animal and Human Health
The application of molecular typing and genotyping of *M. tuberculosis* in animals has significant implications for both animal and human health:

Improved Epidemiological Understanding: Genotyping has improved our understanding of the epidemiology of *M. tuberculosis* in animals, which has helped us develop more targeted control measures.

Revelation of Zoonotic Potential: The discovery of the zoonotic potential of *M. tuberculosis* has been made possible by several Molecular Studies, including genetically similar or identical strains found in humans and animals near one another.

Insight into Evolutionary Adaptations: We have gained a deeper understanding of evolutionary adaptations due to comparative genomic analyses that have revealed genetic adaptations that may facilitate the survival of *M. tuberculosis* in different animal hosts, contributing to our understanding of the co-evolution of hosts and pathogens.

Enhanced Outbreak Investigations: Outbreak investigations have been greatly enhanced by molecular typing, allowing rapid detection of the source of an outbreak in both domestic and wild animal populations.

Basis for Targeted Interventions: The development of genotype-specific information has laid the foundation for the development of more targeted interventions, including genotype-specific diagnostics and vaccines that could be developed based on this information.

Final Recommendations
In order to advance our understanding and control of this pathogen, molecular typing and genotyping studies of *M. tuberculosis* in animals have provided several crucial recommendations. Standardizing protocols for *M. tuberculosis* genotyping in animals is essential to facilitate global comparisons and data sharing, encompassing guidelines for sample collection, processing, and analysis. As part of this standardization, whole genome sequencing should be promoted for *M. tuberculosis* typing in animals, since this high-resolution approach can provide unprecedented insights into transmission dynamics and evolutionary adaptations. It may also be possible to develop rapid, species-specific diagnostic tests by

investing in research to identify genetic markers specific to *M. tuberculosis* strains adapted to particular animal hosts. The development of sophisticated bioinformatics tools that can analyze complex genotyping data from multiple sources, including environmental samples, is essential to supporting these efforts, including the establishment of a global database of *M. tuberculosis* genotypes isolated from various animal species. Molecular epidemiology capacity must be built in high-burden regions to implement these recommendations. Veterinary and wildlife health sectors should invest in training programs for *M. tuberculosis* genotyping and molecular epidemiology. Furthermore, by monitoring animal populations genetically for *M. tuberculosis*, existing tuberculosis control programs can detect emerging strains and patterns of transmission early. To accelerate progress in this field, international research networks focused on the molecular epidemiology of *M. tuberculosis* in animals are needed to foster collaboration and knowledge sharing across disciplines and geographical boundaries. In managing this complex zoonotic pathogen, we can leverage the power of genomics and molecular epidemiology by implementing these recommendations. To develop more effective, targeted strategies to control tuberculosis at the human-animal interface, it will be critical to apply and advance these molecular tools. By providing critical insights into strain diversity, transmission dynamics, and host-pathogen interactions, *M. tuberculosis* in animal populations is revolutionizing our understanding of *M. tuberculosis* in animal populations.

References

1. Hlokwe TM, Said H, Gcebe N. Mycobacterium tuberculosis infection in cattle from the Eastern Cape Province of South Africa. BMC Veterinary Research. 2017;13(1):299.
2. De Garine-Wichatitsky M, Caron A, Kock R, Tschopp R, Munyeme M, Hofmeyr M, et al. A review of bovine tuberculosis at the wildlife–livestock–human interface in sub-Saharan Africa. Epidemiology & Infection. 2013;141(7):1342-56.
3. Morales-Arce AY, Sabin SJ, Stone AC, Jensen JD. The population genomics of within-host Mycobacterium tuberculosis. Heredity. 2021;126(1):1-9.
4. Katale BZ, Mbugi EV, Keyyu JD, Fyumagwa RD, Rweyemamu MM, van Helden PD, et al. One Health approach in the prevention and control of mycobacterial infections in Tanzania: lessons learnt and future perspectives. One Health Outlook. 2019;1(1):2.
5. Thomas J, Balseiro A, Gortázar C, Risalde MA. Diagnosis of tuberculosis in wildlife: a systematic review. Veterinary Research. 2021;52(1):31.
6. Vilchèze C. Mycobacterial cell wall: a source of successful targets for old and new drugs. Applied Sciences. 2020;10(7):2278.

