Systematic review and meta-analysis of Mycobacterium avium subsp. paratuberculosis as environmental trigger of multiple sclerosis

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Highlights

- MAP (Mycobacterium avium subsp. paratuberculosis) has role in MS (multiple sclerosis).
- Efforts channelled to unravel the role of MAP in MS is still limited.
- A total of 2538 MAP-related MS patients' data from 12 studies was meta-analysed.
- MAP was associated with a significant risk of multiple sclerosis.
- MAP and MS link valued at12.76 odds ratios (OR)(antibody) & 5.27OR (DNA).
- Timely screening of MAP in MS cases may benefits therapeutics in its treatment.

Abstract

Mycobacterium avium subsp. paratuberculosis (MAP) has been identified as one of the environmental agents that causes multiple sclerosis (MS). The global prevalence of MS has been upsurging over the years; however, efforts to divulge the role of MAP in MS have been limited. As a result, the present study aimed at assessing the odd ratios (ORs) associated MAP with the risk of MS. MAP-related MS data were obtained from 6 databases using the terms 'multiple sclerosis' or 'MS' and 'paratuberculosis' without regard for time or language restrictions following PRISMA standards. A total of 2,538 participants' data from 12 studies presenting anti-MAP antibodies and MAP DNA from 4 studies were fitted in random-effects (RE) and fixed-effects (FE) meta-analytic models. Furthermore, the between-study heterogeneity was measured using I^2 -values with a significant limit set at an $I^2 > 75\%$.

Analytical rigor and publication bias was determined using leave-one-out-analytics, Egger's tests, and p-curve analysis. In the FE and RE models, anti-MAP antibodies data significantly associated MS risk with MAP as 10.71 OR (95%-CI [7.78; 14.74], p-value < 0.0001) and 12.76 OR (95%-CI [8.13; 20.02], p-value < 0.0001) respectively, with an I^2 value of 34.9% (95%-CI [0.0%; 67.2%]; p-value = 0.11). Similarly, the MAP DNA dataset in FE significantly present MS risk due to MAP as 5.53 OR (95%-CI [3.54; 8.66], p-value< 0.0001) while, RE showed 5.27 OR (95%-CI [3.22; 8.60], $p = 0.0017$), with an I^2 -value = 0.0% (95%-CI [0.0%; 84.7%]; p-value = 0.71). Eggers' test, on the other hand, found publication bias in anti-MAP antibodies data (intercept = 1.61, 95% CI: 0.45 – 2.77, $t = 2.72$, $p = 0.021$), but not in MAP DNA dataset (intercept = -5.57, 95% CI: -20.44 – 9.29, $t = -0.74$, $p = 0.54$). The robustness of the meta-analyses was demonstrated by all sensitivity analyses. In addition, there is no evidence of p-hacking observed (right-skewness test ($P_{Full} < 0.001$, $P_{Half} < 0.001$; statistical power ≥ 94% (95%-CI: 72.5%-99%)). In conclusion, the synthesis revealed a strong association between MAP and MS, indicating that MAP is a significant environmental agent that may trigger MS. Thus, early screening of MAP in MS cases may assist in the therapeutic approach to its management/treatment. Therefore, future studies should be tailored towards the role of MAP in the severity of MS phenotypes, as well as address global data gaps and low disease surveillance.

Keywords: Neuroinfection; Autoantibodies; Neuroimmune; Neuroinflammatory; Neurodegeneration; Myelin damage; Autoimmune disorde

1 Introduction

Multiple sclerosis (MS) is an autoimmune-mediated chronic inflammation, demyelination, and neurological disease characterized by an axonal loss in the central nervous system (Oh et al., 2018). MS causes lesions in the gray and white matter of the spinal cord and the brain that range from focal to diffuse (Lassmann, 2019). MS manifests itself in adulthood, typically with a relapsing-remitting (RR) clinical course at the second, third, or fourth decade of life, with women being affected at a rate 2–3 times that of men (Amato et al., 2017; Lassmann, 2019). RR further converts to a (slow) secondary progressive (SP) course after 10–15 years.

MS begins as a primary progressive (PP) course at a later age, such as the fifth decade of life (Scalfari et al., 2016; Zeydan and Kantarci, 2018; Amato et al., 2017). All MS phenotypes are characterised with cognitive impairments (Johnen et al., 2017; Gouveia et al., 2017; Ruano et al., 2017), the prevalence of which is approximately 33% in patients under the age of 18 years (Julian et al., 2013; Amato et al., 2016) and 34% to 65% in adults (Achiron et al., 2013; Olazar´an et al., 2009; Rao et al., 1991).

MS affects approximately 2.3 million people globally (Global Burden of Disease (GBD), 2015). MS is becoming more prevalent in both high-income and low-income countries (Koch-Henriksen and Sorensen 2010; Browne et al., 2014; Dobson and Giovannoni, 2019). According to the epidemiological data, the global median prevalence of MS is 33/100 thousand people, with North America (140/100 thousand people) and Europe (108/100 thousand people) having the highest prevalence, while, Asia (2.2/100 thousand people) and sub-Saharan African nations (2.1/100 thousand people) experiencing the lowest prevalence (Belbasis et al., 2015). However, there are intra-continental differences in MS prevalence in different regions of Asia, with

85.80/100 000 people, 0.77/100 000 people, and 18.6/100 000 people in Iran, Hong Kong, and northern Japan, respectively (Eskandarieh et al., 2016; Houzen et al., 2018).

