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BACTERIOLOGY (N BOREL, SECTION EDITOR)

Mycobacterium avium subsp. *paratuberculosis* – An Overview of the Publications from 2011 to 2016

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Abstract
Purpose of review The goal was to evaluate the trend in publications related to *Mycobacterium avium* subsp. *paratuberculosis* (MAP) published between 2011 and mid-2016.
Recent findings The publication search was performed using the Web of Science® (WOS) database. Papers were split into categories dealing with i) methods for MAP detection, ii) immune responses to MAP, iii) MAP hosts, iv) prevalence and control, v) food and vi) zoonosis. In 2013, the highest number of papers was published in almost all categories. In all years, papers focused on methods, immunity or hosts predominated. Reports on zoonotic aspects of MAP were mostly published in 2011. Possible connection of MAP with other autoimmune, but non-intestinal diseases was suggested after 2011. MAP was also discovered in new animal species.
Summary It is evident that MAP still plays an important role in many animal species. Its connection with Crohn's disease and other autoimmune diseases is still under discussion.

Keywords *Mycobacterium avium* subsp. *paratuberculosis* · Paper · Search · Epidemiology · Food · Zoonosis

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Introduction

Paratuberculosis or Johne's disease is a disease with a long history. "Wasting or consumptive" disease was first reported in 1807 in cattle by Edward Skellet. The changes in the intestine of infected cattle were described by Johne and Frothingham in 1895. They proposed the name "pseudotuberculous enteritis" for the disease. Awareness of paratuberculosis increased during the following decades. In 1923, *Mycobacterium paratuberculosis* was officially named as the causative agent of paratuberculosis. In the early 1900s, paratuberculosis was described in cattle in the USA. From the 1970s onwards paratuberculosis has been distributed worldwide. In the beginning of the 1900s, Dr. Dalziel published his hypothesis that *Mycobacterium avium* subsp. *paratuberculosis* (MAP) may have zoonotic potential. This interest was re-stimulated by a review by Chiodini [1].

Paratuberculosis was first reported in cattle, but all ruminant and non-ruminant species can be infected. The typical symptoms include diarrhoea, weight loss, emaciation, and decreased milk production. These symptoms, however, occur in animals in the late stage of infection. The late stage of the disease is preceded by a significantly longer period of "silent" and inapparent infection [2]. Animals without clinical signs shed MAP through faeces into the environment and are a source of infection for healthy animals. Apart from this, vertical transmission and feeding of contaminated milk and colostrum represent other important routes of infection [2]. In wildlife ruminants and mammals the clinical phase is mostly featureless or entirely absent [3].

Paratuberculosis causes significant financial losses for cattle farmers [4•]. These losses are mainly connected with lower milk yield, decreased meat production and associated costs (veterinarians, laboratory, slaughterhouse, herd restoration) [4•]. Treatment of paratuberculosis is not possible. With the aim of limiting the spread of infection, paratuberculosis control programmes (PCP) have been implemented. In most countries, the implementation of PCP is voluntary. The success of a PCP depends both on its effectiveness and on the consistency of the herd owner in implementing the programme [5]. Vaccination against paratuberculosis can be effective, but must be accompanied by management changes in the herd [6].

Apart from economic losses, the presence of paratuberculosis in farms for food production is a major concern due to the possible association of MAP with Crohn's disease. Crohn's disease is a chronic, systemic disease of the intestinal tract. Due to the similarity of symptoms with those in ruminants, MAP has been implicated in the pathogenesis of Crohn's disease in humans. This theory has many supporters, but also opponents, which mean that the situation is still unclear. Similarly as in animals, humans can be infected through food derived from infected animals (milk, meat) or water contaminated with faeces from MAP-positive animals. Apart from Crohn's disease, the possible connection of MAP and Blau syndrome, or type 1 diabetes mellitus was suggested.

