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Commentary

Why is the Obvious not Obvious, it is Johne's Disease (Paratuberculosis) Not Crohn's Disease

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Introduction

It is not clear why the obvious is not obvious until one reviews the chronicle of events leading to the debate over whether *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) is the causative agent of an inflammatory enteritis that occurs in cattle and other species including humans, incorrectly referred to as Crohn's disease (CD) instead of Johne's disease (JD), paratuberculosis (Ptb). First is understanding how information has been assimilated on *Map* following discovery that *Map* is the causative agent of Johne's disease (JD) in cattle. Second is understanding the difficulties encountered in documenting *Map* is the causative agent of an inflammatory enteritis in humans that emerged in parallel with the introduction and spread of *Map* in livestock and associated environments. The third is understanding the mechanisms of immunopathogenesis of JD and progress in of control of *Map* infection in humans and livestock.

Discovery and characterization of *Mycobacterium avium* subsp. *paratuberculosis*

There is no recorded history to establish when *Map* and other mycobacterial pathogens were first introduced into livestock during the dawn of civilization. Similar to other mycobacterial pathogens, infection with *Map* leads to development of a latent infection under immune control. Immune control may persist or become compromised by poorly understood factors leading to clinical disease, regardless of species. Lack of understanding of infectious diseases during the transition from hunting and foraging to farming and herding led to establishment of sub-clinically infected animals that served as the original source of exposure and infection with *Map*. Studies suggest this historical event might have occurred in the Mediterranean Basin. Goats, sheep, and cattle were domesticated in this broad geographic region ~10,500 years BP [1-3]. A gap in knowledge remains in the types of mycobacteria present in the environment during this era. Comingling would have exposed animals and humans to whatever was present. Recorded history began at a later date. A debilitating intestinal enteritis of unknown etiology was recognized in cattle well before methods were developed to identify the causative agent. As summarized in an excellent history of *Map* https://johnes.org/history/1910-1930.html, the first description of an agent associated with an enteritis in cattle at the clinical stage of disease was reported in 1895 by a veterinarian in Germany, HA Johne, in collaboration with a visiting investigator from the United States, L Frothingham, who identified an acid fast bacterium in tissues from a malnourished emaciated cow with persistent diarrhea [4]. In contrast with *Mycobacterium tuberculosis* (*Mtb*) it proved difficult to culture. It also did not cause disease in guinea pigs, used to diagnose tuberculosis in cattle and humans. Subsequent investigations in the early 1900s revealed cattle infected with *Map* could be distinguished from cattle infected with *M. bovis* (*Mbv*) by difference in response to a skin test elicited by an antigenic extract, tuberculin, prepared from *Mbv* and *Mycobacterium avium* subsp. *avium* (*Maa*), the causative agent of mycobacterial infection in birds. Although positive in the skin test with avian tuberculin, it could not be cultured under methods used to culture *Maa*,

distinguishing *Map* from *Maa*. A final opportunistic observation led to the discovery that *Map* could be grown when culture media were supplemented with a growth factor no longer produced by *Map*, bacterin. The ability to grow *Map* in culture was followed in ensuing years by the further characterization and classification of *Map* as a member of a much larger phylogenetically related group of mycobacteria, the *M. avium* complex (MAC) [5,6]. Of importance, the grouping includes members considered to be nonpathogenic, opportunistic, and totally pathogenic with a broad host range. Comparative analysis of *Maa* with *Map* indicates a separation of the two lineages occurred with *Map* forming a separate branch [7,8]. Sequencing of the genome [9,10] facilitated conduct of an extensive series of studies to delineate the genomic relation of *Map* obtained from different species. The finding of two major groupings of *Map* that distinguish variants isolated from sheep (S) and cattle (C) indicate introduction of the founding form of *Map* into livestock occurred early during the domestication of livestock [11]. As with *M. tuberculosis* (*Mtb*), the founding form of *Map* could have been introduced from humans into livestock [12]. This supposition would suggest the mutation that gave rise to the S and C forms of *Map* occurred at an early date during the dawn of civilization. Humans would have been the initial host and source of infection during domestication of livestock. This is an interesting possibility and challenge for interpreting findings from recent investigations. Current studies have focused on determining the host range susceptibility to infection with *Map* and documenting interspecies transmission. Methods that distinguish the genetic signature of isolates obtained from domestic species and wildlife reservoirs has provided evidence of interspecies transmission [13]. Evidence of human susceptibility to *Map* from interspecies transmission has also been documented [14,15]. The salient finding from the cumulative studies is that susceptibility to infection by members of both the *Mycobacterium tuberculosis* complex (MTBC) of pathogens and the MAC is not unique. What has not been fully appreciated is that infection leads to development of an immune response that may induce sterile immunity or a response that controls but does not eliminate the pathogen. Depending on the host, the immune response may be sufficient to control infection for a lifetime or until the immune response is compromised. This is the case for tuberculosis and paratuberculosis (JD). Both diseases have become major disease problems worldwide because latency is the usual outcome of exposure. With available methods of detection it is not possible to detect animals at the early stage of infection as well as during the early stages of latency. This has led to the inadvertent spread of *Map* through introduction of infected animals into herds of animals previously unexposed to *Map*, with the best example being the introduction and spread of *Map* in the Czech Republic after the fall of the Soviet Union in 1989 [16]. Prevalence in dairy herds currently provides a continuous source of exposure.