7. Palanivel J, Sounderrajan V, Thangam T, Rao SS, Harshavardhan S, Parthasarathy K. Latent Tuberculosis: Challenges in Diagnosis and Treatment, Perspectives, and the Crucial Role of Biomarkers. Current Microbiology. 2023;80(12):392.
8. Atavliyeva S, Auganova D, Tarlykov P. Genetic diversity, evolution and drug resistance of Mycobacterium tuberculosis lineage 2. Frontiers in Microbiology. 2024;15:1384791.
9. Bhat KH, Yaseen I. Mycobacterium tuberculosis: macrophage takeover and modulation of innate effector responses. Mycobacterium-Research and Development. 2018.
10. Peña JC, Ho W-Z. Non-Human Primate Models of Tuberculosis. Microbiology Spectrum. 2016;4(4):10.1128/microbiolspec.tbtb2-0007-2016.
11. Fonseca KL, Rodrigues PNS, Olsson IAS, Saraiva M. Experimental study of tuberculosis: From animal models to complex cell systems and organoids. PLOS Pathogens. 2017;13(8):e1006421.
12. Matucci A, Maggi E, Vultaggio A. Cellular and humoral immune responses during tuberculosis infection: useful knowledge in the era of biological agents. The Journal of Rheumatology Supplement. 2014;91:17-23.
13. Gupta U, Katoch V. Animal models of tuberculosis. Tuberculosis. 2005;85(5-6):277-93.
14. Ahmad I, Raji YE, Hassan L, Samaila A, Aliyu B, Zinsstag J, et al. Systematic review and meta-analysis of tuberculosis in animals in Nigeria. Heliyon. 2023;9(6).
15. Rajbhandari RM, de la Fuente J, Karmacharya D, Mathema S, Maharjan B, Dixit SM, et al. Understanding Mycobacterium tuberculosis complex in elephants through a One Health approach: a systematic review. BMC Veterinary Research. 2022;18(1):262.
16. Chandranaik BM, Shivashankar BP, Umashankar KS, Nandini P, Giridhar P, Byregowda SM, et al. Mycobacterium tuberculosis Infection in Free-Roaming Wild Asian Elephant. Emerging Infectious Disease journal. 2017;23(3):555.
17. Zheng W, Diao NC, Wang Q, Wang CY, Su N, Yin JY, et al. Worldwide Swine Tuberculosis-Positive Rate and Associated Risk Factors, 1966-2020: A Systematic Review and Meta-Analysis. Vector Borne Zoonotic Dis. 2024;24(4):181-95.
18. Meiring C, van Helden PD, Goosen WJ. TB Control in Humans and Animals in South Africa: A Perspective on Problems and Successes. Front Vet Sci. 2018;5:298.
19. Vågene Å J, Honap TP, Harkins KM, Rosenberg MS, Giffin K, Cárdenas-Arroyo F, et al. Geographically dispersed zoonotic tuberculosis in pre-contact South American human populations. Nat Commun. 2022;13(1):1195.
20. Donoghue HD. Paleomicrobiology of Human Tuberculosis. Microbiol Spectr. 2016;4(4).
21. Anderson BD, Barnes AN, Umar S, Guo X, Thongthum T, Gray GC. Reverse Zoonotic Transmission (Zooanthroponosis): An Increasing Threat to Animal Health. In: Sing A, editor. Zoonoses: Infections Affecting Humans and Animals. Cham: Springer International Publishing; 2022. p. 1-63.
22. Stephens N, Vogelnest L, Lowbridge C, Christensen A, Marks GB, Sintchenko V, et al. Transmission of Mycobacterium tuberculosis from an Asian elephant (Elephas maximus) to a chimpanzee (Pan troglodytes) and humans in an Australian zoo. Epidemiology and Infection. 2013;141(7):1488-97.
23. Devi KR, Lee LJ, Yan LT, Syafinaz A-N, Rosnah I, Chin VK. Occupational exposure and challenges in tackling M. bovis at human–animal interface: a narrative review. International Archives of Occupational and Environmental Health. 2021;94(6):1147-71.
24. Verhagen LM, van den Hof S, van Deutekom H, Hermans PW, Kremer K, Borgdorff MW, et al. Mycobacterial factors relevant for transmission of tuberculosis. Journal of Infectious Diseases. 2011;203(9):1249-55.
25. Martinez L, Shen Y, Mupere E, Kizza A, Hill PC, Whalen CC. Transmission of Mycobacterium tuberculosis in households and the community: a systematic review and meta-analysis. American journal of epidemiology. 2017;185(12):1327-39.

