A review of tuberculosis and parasitic disease co-infection in ungulates, with regard to the potential threat to European bison (*Bison bonasus*)

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Abstract

Bovine tuberculosis (BTB) is a dangerous zoonosis which presents a serious problem for endangered species such as European bison (*Bison bonasus*). Little is known about the influence of parasitic co-infections on the course and diagnosis of tuberculosis in animals. The best known co-infection in cattle is *Fasciola hepatica* and *Mycobacterium bovis*. The aim of this study was to review the most recent literature regarding tuberculosis and parasite co-infection in ungulates and relate the results to European bison. Our findings indicate that any comprehensive diagnosis of BTB should include parasitological monitoring, and the possible impact of such invasions on cellular response-based tuberculosis tests should be taken into account. The diagnosis of BTB is complex, as is its pathogenesis, and parasitic infestations can have a significant impact on both. This should be taken into account during further research and monitoring of tuberculosis in European bison.

Key words: bovine tuberculosis, co-infection, *Fasciola hepatica*, helminths, *Mycobacterium bovis*, *Mycobacterium caprae*, parasites

Introduction

Bovine tuberculosis (BTB) is a re-emerging disease in both humans and animals (Olea-Popelka et al. 2017), and tuberculosis is known to co-occur with parasitic diseases in humans, especially in developing countries (Kumar et al. 2001, Pandey et al. 2005, Das et al. 2006). In humans, such co-infections have been noted with *inter alia* leishmaniasis, trichomoniasis, malaria, toxoplasmosis, toxocarasis, schistosomiasis, hydatosis, echinococcosis and giardiasis (Li et al. 2013). However, within animals, the problem has been only reviewed in the case of *Fasciola hepatica* (Lucena et al. 2017, Howell et al. 2019). Macroparasite infections can affect the host immune response to microparasite infections including the host’s susceptibility and the course...
and severity of the disease (Su et al. 2005, Ayash-Rashkovsky et al. 2007). Th1 cells produce cytokines that enhance immune mechanisms directed against intracellular microparasites, and Th2 cells produce cytokines which are involved in the immune response against macroparasites (Mosmann and Sad 1996). It is known that co-infections with Fasciola spp. or strongyles may affect the host immune response by inducing a strong Th2 cell response, thus inhibiting the Th1 cell response to tuberculin.

Considering the public health implications of co-infection, as well as its potential diagnostic impact, the aim of this article was to review cases of any parasitosis and tuberculosis co-infections in ungulates, with particular consideration paid to the potential threat to European bison (Bison bonasus). This species is beset by numerous cases of BTB (Radulski et al. 2019) and most methods of ante-mortem testing in this species lack validation; therefore, there is a need to better understand the possible impact of parasite invasions on the course of BTB, and the possibility of its control and diagnostics. As no such studies have been published on co-infections in European bison, the present review focuses on closely-related species, such as cattle and buffalo, and those that inhabit a similar niche, such as wild boars.

**Materials and Methods**

PubMed and Google scholar (any date to October 2021) were searched for studies of co-infection of tuberculosis and parasitic diseases in animals. The following keywords were used for the search: bovine tuberculosis together with each of the terms parasite, fasciolosis, nematodes, co-infection, animals, as well as Mycobacterium and parasites, co-infection. The references of these articles were also reviewed to identify potential additional articles.

Papers which were not written in English or Polish were excluded. No formal meta-analysis or statistical analysis was conducted due to insufficient results. The data were extracted independently by two reviewers. When analyzing the results, the following themes were included: the influence of co-infections on the diagnosis of BTB, the advancement of gross lesions, the influence of co-infection on other species of parasites, and the health and mortality of the host. As the review on Fasciola hepatica and BTB is quite recent, we focused primarily on other possible co-infections.

**Research findings**

Over 100 papers were retrieved and 38 papers were finally included in the analysis. Only a few animal species matched the search criteria: cattle, African buffalo, and wild boar. The parasites considered were Trematoda (e.g. Fasciola hepatica, Schistosoma spp., Paramphistomum spp.), strongyle nematodes (e.g. Metastrongylus spp.), coccidia and tick-borne parasites (e.g. Anaplasma spp.). The influence of mutual infections of BTB and parasites has been described for mortality rate, course and severity of BTB lesions, efficiency of diagnostic methods of BTB, immune response of the host and gastrointestinal microbiota of infected animals (Fig. 1).