MS is characterised by a complex multifactorial etiopathogenesis, with several interconnected factors that increase human susceptibility to the disease, including genes and environmental triggers such as ultraviolet B light (UVB) exposure, vitamin D, obesity, Epstein–Barr virus (EBV) infection and smoking (Ascherio, 2013). Previous studies have linked Mycobacterium avium subsp. paratuberculosis (MAP) to the development of MS and other autoimmune diseases such as Hashimoto's thyroiditis, rheumatoid arthritis, diabetes, Crohn's disease, neuromyelitis optica spectrum disorder, and sarcoidosis is associated with via a molecular mimicry mechanism (Bo et al., 2018, 2019a, Cossu et al., 2011a, 2012, 2013a; D'Amore et al., 2010; Dow and Ellingson, 2010; Niegowska et al., 2016; Sechi et al., 2005; Sechi and Dow, 2015; Bo et al., 2019b;Thomas, 2008; Mameli et al. 2013; Masala et al., 2014).

Several studies have found MAP DNA or humoral antibodies (abs) against MAP in patients with the aforementioned autoimmune diseases compared with healthy controls (Mameli et al. 2013; Masala et al., 2014; Bo et al., 2019a; Cossu et al., 2011a, 2012, 2013a; Niegowska et al., 2016; Sechi and Dow, 2015). Also, anti-MAP abs/MAP DNA have been demonstrated explicitly in MS patients compared to controls (Cossu et al., 2011a, 2012, 2013a,b, 2015, 2016; Frau et al., 2013; Mameli et al., 2014, 2016a; Slavin et al., 2018; Yokoyama et al., 2017). However, there is a lack of conclusive evidence that MAP plays a role in the development of MS. Therefore, the present study aimed to assess the odds ratios (ORs) indicating the risk of MS caused by MAP infections.

2 Materials and methods

2.1 Study design

Research articles on MAP-associated multiple sclerosis cases were systematically searched in ProQuest, PubMed, Web of Science (WoS), Google Scholar, Scopus, and EBSCOhost (including CINAHL, MEDLINE, APA PsycInfo, etc.). The title-specific search algorithm, 'multiple sclerosis' or 'MS' in combination with 'paratuberculosis' was employed according to different provisions available in each database without temporal restrictions (details in appendix 1). Mainly, the search and/or retrieval was executed according to the "Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines" (Moher et al., 2009). To accommodate new records, record download was performed on 29 October 2021 with a repeat on 2 November 2021 (GMT +2) to accommodate new records. The email-alert-tracking of records in all the databases was further utilized. Endnote version 20 was used to de-duplicate documents. The search was carried out by one of the authors, (E.T.C.), who searched and screened the titles/abstracts for inclusion based on the criteria outlined in the subsequent section. Following title/abstract review, the full-text of eligible articles was retrieved for data extraction using a predesigned form guided by outlined criteria and desired data in Sections 2.2 and 2.3 , respectively. For additional records, the reference list of all the potentially eligible studies was reviewed. The full search strategy and PRISMA schema for the study (Fig. 1) are documented in Appendix (1).