26. LoBue P, Enarson D, Thoen C. Tuberculosis in humans and animals: an overview [Serialised article. Tuberculosis: a re-emerging disease in animals and humans. Number 1 in the series]. The International Journal of Tuberculosis and Lung Disease. 2010;14(9):1075-8.
27. Lombard JE, Patton EA, Gibbons-Burgener SN, Klos RF, Tans-Kersten JL, Carlson BW, et al. Human-to-Cattle Mycobacterium tuberculosis Complex Transmission in the United States. Frontiers in Veterinary Science. 2021;8.
28. Scanga CA, Flynn JL. Modeling tuberculosis in nonhuman primates. Cold Spring Harbor perspectives in medicine. 2014;4(12):a018564.
29. Pesciaroli M, Alvarez J, Boniotti M, Cagiola M, Di Marco V, Marianelli C, et al. Tuberculosis in domestic animal species. Research in veterinary science. 2014;97:S78-S85.
30. Lécu A, Ball R. Mycobacterial infections in zoo animals: relevance, diagnosis and management. International Zoo Yearbook. 2011;45(1):183-202.
31. XU C-h, ZHOU X-m, FAN W-x, ZHAO Y-l. Current situation and prospect of prevention and control of tuberculosis in humans, livestock and poultry. Chinese Journal of Antituberculosis. 2021;43(10):993.
32. Macedo Couto R, Ranzani OT, Waldman EA. Zoonotic Tuberculosis in Humans: Control, Surveillance, and the One Health Approach. Epidemiologic Reviews. 2019;41(1):130-44.
33. Ghazvini K, Khoshbakht R, Tadayon K, Mosavari N, BahramiTaghanaki HR, Mohammadi GR, et al. Genotyping of Mycobacterium tuberculosis complex isolated from humans and animals in northeastern Iran. Scientific Reports. 2023;13(1):6746.
34. Driscoll JR. Spoligotyping for molecular epidemiology of the Mycobacterium tuberculosis complex. Molecular Epidemiology of Microorganisms: Methods and Protocols. 2009:117-28.
35. Carugati M, Zanini F, Schiroli C, Gori A, Franzetti F, Hanekom M, et al. Mycobacterial Interspersed Repetitive-Unit–Variable-Number Tandem-Repeat Analysis and Beijing/W Family of Mycobacterium tuberculosis. Journal of clinical microbiology. 2011;49(7):2780-1.
36. Nelson KN, Talarico S, Poonja S, McDaniel CJ, Cilnis M, Chang AH, et al. Mutation of Mycobacterium tuberculosis and implications for using whole-genome sequencing for investigating recent tuberculosis transmission. Frontiers in Public Health. 2022;9:790544.
37. Comín J, Otal I, Samper S. In-depth Analysis of IS 6110 Genomic Variability in the Mycobacterium tuberculosis Complex. Frontiers in Microbiology. 2022;13:767912.
38. Brites D, Loiseau C, Menardo F, Borrell S, Boniotti MB, Warren R, et al. A New Phylogenetic Framework for the Animal-Adapted Mycobacterium tuberculosis Complex. Frontiers in Microbiology. 2018;9.
39. Collins JD. Tuberculosis in cattle: strategic planning for the future. Veterinary microbiology. 2006;112(2-4):369-81.
40. Michelet L, Conde C, Branger M, Cochard T, Biet F, Boschiroli ML. Transmission network of deer-borne Mycobacterium bovis infection revealed by a WGS approach. Microorganisms. 2019;7(12):687.
41. Zachariah A, Pandiyan J, Madhavilatha GK, Mundayoor S, Chandramohan B, Sajesh PK, et al. Mycobacterium tuberculosis in Wild Asian Elephants, Southern India. Emerging Infectious Disease journal. 2017;23(3):504.
42. Silva-Pereira TT, Soler-Camargo NC, Guimarães AMS. Diversification of gene content in the Mycobacterium tuberculosis complex is determined by phylogenetic and ecological signatures. Microbiology Spectrum. 2024;12(2):e02289-23.
43. Ghodbane R, Drancourt M. Non-human sources of Mycobacterium tuberculosis. Tuberculosis. 2013;93(6):589-95.
44. Wirth T, Hildebrand F, Allix-Béguec C, Wölbeling F, Kubica T, Kremer K, et al. Origin, spread and demography of the Mycobacterium tuberculosis complex. PLoS pathogens. 2008;4(9):e1000160.