**Influence of co-infections on mortality rate, course and severity of BTB lesions**

To effectively manage and control BTB, a zoonotic disease spread between livestock and wildlife, it is necessary to identify the host reservoir (Gortazar et al. 2007). It has been shown that the wild boar (Sus scrofa) is a wildlife reservoir of BTB which might facilitate the transmission of the disease to other species (Naranjo et al. 2008). Infestation with Metastrongylus spp. has been found to be positively correlated with the severity of BTB in wild boar (Risco et al. 2014). In addition, co-infection of Mycobacterium bovis with other pathogens, e.g. helminths, may reactivate the progression of BTB in previously-infected hosts (Diedrich et al. 2011) or accelerate the progression of lesions in recently-infected hosts (Elias et al. 2005a). Moreover, co-infection with Metastrongylus spp. may affect the progression of BTB in wild boar and increase the nasal shedding of Mycobacterium tuberculosis, especially in animals with generalized TB patterns (Risco et al. 2019). Interestingly, in a more recent study of the immune response in wild boar, Lopes et al. (2021) failed to confirm any association between Metastrongylus spp. infection and BTB lesion patterns. Importantly, macroscopic BTB lesions were detected in only 10.3% of BTB-positive animals; this remains a serious concern for preliminary diagnostics (Lopes et al. 2021).

In addition, it has been suggested that deworming may help reduce the prevalence of BTB in exposed populations of wild boar (Risco et al. 2014); however, the effectiveness of antiparasitic treatment as a disease control measure appears to differ between wildlife and humans. Although limiting the parasite burden may reduce host morbidity caused by parasitic diseases, the influence on co-infections and their outcome remains unclear (Fenton 2013).

Although no studies have been performed on the influence of gastrointestinal nematodes on the progression of BTB in wild boar, this relationship has been described in other species e.g. African buffalo (Syncerus caffer) (Ezenwa et al. 2010, Risco et al. 2014).

African buffalo co-infected with gastrointestinal nematodes and BTB demonstrated higher mortality...
A review of tuberculosis and parasitic disease co-infection... than those with a single infection or uninfected hosts, which would limit the number of co-infected animals and reduce exposure to BTB among susceptible buffalo. The authors suggest that co-infections may interfere with the pattern of BTB progression, and that parasitic infections could influence detectability of BTB (Jolles et al., 2008). A different study on African buffalo found that parasite resistance and anthelmintic treatment have different influences on the progression of BTB infection. Firstly, more severe disease progression and higher mortality rates have been observed for parasite-resistant buffalo than for individuals treated with anthelmintics. Secondly, parasite resistance was found to influence the pathological changes associated with BTB in the lungs, while anthelmintic treatment resulted in changes in the lymph nodes; this implies that BTB progression is based on a diverse range of mechanisms, and different ones govern parasite resistance and treatment (Ezenwa et al. 2021). Another study on free-ranging African buffalo found anthelmintic treatment to have no influence on the probability of BTB infection, although it was useful in predicting mortality risk after BTB infection: the dewormed animals demonstrated a ninefold greater survival rate compared to BTB-infected buffalo.

**Influence of co-infections on diagnostics of BTB**

A preliminary article by Ameni and Medhin (2000) indicated that heifers infected with gastrointestinal parasites reacted more strongly to Tuberculin Skin Test (TST) than those that were not. In addition, while the heifers infected with *Fasciola* spp. or strongyle showed a significantly lower response to TST than uninfected animals, no such lowered response to TST was observed for those infected with *Paramphistomum* spp., coccidia or schistosome.