First recorded report of an inflammatory ileitis associated with infection with *Map*

As it became apparent that *Map* is the causative agent of an emerging chronic wasting disease in cattle, an astute observation was made and reported in 1913 by a surgeon in Scotland, TK Dalziel, that a new form of enteritis was beginning to appear with similarity to an enteritis caused by *Mtb* in humans [17]. Although the pathology looked identical to tubercular enteritis, no bacteria could be identified by microscopy. Also, the diagnostic use of guinea pigs to determine whether the causative agent was *Mtb* yielded negative results. This led to the consideration that another mycobacterial pathogen might be the causative agent. Based on studies reported by a veterinarian, McFadyan, in the Journal of Comparative Pathology and Therapeutics, Dalziel proposed the enteritis might be attributable to a mycobacterium, referred to as pseudotuberculosis, the causative agent of enteritis in cattle [17]. By visual inspection, the gross appearance of affected tissues from cattle and humans appeared indistinguishable, strongly supporting the proposition that the same mycobacterium might be the causative agent in cattle and humans. The inability to detect *Map* in feces or tissue, however, raised issues as to whether *Map* was the causative agent of this new unique enteritis beginning to appear in humans. Further characterization of this form of enteritis occurred during the ensuing years led by B. B. Crohn, a major participant in the development of the specialty gastroenterology. He and his associates described the clinical features of the emerging new form of enteritis that set it apart from other disorders involving the gastrointestinal track (reprinted article first published in 1932 [18]). He and his colleagues were aware of the suggestion made by Dalziel that a mycobacterium might be the causative agent, but they, like other contemporary investigators, were unable to detect a mycobacterium in feces or tissue with available technologies. This gave rise to the supposition that this new form of enteritis, which became known as

Crohn's disease (CD), was attributable to other causative factors. This thought became entrenched in the minds of the medical community without any recorded evidence that all available methods were used to rule out the possibility that *Map* was the causative agent of the new form of enteritis such as use of the avian tuberculin test or antibody tests used to detect antibodies to *Map* in cattle with JD. It appears they were not aware of the finding that *Map* could be cultured in media supplemented with bacterin.

The seminal evidence that *Map* might actually be the etiologic agent of the emerging form of ileitis in humans was reported decades later by R. Chiodini, a graduate student at the time, who isolated a mycobacterium from patients with the clinical features of CD [19,20]. Unlike *Map* isolated from cattle, the initial isolates obtained from humans were a spheroplastic form of a mycobacterium without a cell wall, not detectable by acid fast staining [21]. On culture, however, the isolates regained the capacity to form a cell wall (a known characteristic of mycobacteria reviewed in [22]). A preliminary report by Van Kruinigan., *et al.* demonstrated passage of one of the isolates, Linda, into goats led to development of a clinical enteritis identical to JD [23]. Concurrent studies by Mc Fadden., *et al.* revealed the unclassified mycobacterium isolated from humans was *Map* [24,25]. Difficulty by other contemporary investigators to isolate *Map* from patients diagnosed with CD strengthened the view that *Map* is not the causative agent of CD. However, many of the technological difficulties in detecting *Map* in patients with clinical enteritis diagnosed as CD have been overcome since Chiodini's initial findings. A successive series of studies have demonstrated the presence of antibody to *Map* antigens in serum from patients with CD [26], then presence of *Map* in blood from patients with CD [27] and concurrently, detection of *Map* DNA in affected tissues from the intestine [28-33]. Although the investigators were attempting to link the presence of *Map* with the occurrence of CD they were actually providing data to show the patients had JD caused by *Map*. A review by Naser provides an excellent summary of many of these studies [34]. One of the apparent confounding issues during this era of investigation was the finding of *Map* in patients with other diseases and subjects with no clinical disease, enlisted in studies as controls. This was interpreted as evidence that *Map* is not the causative agent of CD. What was overlooked is the similarity of the pathogenesis of ileitis caused by *Map* with pathogenesis of tuberculosis caused by *Mtb* in humans. Infection with both pathogens leads to development of a latent infection under immune control. Disruption of protective immunity leads to development of clinical disease. An extensive study by Singh., *et al.* in India where *Map* is endemic in livestock and a contaminant in the environment have shown humans can be infected with *Map* regardless of health status, with some subjects presenting with an ileitis caused by infection with *Map* [33].