In the past, the Web of Science® (WOS) portal was utilised to analyse publications regarding mycobacteria in food and feed [7] or in water, soil and plants [8]. The aim of this paper was to assess the trend in scientific publications regarding paratuberculosis. In our analysis we attempted to cover the most important issues of paratuberculosis research, such as methods of detection, immune response of hosts to the agent, epidemiology and paratuberculosis as a zoonosis. The search was limited to papers published after 2011 as a similar analysis was not carried out after this year.

Web of Science Database

The Web of Science® (WOS) Core Collection database (Thomson Reuters) was used to retrieve the publications relating to MAP. The WOS provides access to the following indexes: Science Citation Index (SCI), Social Sciences Citation Index (SSCI), Arts & Humanities Citation Index (A&HCI), Conference Proceedings Citation Index, Emerging Sources Citation Index, Current Chemical Reactions and Index Chemicus. The search encompassed papers published during the period of 2011–2016 (August the 5th). The search within WOS was split into certain categories: i) methods of detection, ii) immune response, iii) MAP hosts, iv) prevalence and control, v) food and vi) zoonosis. A specific search profile was used for each category as a "Topic" word (menu in WOS database). The results were then refined (utility "Refine") using specific sub-keywords (Table 1). The "Analyse results" utility was applied when comparing the number of papers published in individual years in each category.

The "topic" keywords were selected to retrieve the maximum number of papers published in each category from 2011 to mid- 2016. However, for this reason, many papers were duplicated among certain categories. As the keywords for search profiles were rather general, we are aware that some non-relevant publications could be retrieved.

From each category, the most important papers or papers reporting novel information were selected and are discussed in the text.

Paratuberculosis and Johne's Disese

In general, 1464 results were retrieved when "paratuberculosis or johne*" keywords were submitted to the WOS database. To find papers defined by these keywords together with other themes, specific search profiles were created (Table 1).

Methods for MAP Detection

Culture remains the gold standard for MAP detection; accordingly, papers dealing with culture represented the major group of retrieved publications (Table 1). Between 2011 and 2016, the papers were mostly oriented towards the i) development, innovation and validation of cultivation methods [9, 10], ii) comparison of culture with other methods [11] and iii) ability to culture MAP from different matrices [12, 13]. Despite this, MAP detection using culture is increasingly being replaced by other, more modern methods, especially PCR.

Nowadays, the PCR method is involved in practically all areas of MAP research and has found indispensable application in epidemiology, development of new methods of DNA isolation [14], or development, standardization and improvement of new or already existing PCRs [15, 16]. Newly developed PCR-based methods (e.g. high-resolution melting for analysis of short sequence repeats) can be introduced for paratuberculosis detection [17, 18]. Diagnosis based on PCR can be used on a wide variety of matrices such as milk [19], dairy products [20], meat [21], water [22] or environmental samples [23]. Further refining of papers containing PCR showed, that most PCR articles dealt with faeces (167 results), milk (145), tissue (99), environment (76) and water (35).

Using the keyword "ELISA" 290 articles were found (Table 1). For diagnosis of paratuberculosis, ELISA is most commonly utilized due to its ability to analyse many samples in a short time for a low price. It was also applied to determine the in-herd prevalence based on samples of bulk tank milk [24], or individual milk [25]. In most retrieved publications, ELISA was, however; only mentioned as one of several methods used for diagnosis of MAP in the infected sample/animal [26].

Due to the number of papers found, it seems that there is an increasing focus on the study of gene expression in MAP (Table 1; 216 articles). The gene expression finds its application mainly in the study of differences between examined (treated)

Table 1 Search categories, search profiles and numbers of papers found in the Web of Science® (WOS) database for the period 2011–2016 (August the 5th)