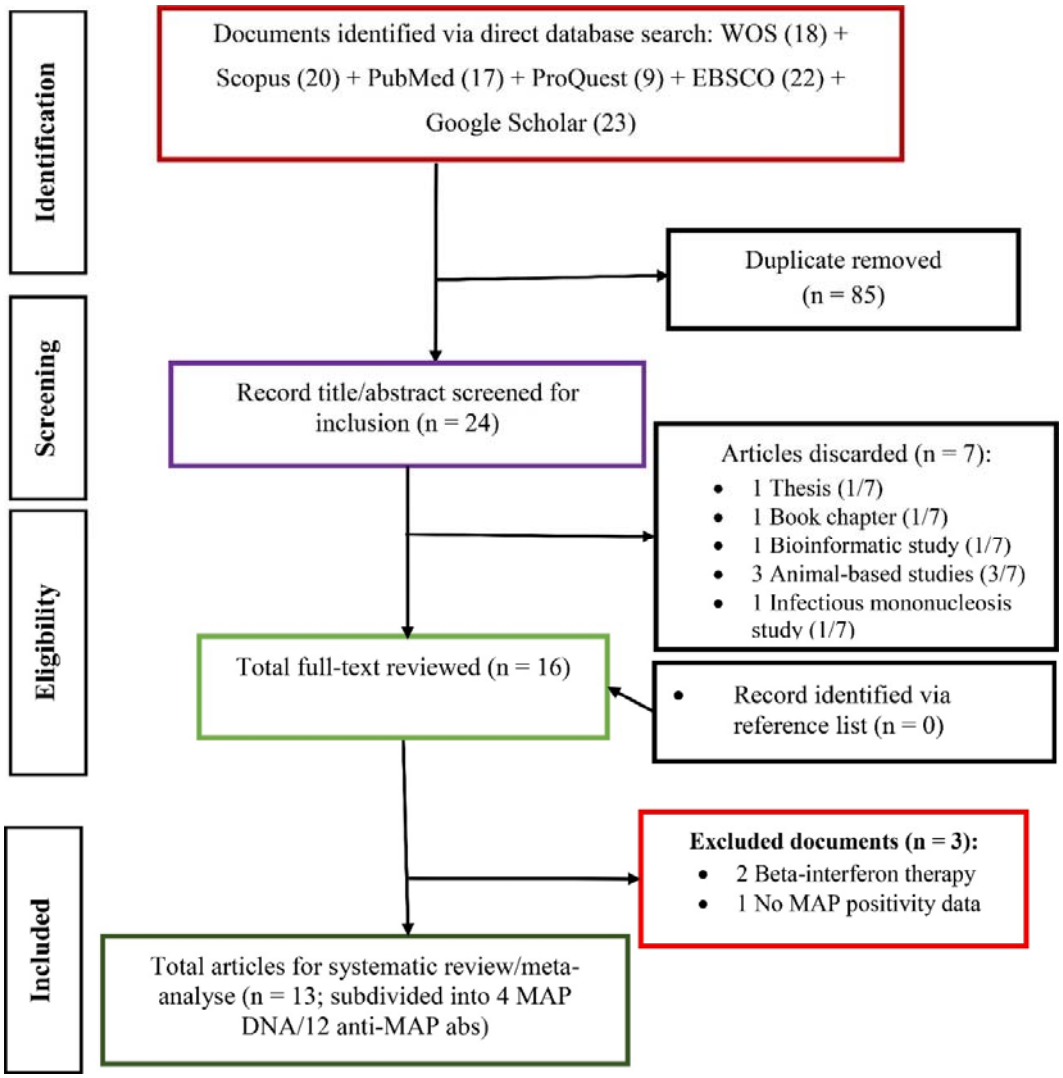

Fig. 1. Schema for selecting studies on MAP-associated multiple sclerosis.

2.2 Inclusion criteria

A study was considered as eligible if it assayed MAP abs/DNA in multiple sclerosis conditions, and detailed the following associated data: 1. number of patients vs. controls (Cs). 2. The number of positive patients vs. Cs; or in the case of a prevalence study, the sample size and number of positive sample(s), and; 3. Adequately describe the assay method used to detect MAP. It should be noted that MAP infection was defined as the presence of anti-MAP abs and/or IS900 PCR positivity, with no distinction made between asymptomatic from symptomatic cases.

2.3 Data extraction

The data extracted from the included studies included authors' name, publication year, country, number of patients and Cs, MAP positivity in MS patients and Cs, MAP assay method, and total MS patients' population versus MAP positivity in the prevalence study. Other variables extracted include MAP antigen assayed, antigens of other pathogens assayed, MS patients age,

Cs age, MS stage (MS_k), MS_{DIA} (MS diagnostic definition/criteria), number of male and female MS patients (M/F_{ms}), number of male and female Cs (M/F_{cs}), EDSS (Expanded Disability Status Scale), age at MS onset (MS_{onset}), and DD (duration of MS disease). Data was extracted by 5 authors (T.A.O., M.A.A., A.O.F., B.E.I., and C.D.I), while data validation was carried out by 2 authors (E.T.C. and T.A.O.) who resolved any variance by discussion.

2.4 Statistical analysis

From the eligible studies, anti-MAP abs data (2302 observations (o) and 455 events (e)) from 12 studies and MAP DNA data ($o = 1242$, $e = 166$) from 4 studies were separately fitted using the Mantel-Haenszel approach in both random-effects (RE) and fixed-effects (FE) meta-analytic models with a 0.1 continuity correction in all studies to obtain the pooled OR (Mantel and Haenszel, 1959; Robins et al., 1986; Schwarzer et al., 2015). The Sidik-Jonkman estimator was used for tau-squared (τ^2) calculation and adjusted by the Hartung-Knapp approach (IntHout et al., 2014). Furthermore, the Q-statistic and I^2 -statistic were used in quantifying the heterogeneity between studies with a considerable limit set at an $I^2 > 75\%$ (Higgins and Thompson, 2002). The robustness of the analyses and publication bias was further assessed using a random-effects leave-one-out-meta-analytics (Viecht-bauer, 2010a) and funnel plot/Egger's tests (Egger et al., 1997), respectively. Additionally, the effects of publication bias/p-hacking on the pooled OR (effect size) was tested using the p-curve analysis when $I^2 \leq 50\%$ (Simonsohn et al., 2015; van Aert et al., 2016). The primary pooled OR results were summarized using forest plots. All analyses were carried out in R (version 4.1.0.) using cascades of functions from the metafor version 3.0–2 (Viechtbauer and Cheung, 2010b), meta version 4.18–2 (Balduzzi et al., 2019), dmetar version 0.0.9000 (Harrer et al. 2019), and PerformanceAnalytics version 2.0.4 (Peterson and Carl, 2020) packages.

3 Results

The systematic search of MAP-related MS studies yielded 109 articles, 24 of which had title/abstracts that were considered for inclusion (Fig. 1). Data from 13 studies were analysed in total, with anti-MAP abs from 12 studies (Cossu et al., 2013a,a,b, 2015, 2016; Frau et al., 2013; Mameli et al., 2014, 2016a; Yokoyama et al., 2017) and MAP DNA data from 4 studies (A. Cossu et al. 2011; Cossu et al. 2013a; Cossu et al. 2013b; Frau et al. 2013)) were included (Table 1 and Fig. 1). The studies included data from 2 continents (Asia and Europe, Table 1), among which, 10 (76.92%) and 3 (23.08%) studies were conducted in Italy and Japan, respectively (Table 1). The studies were carried out between 2011 – 2018.