Diagnosis and control of JD (Ptb) in humans and livestock

Cumulative studies indicating *Map* is a member of the MAC that has coevolved with humans starting sometime during the dawn of civilization provides a different frame of reference for moving forward with approaches to control infection with *Map* [1,35,36]. For future studies it will be more appropriate to refer to infection with *Map* as paratuberculosis (Ptb) not JD, except for historical reference to the first description of *Map* by Johne and Frothingham [4]. For description of humans clearly infected with *Map* presenting with a clinical enteritis, CD is a misnomer. There may be other forms of ileitis caused by other factors. These forms of ileitis must be documented and distinguished from ileitis caused by *Map* to formulate a correct therapy. The current problem with Ptb, is attributable to movement of latently infected livestock into *Map* free areas best illustrated by recent events in the Czech Republic [16]. The dairy industry has been significantly impacted by introduction of latently infected cows into *Map* free dairy herds worldwide, increasing exposure of the general public to *Map* through dairy and meat products as well as contaminated environments e.g. Singh., *et al* [33].

A foundation has been established in recognition that *Map* is zoonotic and the causative agent of an inflammatory enteritis in humans https://humanpara.org/the-long-road-to-the-development-of-a-peptide-based-vaccine-for-mycobacterium-avium-paratuberculosis/. It has provided a forum for review of the current status of research on Ptb in humans and livestock and means of control. Past efforts to detect latently infected animals before they begin to shed bacteria have not been successful, indicating vaccination is the only way to clear *Map* from livestock. Studies with killed vaccines have shown it should be possible to develop a vaccine that elicits a sterile immunity that prevents shedding of bacteria [37-40]. Recent studies suggest efficacy might be improved with use of a live attenuated vaccine [41,42] or a peptide based vaccine [43,44]. An efficacious vaccine would aid in removal of one mycobacterial pathogen affecting animals and humans. Establishment of standard methods of diagnosis of Ptb in humans is essential. Other members of the MAC can also infect animals and humans and cause disease [45-49]. None of the methods currently used in research have been standardized for use in diagnosis of Ptb in humans. However, verification that *Map* is a zoonotic pathogen has increased attention on the need of standard methods for diagnosis.

Because of the ongoing uncertainty that *Map* is the causative agent of a major form of inflammatory ileitis in humans two strategies are being employed for therapy, one focused on suppression the immune response with anti-inflammatory drugs and antibodies based on the assumption that ileitis is attributed to an autoimmune disorder and the other focused on use of combinations of antibiotics on the assumption that the ileitis is caused by a pathogen, *Map*. It is not clear if any comparative studies are in progress to determine the efficacy of any of the anti-inflammatory therapies for treatment of inflammatory enteritis. There is a major study underway to determine the efficacy of antibiotics sponsored by RedHill Biopharma Limited, Tel Aviv, Israel, 'Efficacy and Safety of Anti-*Map* Therapy in Adult Crohn's Disease (MAPUS)' https://clinicaltrials.gov/ct2/show/NCT01951326?term=crohns+disease%2C+MAP+USrank=1. Human Para Foundation provides a forum further discussion of the role of *Map* in phylogenetic history of humans during the domestication of livestock https://humanpara.org/the-long-road-to-the-development-of-a-peptide-based-vaccine-for-mycobacterium-avium-paratuberculosis/. The bringing together of clinicians and research scientists should facilitate development of methods to eliminate *Map* from the food stream and development of effective therapies to treat humans with Ptb [50].

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Conflict of Interest

I declare no conflicts of interest.

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