Category	Search profile[a,b]	No. of publications	Refining keywords	No. of publications
Methods	((paratuberculosis or johne*) and (cultiv* or *PCR* or sequenc* or cultur* or assay* or elisa or immuno* or chemic* or express* or transcript* or magnet*))	1064	cult* *PCR* ELISA express* VNTR* magnet*	496 412 290 216 33 29
Immunity	((paratuberculosis or johne*) and (immun* or vaccin* or receptor* or antigen* or elisa* or antibod* or interferon* or macrophag* or lymphocyt* or response* or react* or serolog* or blood* or host* or assay*))	1090	antibod* antigen* vaccin*	292 205 169
Host	((paratuberculosis or johne*) and (ruminant* or animal* or cow* or cattle* or goat* or sheep* or mammal* or wildlife or deer*))	1050	(cattle or cow* or calv* or bull*) (sheep* or goat*) (mammal* or wild* or deer*)	782 242 186
Prevalence and control	((paratuberculosis or johne*) and (distribut* or control* or prevalenc* or spread* or program* or measur* or cost* or finan*))	858	(control* and program*) (herd* and level*) cost* or financ*	148 118 94
Food	((paratuberculosis or johne*) and (food* or meat* or water* or milk* or cheese* or yoghurt*))	426	milk* water* meat* cheese* yoghurt*	338 74 27 19 1
Zoonosis	((paratuberculosis and crohn*) and (zoonos* or patient* or hospital* or medic* or gut* or colit*))	165	diabet* scleros* autism* Parkins*	20 9 3 2

*asterisk can be replaced by any letter during the search

[a] searched as a "Topic" word (menu in WOS)

[b] paratuberculosis (as disease) as well as *Mycobacterium avium* subsp. *paratuberculosis* are retrieved when "paratuberculosis" as a search word is entered into WOS

and control group of animals/samples [27] finding of biomarkers [28], or in the study of the immune response [29].

The VNTR method was used to gain a better understanding of the biodiversity of MAP in infected animals/herds [30], characterisation of MAP isolates from infected animals [31] or in the search for new polymorphisms [32]. Although the method can provide deep insights into the epidemiology of paratuberculosis, limitations still exist due to the inability to identify closely and distantly related isolates [33].

Isolation methods based on magnetic separation improve the process of DNA extraction through the inclusion of multiple/repeated washing steps which enable DNA isolation from matrices containing large amounts of inhibitory compounds. Milk and faeces, the most typical biological samples for MAP DNA isolation, belong to such problematic matrices [34, 35]. The method of magnetic separation was further used when distinguishing between live and dead cells using propidium monoazide [36]. An interesting connection between magnetic separation and serodiagnosis of paratuberculosis was described by Wadhwa *et al.* [37].

Immune Response to MAP

Based on the search results, the category of "immunity" was the widest (1090 results; Table 1, Fig 1). Within this category three sub-searches were applied (Table 1). The findings using the "vaccin*" keyword (169 results) were further separated based on the animal of interest. In small ruminants, vaccination has still been solved [38]. Apart from paratuberculosis, vaccination programs and trends in the development of new vaccines against other significant diseases in small ruminants is well reviewed in Lacasta *et al.* [39]. Vaccination in dairy herds mostly takes place as part of paratuberculosis control programs [40] or as part of research regarding the efficacy of vaccination [41]. From the diagnostic point of view, the