Furthermore, Table 1 presents information on the antigens assayed, demographic and symptomatic characteristics of the participants in the MAP-related MS studies meta-analysed. The MAP antigens tested included MAP 0106 ($n = 1$), MAP 4027 ($n = 1$), MAP DNA IS900 ($n = 4$), MAP HSP70 ($n = 1$), $MAP\_0106c12_{1–132}$ ($n = 2$), MAP_2,694,295–303 ($n = 3$), $MAP\_4027_{18–32}$ ($n = 3$), MAP_5p ($n = 1$), $MAP12_{1–132}$ ($n = 1$), MAP2694 ($n = 4$), $MAP2694_{97–105}$ ($n = 1$) and PtpA/PknG ($n = 1$) and other pathogens/antigens assayed were 3 EBV (Epstein-Barr Virus) antigens (BOLF1_305–320, EBNA1400–413; EBNA1) and 1 Helicobacter pylori antigen (HP986). Also, the average age of MS patients and Cs in the studies ranged from 38.0 ± 11.0 – 44.5 ± 11.25 and 37.0 ± 6.5 to 71±11 years, respectively. In the MS cases, the EDSS ranged from 1.8 ± 1.8 (0–8) to 2 ± 2(0–6.5). Similarly, the disease duration and MS onset recorded ranged from 6.0 ± 5.4 – 14.1 ± 11.6 years and 29.0 ± 10.5 to 32.7 ± 9.9 years, respectively.

Table 1
Explanatory, demographic and symptomatic characteristics of the participants in the MAP-related MS studies included in the meta-analysis.

Sn	Author	Em	Nm	Ec	Nc	method	Sample	Nation, continent	MAP antigen	other antigens	MS patients age (years)	HC age (years)	MSk	MS DIA	M/ Fms\ M/ Fhc	EDSS [mean (range)]	DD/ MS onset
1	Cossu et al., 2013a	23	100	7	100	PCR	Blood/ serum	Italy, Europe	MAP DNA IS900	NT	40±10.8	46	82%RR, 12.5% SP, 5.5% PP.	RMC	30/ 70\ 35/ 65	2 ± 2 (0–6.5)	12 ± 9.7/ 31± 10.8
1	Cossu et al., 2013a	36	100	3	100	ELISA	Blood/ serum	Italy, Europe	MAP2694	NR	40±10.8	46	82%RR, 12.5% SP, 5.5% PP.	RMC	30/ 70\ 35/ 65	2 ± 2 (0–6.5)	12 ± 9.7/ 31± 10.8
2	Cossu et al. 2014	14/22	47	2/2	42	ELISA	Blood/ serum	Italy, Europe	MAP2694/ MAP269497–105	NR	44.5 ± 11.25	NR	90%RR, 10%SP	RMC	21/ 26\ 20/ 22	NR	14.1 ± 11.6/ 30.4 ± 29.9
3	Cossu et al., 2015	32/ 40EBV+MS \ 2/7EBV– MS	47	2/ 40EBV+ HCs\6/ 13EBV– HCs	53	ELISA	Blood /serum	Italy, Europe	MAP_4027.18–32	$BOLF1_{305–320}$ (40 (85%) MS EBV+; 40 (75%) HCs EBV+)	41 ± 11	NR	42 (89.4%) RR, 2 (4.2%), SP, 3 (6.4%) PP	RMC	11/ 36\	1.8 ± 1.8 (0–8)	7 ± 6.9/ 32 ± 9
4	Cossu et al., 2012p	27	119	7	117	PCR	Blood/ serum	Italy, Europe	MAP DNA IS900	EBV (17 (17.3%)); HP986 (5 (4%))	41± 11.8	NR	80.5% RR, 12.2% SP, 7.3% PP	RMC	39/ 80\	2 ± 2 (0–6.5).	10± 9.8/ 31± 9.8
5	Cossu et al., 2013be	62	268	15	231	ELISA	Blood/ serum	Italy, Europe	MAP HSP70	NR	39 ± 10.7	44.0 ± 7.0	84%RR, 11%SP, 5%PP	RMC	85/ 183\ 71/ 160	2.0 (0–6.5)	9.8 ± 10.3/ 29.0 ± 10.6
6	Cossu et al., 2016	15	50	1	50	ELISA	Blood/ serum	Japan, Asia	MAP_0106c121–132, MAP_402,718–32, and MAP_2,694,295–303	NR	41.0 ± 11.2	41.2 ± 11.6	44 (88%) RR, 4 (8%)SP, 2 (4%) PP	RMC	13/ 37\ 13/ 37	md2 (0 – 7)	8.36±6.8/ 32.7 ± 9.9
7	A. Cossu et al. 2011p	21	50	7	56	PCR	Blood/ serum	Italy, Europe	MAP DNA IS900/	NT	44.5 ± 11.25	47.7 ± 12.7	42RR, 6SP, 2PP	MC05	22/ 28\ 26/ 30	NR	14.1-+11.6/ 30.4-+29.9
7	A. Cossu et al. 2011e	6	50	1	56	ELISA	Blood/ serum	Italy, Europe	MAP2694	NT	44.5 ± 11.25	47.7 ± 12.7	42RR, 6SP, 2PP	MC05	22/ 28\ 26/ 30	NR	14.1-+11.6/ 30.4-+29.9
8	Mameli et al., 2016a	37//30	43	15//3	33*#	ELISA	Blood /serum	Italy, Europe	MAP 0106/ MAP 4027	EBV proteins EBNA1 (26/ 43MS, 6/ 33OND)	39± 14.0	17 IND (12/5| 39 ± 23.1), 11 NIND (5/6| 41± 27.1) 5 UND (3/	39 RR, 4 SP	RMC	19/ 24 \33\ 19/ 14	1.9 (0–7.0)	**6 ± 5.4/ 29.0 ± 10.5**

(*continued on next page*)

Table 1 (*continued*)