Fasciolosis is known to lead to losses in livestock production, and is also considered to be a re-emerging disease in humans. In animals, the disease most often occurs in cattle, sheep and goats (Beesley et al. 2018). *Fasciola hepatica* (liver fluke) infection has been found to result in false negative TST and interferon-gamma release assays (IGRA) results in the Bovidae (Kita et al. 2013, Garza-Cuartero et al. 2016). In studies conducted in England and Wales, it was noted that 1/3 of cattle positive for both *M. bovis* and *F. hepatica* showed a false negative TST result, implying they had significantly lower sensitivity for TST (Claridge et al. 2012). In cattle experimentally infected with *M. bovis* bacille Calmette-Guérin (BCG) and *F. hepatica*, negative results were obtained for seven out of nine animals for TST, and eight out of nine animals for IGRA. In calves, those infected with *F. hepatica* demonstrated 42% lower sensitivity to TST compared to uninfected calves (Flynn et al. 2007). However, a recently-published review concluded that while *F. hepatica* infections may have an impact on diagnosis with both TST and IGRA, the practical significance is rather low, with greater effects being observed on older and dairy cattle (Howell et al. 2019).

Interestingly, it turns out that *F. hepatica* infection may affect the immune response not only on the course of BTB, but also on paratuberculosis (Byrne et al. 2017, Lucena et al. 2017). Although little is known about BTB co-infection with other *Fasciola* species, it can be assumed that the phenomena are similar. One study found an association between the occurrence of BTB gross lesions and the presence of *Fasciola* infection, and shows that *F. gigantica*-positive cattle demonstrate a slightly lower IFN-γ response to testing (Kelly et al. 2018).

**Influence of co-infections on immune response**

Parasites may regulate the host’s immune response, ensuring themselves niches supporting their survival (Maizels et al. 2004). Helminths induce a strong Th2 response directed against macroparasites and inhibit the Th1 response, which can impair immunity to intracellular microparasites (Mosmann and Sad 1996, Else and
Finkelman 1998). Studies on cattle indicate that infection with Fasciola spp. or strongyle induce a strong Th2 cell response, thus inhibiting the Th1 cell response to tuberculin, and also indicate that animals should be dewormed before TST is conducted (Ameni and Medhin 2000). The false negative results to TST and IGRA tests could result from the simultaneous increase in IL-4, IL-10 and TGF-B level in the blood, together with a reduction in gamma interferon, known to play a key role in the cellular immune response in tuberculosis (Flesch and Kaufman 1993). The production of IL-4 by Th2 lymphocytes is known to play a significant role in the immune response to F. hepatica, which is also characterized by an elevated humoral immune response stimulated by the production of IgG-1 and IgG-2 (Brady et al. 1999).

Wildlife are commonly infected with multiple pathogens at a time, and such interactions occurring between pathogens could influence disease outcomes. An early study of the immune-mediated link between parasitic and bacterial infection in a free-ranging wildlife population revealed cross-regulated immunity between gastrointestinal nematodes and BTB in African buffalo (Syncerus caffer). In African buffalo, helminth infection appears to affect the host immune response by stimulating a strong Th2 response while impairing the Th1 response (Ezenwa 2010), in a similar way as previously described for cattle (Ameni and Medhin 2000). The tested buffalo that were more resistant to nematode infection tended to demonstrate a lowered Th1 response. Successful anthelminthic treatment was found to boost the Th1 response. This appeared to alter the BTB dynamics, preventing M. bovis from invading the buffalo population. This indicates that nematodes play a significant role in BTB disease dynamics (Ezenwa 2010). The mortality rate of buffalo infected with BTB was higher than in dewormed animals, which also presented stronger gamma interferon responses than the infected control groups, which may be attributed to the increased Th1 immunity conferred by anthelmintic treatment. This suggests that anthelmintic treatment might enhance the spread of M. bovis in the population by increasing the survival time of BTB-positive animals (Ezenwa and Jolles 2015, Ezenwa 2016).

BTB can also be managed by the induction of alternatively-activated macrophages (AAMs) in the lungs during parasite co-infection (Potian et al. 2011, Monin et al. 2015). In addition to enhancing host resistance to parasites (Anthony et al. 2006, Kreider et al. 2007), AAMs express arginase-1, an enzyme which affects the outcome of BTB infection, even in the absence of parasite infection (El Kasmi et al. 2008, Monin et al. 2015, Ezenwa et al. 2021).

Furthermore, as described for other animals, the interaction between M. bovis and Metastrongylus spp. may impair the efficacy of the vaccine against BTB in wild boar (Garrido et al. 2011) due to the suppression of the Th1 immune response by helminth infection (Borkow and Bentwich 2004, Elias et al. 2005b).