45. Mutayoba BK, Michael Hoelscher, Heinrich N, Joloba ML, Lyamuya E, Kilale AM, et al. Phylogenetic lineages of tuberculosis isolates and their association with patient demographics in Tanzania. BMC Genomics. 2022;23(1):561.
46. Abd El-Rahman OA, Rasslan F, Hassan SS, Ashour HM, Wasfi R. The RND Efflux Pump Gene Expression in the Biofilm Formation of Acinetobacter baumannii. Antibiotics (Basel). 2023;12(2).
47. Wiens KE, Woyczynski LP, Ledesma JR, Ross JM, Zenteno-Cuevas R, Goodridge A, et al. Global variation in bacterial strains that cause tuberculosis disease: a systematic review and meta-analysis. BMC Medicine. 2018;16(1):196.
48. Wirth T, Hildebrand F, Allix-Béguec C, Wölbeling F, Kubica T, Kremer K, et al. Origin, Spread and Demography of the Mycobacterium tuberculosis Complex. PLOS Pathogens. 2008;4(9):e1000160.
49. Smith CM, Baker RE, Proulx MK, Mishra BB, Long JE, Park SW, et al. Host-pathogen genetic interactions underlie tuberculosis susceptibility in genetically diverse mice. eLife. 2022;11:e74419.
50. Vikas Saket K, Kachhi R, Singh P. Tuberculosis in animals and humans: Evolution of diagnostics and therapy. Asian J of Ani and Vet Advan. 2017;12:177.
51. Erwin PC, Bemis DA, Mawby DI, McCombs SB, Sheeler LL, Himelright IM, et al. Mycobacterium tuberculosis Transmission from Human to Canine. Emerging Infectious Disease journal. 2004;10(12):2258.
52. Murai K, Tizzani P, Awada L, Mur L, Mapitse N, Caceres P. Bovine tuberculosis: global distribution and implementation of prevention and control measures according to WAHIS data. WOfAH OIE, Editor; 2019.
53. Ayantunde AA, Asse R, Said MY, Fall A. Transhumant pastoralism, sustainable management of natural resources and endemic ruminant livestock in the sub-humid zone of West Africa. Environment, development and sustainability. 2014;16:1097-117.
54. Mokrousov I. Major impact of massive migration on spread of Mycobacterium tuberculosis strains. Human Migration: Biocultural Perspectives. 2021;1.
55. Rush ER, Dale E, Aguirre AA. Illegal wildlife trade and emerging infectious diseases: pervasive impacts to species, ecosystems and human health. Animals. 2021;11(6):1821.
56. Bushmitz M, Lecu A, Verreck F, Preussing E, Rensing S, Mätz-Rensing K, et al. Guidelines for the prevention and control of tuberculosis in non-human primates: Recommendations of the European Primate Veterinary Association Working Group on Tuberculosis. Journal of Medical Primatology. 2009;38(1):59-69.
57. Conteddu K, English HM, Byrne AW, Amin B, Griffin LL, Kaur P, et al. A scoping review on bovine tuberculosis highlights the need for novel data streams and analytical approaches to curb zoonotic diseases. Veterinary Research. 2024;55(1):64.
58. Allix C, Supply P, Fauville-Dufaux M. Utility of Fast Mycobacterial Interspersed Repetitive Unit—Variable Number Tandem Repeat Genotyping in Clinical Mycobacteriological Analysis. Clinical Infectious Diseases. 2004;39(6):783-9.
59. Sekizuka T, Yamashita A, Murase Y, Iwamoto T, Mitarai S, Kato S, et al. TGS-TB: total genotyping solution for Mycobacterium tuberculosis using short-read whole-genome sequencing. PloS one. 2015;10(11):e0142951.
60. Rajbhandari RM, Napit R, Manandhar P, Raut R, Gurung A, Poudel A, et al. Phylogenomic analysis supports Mycobacterium tuberculosis transmission between humans and elephants. Frontiers in Veterinary Science. 2023;10:1133823.
61. Doughty EL, Sergeant MJ, Adetifa I, Antonio M, Pallen MJ. Culture-independent detection and characterisation of Mycobacterium tuberculosis and M. africanum in sputum samples using shotgun metagenomics on a benchtop sequencer. PeerJ. 2014;2:e585.