Sn	Author	Em	Nm	Ec	Nc	method	Sample	Nation, continent	MAP antigen	other antigens	MS patients age (years)	HC age (years)	MSk	MS DIA	M/ Fms\ M/ Fhc	EDSS [mean (range)]	DD/ MS onset
9	Yokoyama et al. 2018	12	46	0	29*	ELISA	Blood/ serum	Japan, Asia	MAP_2,694,295–303; MAP_5p	NT	38.5 ± 10.3	2|71± 11) *48.6 ± 17.1	36RR, 2SR, 1PP, 5CIS, 2RIS	RMC	11/ 35\ 7/22	2(0–7)	6.9 ± 6/ 31.3 ± 7.9
10	Yokoyama et al., 2017	15	50	1	50	ELISA	Blood/ serum	Japan, Asia	MAP_0106c121–132; $MAP_{402,718–32}$; MAP_2,694,295–303	NR	NR	NR	NR	NR	NR	NR	NR
11	Mameli et al., 2014	12/48 EBV+	53	1/34 EBV+	53	ELISA	Blood /serum	Italy, Europe	MAP121–132	EBNA1400–413	38.0 ± 11.0	37.0 ± 6.5	44RR, 6SP, 3PP	RMC10	25/ 28\ 26/ 27	2.0 (1.5–2.8)	NR
12	Slavin et al., 2018	43/43	72	18//8	79	ELISA	Blood /serum	Italy, Europe	PtpA/PknG	NR	39.88	46.78	10 CIS, 62RR	RMC11	18/ 54\ 25/ 54	NR	NR
13	Frau et al., 2013p	68	436	6	264	PCR	Blood/ serum	Italy, Europe	MAP DNA IS900	NT	41± 11	36± 12	367 (84%) RR, 51 (12%) SP, 18 (4%)PP	RMC	134/ 302\	median 2 (0–8.5)	28 (25th:22, 75th:36)
13	Frau et al., 2013e	123	436	10	264	ELISA	Blood/ serum	Italy, Europe	MAP2694	NT	41± 11	36± 12	367 (84%) RR, 51 (12%) SP, 18 (4%)PP	RMC	134/ 302\	median 2 (0–8.5)	NR

Em = MAP+ MS patients; Nm = population MS patients; HC = healthy control; Ec = MAP+ HCs; Nc = population controls; RR (relapsing remitting), 74 SR (secondary relapsing), 62 SP (secondary progressive), 57 PP (primary progressive), 15 CIS (clinically isolated syndrome) and 2 RIS (radiologically isolated syndrome), Helicobacter pylori HP986; EBV (Epstein-Barr Virus), NR/NT = not reported/not tested; MC05 = 2005 McDonald Criteria (Polman et al., 2005); RMC= Revised McDonald Criteria; RMC10 = 2010 Revised McDonald Criteria; RMC11 = 2011 Revised McDonald Criteria; *disease control; *# Other Neurological Disease composed.

Figure S1 depicts the distribution of MS disease conditions among 1381 MS patients included in the eligible studies. The disease stage was reported in 1331 cases (96.4%) of the 1381 MS cases, with 1121 RR (relapsing-remitting), 136 SP (secondary progressive), 57 PP (primary progressive), 15 CIS (clinically isolated syndrome) and 2 RIS (radiologically isolated syndrome) (Table 1).

The forest plot for pooled OR of anti-MAP abs data from MAP-associated MS is shown in Fig. 2 . The risk of MS was significantly associated with MAP infection, with ORs of 10.71 (95%-CI [7.78; 14.74], $z = 14.55$, p-value < 0.0001) and 12.76 (95%-CI [8.13; 20.02], $t = 12.43$, p-value < 0.0001) in the FE and RE models, respectively; with a PI of 3.74 – 43.53 and an I^2 value of 34.9% (95%-CI [0.0%; 67.2%]; $Q_{df=11} = 16.90$, p-value = 0.11). Similarly, the pooled OR of MAP DNA data of MAP-associated MS is depicted in Fig. 3 . While FE significantly present the risk of MS due to MAP as an OR of 5.53 (95%-CI [3.54; 8.66], $z = 7.49$, p-value< 0.0001), RE yielded an OR of 5.27 (95%-CI [3.22; 8.60], $t = 10.77$, $p = 0.0017$). In both cases, the PI of the risk was ORs ranging from 2.08 – 13.33, with heterogeneity value (I^2) of 0.0% (95%-CI [0.0%; 84.7%]; $Q_{d.f.=3} = 1.38$, p-value = 0.71).

Figs. 4 and S2 show funnel plots of pooled anti-MAP abs and MAP DNA data from MAP-related MS studies, respectively. The Eggers' test revealed the presence of publication bias/funnel plot asymmetry in anti-MAP data (intercept = 1.61, 95% CI: 0.45 – 2.77, $t = 2.72$, $p = 0.021$), the test (intercept = −5.57, 95% CI: −20.44 – 9.29, $t = -0.74$, $p = 0.54$) showed the absence of bias in MAP DNA dataset. However, MAP DNA data from 4 studies were pooled, and the Egger's test may lack significant (statistical) power to detect funnel plot asymmetry. Table 2 summarises the comparison of the RE leave-one-out meta-analyses of anti-MAP abs and MAP DNA data with the main RE of MAP-associated MS studies. The results demostrated the robustness of the anti-MAP and MAP DNA meta-analyses. However, leave-one-out analysis identified Cossu et al. 2013b as an influential study; because the pooled OR and heterogeneity level when it was omitted increased from 12.76 (95%-CI [8.13; 20.02], p-value < 0.0001) (i.e., main RE) to 15.59 (95%-CI [11.01; 22.07] < 0.0001) and reduced from 34.9% (95%-CI [0.0%; 67.2%]; $Q_{df=11} = 16.90$, p-value = 0.11) (i.e., main RE) to 0.0% (95%-CI [0.0%; 60.2%], $Q_{d.f.=10} = 5.63$, $p = 0.85$), respectively.