Influence of co-infections on gastrointestinal microbiota of BTB-infected animals

Studies indicate that taxonomic and functional parasite richness increase significantly in BTB-infected African buffalo. The presence of M. bovis appears to alter the parasite composition, leading to an increase in directly-transmitted, quickly-replicating taxa with simple life cycles. This suggests that a specific profile of pathogens may be favored by BTB infection (Beechler et al. 2019). Moreover, buffalo were found to demonstrate a small but significant decrease in strongyles and Anaplasma marginale after BTB infection. The authors suggest that the observed decrease in strongyles may be caused by the coinfected mortality in the buffalo (Beechler et al. 2019).

It has also been found that co-infection with BTB and gastrointestinal nematodes resulted in shifts in microbiota composition, and that these changes depended on both the presence of other pathogens and the duration of the infection; in addition, the duration of BTB infection appeared to have a greater influence on these shifts than BTB alone. Interestingly, it was suggested that the effects of the BTB infection on the microbiota can change over time: microbial diversity increased in the first 12 months of BTB infection and decreased 12-18 months after the infection (Sabey et al. 2020).

Results analysis

The European bison (Bison bonasus) is particularly susceptible to Mycobacterium tuberculosis complex (MTBC) infection; indeed, it has been the most common source of bovine tuberculosis mycobacteria isolation in Poland in recent years (Radulski et al. 2019). Between 1996 and 2013, 45 cases of tuberculosis were confirmed microbiologically in this species in the Bieszczady Mountains in two different herds (Welz et al. 2005). Cases of BTB have also been confirmed in captive European bison, most recently Mycobacterium caprae in the Smardzewice Bison Breeding Center (central Poland) in 2018 (Didkowska et al. 2020, 2021). Hence BTB remains an urgent issue in European bison and, considering its endangered status, this state of affairs requires continuous monitoring. Additionally, it should be emphasized that BTB is a dangerous zoonosis, and effective control in ani-
mals is needed to protect humans in accordance with the principle of ‘One Health’.

The main challenge remains the diagnosis of BTB in European bison. This presents many difficulties, such as those associated with the ante-mortem collection of material and the lack of standardization of methods. In recent years, the algorithm has been extended to include new methods of material collection and testing (Didkowska et al. 2020, 2021), but no studies have examined the influence of parasitic infestation on its effectiveness.

The parasitic fauna of European bison is quite well recognized and its members are shared with livestock and wild Cervidae (Karbowiak et al. 2014a,b) as well as in other wildlife species (Ichikawa-Seki et al. 2017). For example, most nematodes found in buffalo have been previously described in livestock as well as other wild bovid species (Espie 1999, Ezenwa 2003).

No data exist currently regarding parasite and mycobacteria co-infection in European bison; any conclusions have to be drawn on the basis of data obtained from studies in other related species (buffalo, cattle) and those occurring in a common niche (wild boar). The most commonly-used indirect ante-mortem approaches for monitoring BTB in European bison are IGRA and the tuberculin skin test (Anusz et al. 2017). Studies on other species indicate that the results may be influenced by parasite infestation (Howell et al. 2019), therefore, it seems reasonable to assume this may also be the case in European bison.

Co-infection of parasite and TB has not yet been studied in European bison, and undertaking research in this area seems to be fully justified. Therefore, we recommend conducting parasitological diagnostics, with particular emphasis on *F. hepatica* in any animal suspected of carrying MTBC. These findings also seem to imply that European bison with certain parasites may therefore be more susceptible to mycobacterial infection or the development of disease.

With no comprehensive understanding of the association between the coexisting pathogens, deworming programs may have an undesired influence on the severity and spread of diseases (Fenton 2013).

**Conclusion**

Parasitic infestations may have a multidirectional effect on tuberculosis infection in ungulates. Therefore, we recommend implementing parasitic monitoring in parallel with tuberculosis monitoring in European bison. Most importantly, in individuals suspected of carrying *M. bovis* or *M. caprae*, the possible impact of co-infection on diagnostics should be taken into account.

**References**


M. Gałązka et al.
A review of tuberculosis and parasitic disease co-infection...