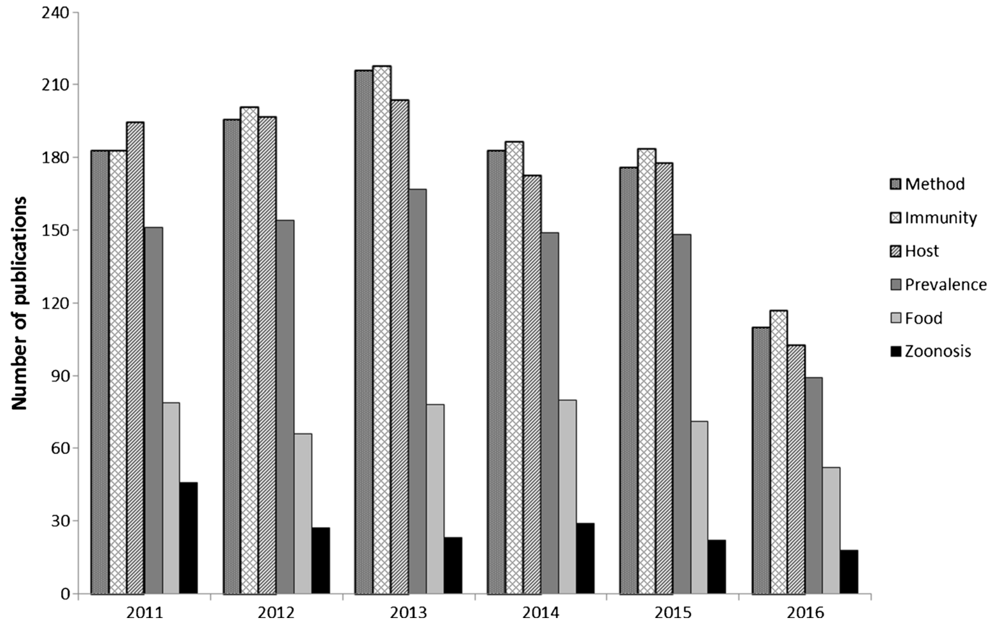

Fig. 1 Papers on *Mycobacterium avium* subsp. *paratuberculosis* (MAP) published between 2011 and 2016 (August 5th). The papers were categorised according to their subject: *i*) methods of MAP detection, *ii*) immune responses to MAP, *iii*) MAP hosts, *iv*) prevalence and control, *v*) food and *vi*) zoonosis

interference of paratuberculosis and bovine tuberculosis in serological tests after vaccination is significant and therefore research on this field should not be neglected [42].

Diagnostics using immunological methods in ruminants requires the discovery of MAP-specific antigens which have been proven to induce the immune response. The significance of this problem is apparent as almost one fifth of articles were refined when using the "antigen" keyword (Table 1). Most of the refined articles referred to the testing of MAP-specific antigens on animals in different infection conditions. Efforts directed towards finding novel MAP antigens (based on secreted proteins, lipopeptids, protoplasmatic antigens, etc.) are ongoing according to papers published between 2011 and 2016 [43]. A slightly larger proportion of papers dealt with antibodies against MAP and antibody response profiles in animals which were naturally or artificially infected by MAP [44].

MAP Hosts

Refining articles using the above search profile resulted in 1050 publications. Most reported the detection of MAP in animals, farms or farm environments, improved methods for agent detection, or the immune response of hosts to paratuberculosis. As all these topics were included in the above categories, only selected papers referring to novel, but also specific and remarkable findings are mentioned in this paragraph. Mortier *et al.* [45] found that not only calves up to six months of age, but also those up to one year old are highly susceptible to MAP infection. Tissue colonisation by MAP in experimentally infected calves can be decreased when gallium is chemoprophylactically applied before and during the period of high susceptibility [46]. On the basis of age and shedding of MAP in faeces, Mitchell *et al.* [47••] suggested three categories of immune control. The phenomenon of passive shedding was studied [48] and different bacterial species were described in the faeces of paratuberculosis infected and non-infected animals [49]. Control programmes are implemented mainly in dairy herds, but are important for beef herds as well [50]. Apart from standard methods, examination of milk filters was suggested as a useful tool for MAP detection before any control strategies [51].

About one quarter of articles (242) were refined when sub-searching for "goat* or sheep*" keywords. More papers dealt with sheep (123 papers) compared to goats (49 papers). The most cited "sheep" papers dealt with the immune response in early stages of infection [52], progression of the disease in naturally exposed animals [53] and culture differentiation of MAP isolates obtained from different regions and hosts [54]. The most cited "goat" papers focused on preparation and evaluation of MAP mutants for vaccine purposes [55•, 56]. The method based on the measurement of volatile organic compounds in the exhaled breath of infected animals appears to be a potentially very interesting tool for paratuberculosis diagnostics [57].

As for the period preceding 2011, a range of current papers (2011–2016) dealt with MAP detection and paratuberculosis infection in wild deer, such as red deer [58, 59], roe deer [58] or elks [60, 61]. Many different animal species–ruminant and non-ruminant–were found to be infected by MAP; most were described in papers published before 2011. However, after 2011, some new hosts of MAP were also revealed (Table 2).