Table S1 depicts the p-curve analysis of the effects of publication bias/p-hacking on the pooled OR of MAP-associated MS meta-analysis. The anti-MAP dataset's right-skewness test (pBinomial = 0.001; zFull = −10.680; $P_{Full} < 0.001$, zHalf = −9.881, $P_{Half} < 0.001$) and MAP DNA (pBinomial = 0.062; zFull = −4.934; $P_{Full} < 0.001$, zHalf = −4.373, $P_{Half} < 0.001$) revealed true association of MAP with high risk of MS as its right-skewness test ($P_{Full} < 0.001$, $P_{Half} < 0.001$) was significant with substantial statistical power of 99% (95%-CI: 96.8%−99%) and 94% (95%-CI: 72.5%−99%), respectively.

4 Discussion

The current study investigated the role of MAP as an environmental trigger of MS. The findings from the present study found limited published works considering the association between MAP and MS. Also, the studies were geographically linked to Italy (Europe) and Japan (Asia). This could have been influenced by the rigorous epidemiological studies and the high prevalence of MS in high-income regions (Stenager, 2019; GBD 2016 MS Collaborators, 2019).

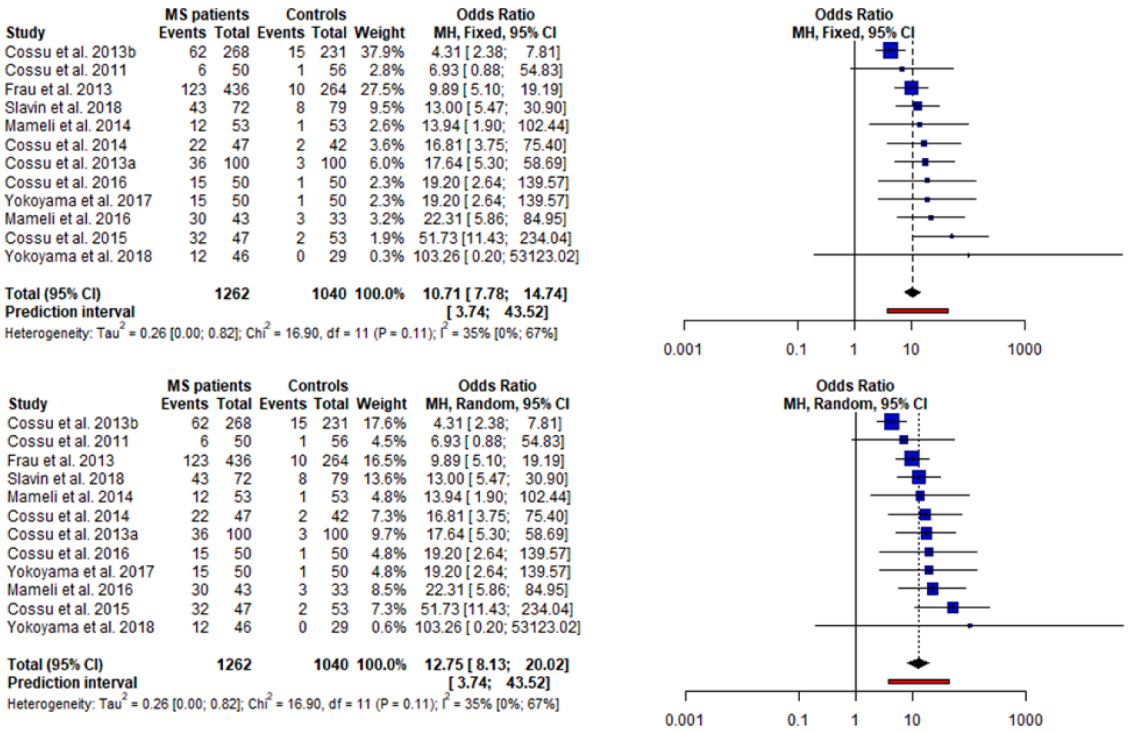

Fig. 2. Forest plots for pooled anti-MAP abs data in MAP-related MS studies. Horizontal lines in the plots represent 95% confidence interval of the observed ORs; the size of each blue square represents the weight of the ORs (effect size); black diamond shape represents the average pooled ORs and its length the 95% CI of the pooled ORs on the x-axis; vertical solid reference line represents the point on the x-axis equal to no effect; vertical dash reference line equals to the pooled ORs; red solid line below the black diamond shape represents the prediction interval for the ORs.