62. Pisu D, Huang L, Narang V, Theriault M, Lê-Bury G, Lee B, et al. Single cell analysis of M. tuberculosis phenotype and macrophage lineages in the infected lung. The Journal of experimental medicine. 2021;218(9).
63. Cobelens F, van den Hof S, Pai M, Squire SB, Ramsay A, Kimerling ME. Which new diagnostics for tuberculosis, and when? Journal of Infectious Diseases. 2012;205(suppl_2):S191-S8.
64. Eddabra R, Ait Benhassou H. Rapid molecular assays for detection of tuberculosis. Pneumonia. 2018;10(1):4.
65. Gazi MA, Islam MR, Kibria MG, Mahmud Z. General and advanced diagnostic tools to detect Mycobacterium tuberculosis and their drug susceptibility: a review. European Journal of Clinical Microbiology & Infectious Diseases. 2015;34:851-61.
66. Gliddon H, Frampton D, Munsamy V, Heaney J, Pataillot-Meakin T, Nastouli E, et al. A rapid drug resistance genotyping workflow for Mycobacterium tuberculosis, using targeted isothermal amplification and nanopore sequencing. Microbiol Spectr 9: e00610-21. 2021.
67. Nahid P, Kim PS, Evans CA, Alland D, Barer M, Diefenbach J, et al. Clinical research and development of tuberculosis diagnostics: moving from silos to synergy. Journal of Infectious Diseases. 2012;205(suppl_2):S159-S68.
68. Yayan J, Franke K-J, Berger M, Windisch W, Rasche K. Early detection of tuberculosis: a systematic review. Pneumonia. 2024;16(1):11.
69. Gill CM, Dolan L, Piggott LM, McLaughlin AM. New developments in tuberculosis diagnosis and treatment. Breathe. 2022;18(1).
70. Choi YJ, Kim Y, Park HJ, Kim D, Lee H, Kim YA, et al. Development of a multiplex droplet digital PCR method for detection and monitoring of Mycobacterium tuberculosis and drug-resistant tuberculosis. Annals of Clinical Microbiology and Antimicrobials. 2024;23(1):29.

Commented [u17]: This paper lacks formality and repeated many similar concepts in several locations which needs re-write