Prevalence and Control

Refining using (herd* and level*) keywords resulted in 118 papers (from 858) between 2011 and 2016. During this period,

Table 2 Detection of *Mycobacterium avium* subsp. *paratuberculosis* in animals other than domestic ruminants determined by microscopy, culture and/or PCR in the period 2011–2016

Species	Country	Citation
Guanaco (*Lama Guanicoe*)	Chile	[62]
Alpaca (Lama Pacos)	Germany	[63]
Eurasian otter (*Lutra lutra*)	Portugal	[64, 65]
Badger (*Meles meles*)	Portugal	[64]
Beech marten (*Martens foina*)	Portugal	[64]
Egyptian mongoose (*Herpestes ichneumon*)	Portugal	[64]
Elk (*Cervus elaphus*)	Canada	[60, 66]
Key deer (*Odocoileus virginianus clavium*)	Florida, USA	[67]
Rusa Deer (*Cervus timorensis rusa*)	Mauritius	[68]
Alpaca (*Vicugna pacos*)	USA	[69]
Chamois (*Rupicapra rupicapra*)	Switzerland	[58]
Tundra Reindeer (*Rangifer tarandus tarandus*)	Scotland	[70]
Wild boar (*Sus scrofa*)	Korea	[71]
Sitatunga antelope (*Tragelaphus spekii*) West African dwarf goat (*Capra aegagrus f. hircus*)	Germany	[72]
Bactrian camel (*Camelus bactrianus*)		
Chapman's zebra (*Equus quagga chapmani*)		
Black-and-white ruffed lemur (*Varecia variegata*)		
Cottontop tamarin (*Saguinus oedipus*)		
Gelada baboon (*Theropithecus gelada*)		
Desmarest's hutia (*Capromys pilorides*)		
Patagonian cavy (*Dolichotis patagonum*)		
Emu (*Dromaius novaehollandiae*)		
Ara parrot		
Snowy owl (*Bubo scandiacus*)		
Vulturine guineafowl (*Acryllium vulturinum*)		
Wallaby (*Macropodidae*)		
Water buffalo (*Bubalus bubalis*)	Brazil	[73]
Camel (*Camelus dromedarius*)	Saudi Arabia	[74, 75]
Fox (*Vulpes vulpes*)	Portugal	[76]
Southern Black Rhinoceros (*Diceros bicornis minor*)	Australia	[77]
Arctic Caribou (*Rangifer Tarandus* Ssp.)	Canada	[78]
Indian monkey (*Rhesus macaques*)	India	[79]
Hare (*Leupus*)	Chile	[80]
	New Zealand	[81]
Brushtail possum (*Trichosurus vulpecula*) Hedgehog (*Erinaceus*)	New Zealand	[81]
Cat (*Fellis*)		
Paradise sheldok (*Tadorna variegata*)		
Rabbit (*Oryctolagus cuniculus*)	New Zealand	[81]
	Spain	[82]

prevalence studies were conducted in different countries using different methods (culture, PCR, ELISA) and matrices (faeces, milk, environmental samples). High between-herd prevalence (HTP) was found, e.g. in cattle in the US (91.1%) [25], Canada (68-76%) [83], Denmark (87-96%) [84] and Northern Italy (around 70%) [85]. In sheep, the HTP was assessed to be 66.8% in Canada [86] and 76% in New Zealand [87]. A HTP of 62.6% was calculated in dairy goats in Canada [86], 74.1% in water buffaloes in Italy [88] and 46% and 42% in New Zealand deer and beef herds, respectively [87].

With the aim of decreasing prevalence levels, PCP have been implemented by individual countries. In most countries, their realisation is voluntary, but in a few countries the implementation of PCP is obligatory. The participation of farmers in voluntary PCP can be supported by governments through incentive measures, e.g. higher milk prices [89]. A survey from selected US farms published in 2015 [90] showed that participation in voluntary PCP was characteristic for farms with larger herds, with high self-assessed knowledge of paratuberculosis and a better understanding of the details of PCP. Control programmes are mainly directed towards cattle [50, 84, 90, 91], but a few were prepared for sheep and goat as well [92, 93].