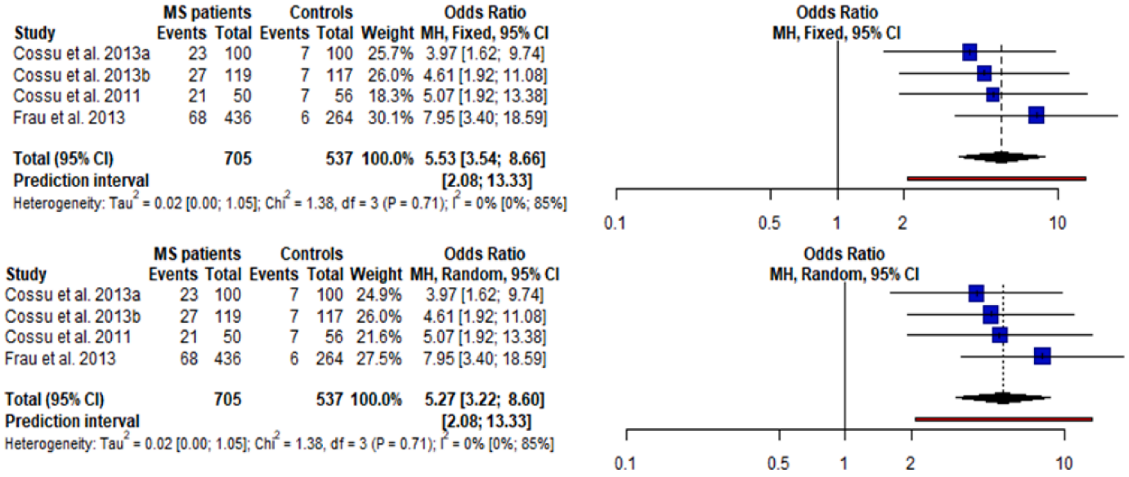

Fig. 3. Forest plots for meta-analysis of the risk of MS linked with MAP infection demonstrated by PCR detection of MAP DNA. Horizontal lines in the plots represent 95% confidence interval of the observed ORs; the size of each blue square represents the weight of the ORs (effect size); black diamond shape represents the average pooled ORs and its length the 95% CI of the pooled ORs on the x-axis; vertical solid reference line represents the point on the x-axis equal to no effect; vertical dash reference line equals to the pooled ORs; red solid line below the black diamond shape represents the prediction interval for the ORs.

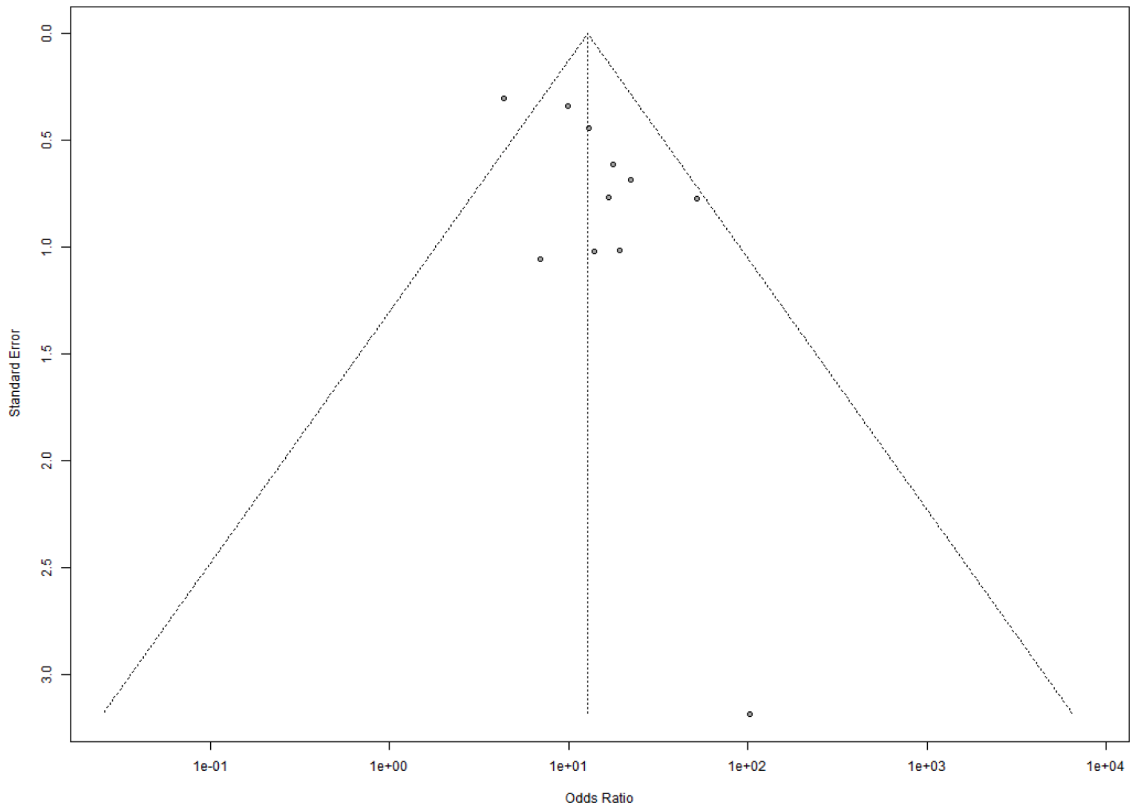

Fig. 4. Funnel plot of pooled anti-MAP abs data in MAP-related MS studies. Eggers' test of the intercept (Eggers' test (intercept = 1.61, 95% CI: 0.45 - 2.77, $t = 2.72$, $p = 0.021$).

Table 2
Comparison between main random-effects meta-analyses of anti-MAP abs, MAP DNA data and leave-one-out sensitivity analyses of MAP-associated MS studies.