Paratuberculosis, mainly in dairy cattle, causes significant financial loses for farmers. Financial losses stem from decreased milk production, weight loss and lower meat production, loss of animals and subsequent replacement costs, veterinary treatment and laboratory testing [4]. After 2011, the financial losses of farmers connected with partuberculosis were estimated in a few publications. The extent of financial losses depended on herd size, level of prevalence, participation of farm in PCP or methods used for testing [26, 94, 95]. The economic benefit of vaccination against paratuberculosis in cattle herds was also calculated [96, 97].

MAP and Food

Out of 426 initially retrieved papers, 338 were found when refined by "milk*", 74 when refined by "water*", 27 by "meat*", 19 by "cheese*" and one by "yoghurt*".

In these papers, detection of MAP in food matrices continued as before. However, a few previously untested foods were also examined, e.g. yogurt, acidophilus milk and kefir [20], goat and sheep cheeses [12] or powdered infant formula [98]. Other authors aimed at procedures directed at the reduction of MAP in food. The presence of probiotic cultures led to a reduction in MAP numbers in fermented milk products [20]. Longer ripening time of cheese [99] and extended cooking time of hamburger patties together with higher temperature [100] led to a decrease in MAP counts. Direct steam injection was successfully applied to MAP-contaminated milk [101].

Zoonosis

Between 2011 and mid-2016, 165 publications related to paratuberculosis and Crohn's disease have been published.

As in the past, testing for the presence of MAP in Crohn's patients continues in recently published studies with more [102, 103], or less successful results [104, 105]. Today, it is recognised that MAP is only one of the possible triggers and that other bacteria can be involved as well [106]. Apart from this, other factors such as genetic susceptibility, commensal microflora, viral infection and environmental factors play a significant role in the development of inflammatory bowel diseases, including Crohn's disease [106–108]. Since 2011, a role for MAP in the development of Crohn's disease was discussed and evaluated in a number of reviews [109–112]. Other groups of reviews focused on the assessment of antibiotic treatment [113], effect of probiotics [114] or nutrition [115] in Crohn's disease therapy and etiopathogenesis. Except for Crohn's disease, potential relationships between MAP and type 1 diabetes [116, 117]; and MAP and multiple sclerosis [118, 119] have also been discussed. Dow [120] hypothesized that MAP may be a trigger of autism. In 2016, MAP DNA was found in some patients with Parkinson's disease; however, the mechanism by which MAP might be involved in the disease is unclear [121•].

Conclusion

In total, 1464 papers were retrieved when searching for papers published between 2011 and 2016 using "paratuberculosis and johne". Most papers (1090, 74.5%) were concerned with "immunity". Almost the same number of publications was obtained for "methods". In both categories, the number of publications increased from 2011 to 2013 and decreased slightly from 2014 onwards. From the diagnostic point of view cultivation is the most commonly used method. Although PCR is still not regarded as an equivalent diagnostic method, the number of publications was very close to those concerned with culture. Within the "host" category, MAP presence was described in new animal species. In spite of this, publications regarding cattle are naturally the most numerous. In spite of the application of paratuberculosis control programmes, the incidence of paratuberculosis around the word remains high. The number of "food" publications was stable during the examined period. Milk was the most commonly studied matrix. The possible connection of MAP with Crohn's disease is still under discussion; further, its connection with other, non-intestinal diseases has also been suggested.

As the number of publications was stable (or slightly increasing) during the examined years, it is evident that mycobacteria are still the subject of intense focus in the spheres of animal and human research.

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Compliance with Ethical Standards

Conflict of Interest Radka Dziedzinska and Iva Slana declare they have no competing interests.

Human and Animal Rights and Informed Consent This article contains no studies with human or animal subjects performed by the authors.

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- Of importance
•• Of major importance

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