Study omitted	*Participants and events	OR [95%-CI]	T	p-value	PI [LB; UB]	I^2-statistic [95%-CI]	Q (d.f.; p-value)
Main anti-MAP abs	$k = 12$, $o = 2302$, $e = 455$	12.76 [8.13; 20.02]	12.4	< 0.0001	[3.74; 43.53]	34.9%[0.0%; 67.2%]	16.90(11; 0.11)
Slavin et al., 2018	$k = 11$, $o = 2151$, $e = 404$	12.89 [7.71; 21.55]	11.1	< 0.0001	[3.39; 49.01]	39.5% [0.0%; 70.2%]	16.53 (10; 0.09)
Cossu et al., 2013a	$k = 11$, $o = 2102$,$e = 416$	12.46 [7.58; 20.50]	11.3	< 0.0001	[3.32; 46.86]	37.6% [0.0%; 69.3%]	16.02 (10; 0.10)
Cossu et al. 2014	$k = 11$, $o = 2213$, $e = 431$	12.63 [7.71; 20.70]	11.4	< 0.0001	[3.35; 47.60]	39.2% [0.0%; 70.1%]	16.45 (10; 0.09)
**Cossu et al., 2013b	$k = 11$, $o = 1803$,$e = 378$	15.59 [11.01; 22.07]	17.6	< 0.0001	[6.36; 38.23]	0.0% [0.0%; 60.2%]	5.63 (10; 0.85)
Frau et al., 2013	$k = 11$, $o = 1602$, $e = 322$	13.51 [8.07; 22.61]	11.3	< 0.0001	[3.65; 49.99]	40.8% [0.0%; 70.8%]	16.90 (10; 0.08)
Cossu et al., 2016	$k = 11$, $o = 2202$, $e = 439$	12.63 [7.77; 20.53]	11.6	< 0.0001	[3.38; 47.21]	39.4% [0.0%; 70.1%]	16.49 (10; 0.09)
A. Cossu et al. 2011	$k = 11$,$o = 2196$, $e = 448$	13.15 [8.14; 21.26]	12	< 0.0001	[3.72; 46.55]	40.4% [0.0%; 70.6%]	16.77 (10; 0.08)
Cossu et al., 2015	$k = 11$, $o = 2202$, $e = 421$	11.08 [7.37; 16.67]	13.1	< 0.0001	[3.88; 31.68]	18.1% [0.0%; 58.1%]	12.21 (10; 0.27)
Mameli et al. 2016	$k = 11$, $o = 2226$, $e = 422$	12.19 [7.51; 19.80]	11.5	< 0.0001	[3.34; 44.58]	35.4% [0.0%; 68.3%]	15.48 (10; 0.12)
Yokoyama et al. 2018	$k = 11$, $o = 2227$, $e = 443$	12.75 [7.90; 20.58]	11.9	< 0.0001	[3.91; 41.57]	40.2% [0.0%; 70.5%]	16.71 (10; 0.08)
Yokoyama et al., 2017	$k = 11$, $o = 2202$, $e = 439$	12.63 [7.77; 20.53]	11.6	< 0.0001	[3.38; 47.21]	39.4% [0.0%; 70.1%]	16.49 (10; 0.09)
Main MAP DNA	$k = 4$, $o = 1242$, $e = 166$	5.27 [3.22; 8.60]	10.8	0.0017	[2.08; 13.33]	0.0% [0.0%; 84.7%]	1.38 (3; 0.71)
Cossu et al., 2013a	$k = 3$, $o = 1042$, $e = 136$	5.78[2.75; 12.17]	10.2	0.0096	[0.35; 95.64]	0.0% [0.0%; 89.6%]	0.86 (2; 0.65)
Cossu et al., 2013b	$k = 3$, $o = 1006$, $e = 132$	5.51[2.24; 13.56]	8.15	0.0147	[0.15; 202.45]	0.0% [0.0%; 89.6%]	1.25 (2; 0.53)
A. Cossu et al. 2011	$k = 3$, $o = 1136$, $e = 138$	5.32[2.13; 13.27]	7.86	0.0158	[0.13; 225.25]	0.0% [0.0%; 89.6%]	1.37 (2; 0.51)
Frau et al., 2013	$k = 3$, $o = 542$, $e = 92$	4.50[3.33; 6.09]	1.47	0.0022	[1.74; 11.63]	0.0% [0.0%; 89.6%]	0.14 (2; 0.93)

* =; k =number of studies combined; o = number of observations; e = number of events; ** = influential study; PI = prediction interval; LB= lower bound, UB= upper bound; OR = odds ratio; d.f. = degree of freedom.

Furthermore, the findings imply that the role of MAP as an environmental trigger of MS is yet to be determined in most countries. While there is a global increase in MS prevalence (Koch-Henriksen and Sorensen 2010; Browne et al., 2014; Dobson and Giovannoni, 2019) as well as paratuberculosis in livestock, which may predispose humans to MAP infections in a variety of ways (Whittington et al., 2019), the role of MAP in MS cannot be overemphasised.

For example, a formal control program for paratuberculosis (MAP) is generally unavailable in 76% of countries in South/Central America, Asia/Africa, and 20% in Europe for various reasons. Among these reasons are a farmer/veterinarian's awareness, a lack of surveillance, a lack of knowledge of the signs/symptoms of the disease, poor diagnostic tests, and farmer fear of stigma, among others (Whittington et al., 2019). Furthermore, recent studies reported a rising prevalence of MS in South Africa (Heine et al., 2020), United Arab Emirates (Etemadifar et al., 2020), Saudi Arabia (AlJumah et al., 2020), Arab/Persian Gulf countries (Etemadifar et al., 2020; Mohammed, 2016) and somewhat neglect in Nigeria (Okubadejo et al., 2014) to mention a few. An initiative to unravel the role of MAP in MS and other infectious agents could help in the management of the disease.

Although different MAP antigens were used in the diagnosis of MAP in MAP-related MS across the studies, no noticeable effects on the pooled OR/risk of MS due to MAP were observed in this study (Figs. 2 and 3). However, the study that targeted MAP HSP70 antigen was identified as an influential study (Cossu et al., 2013b) (Table 2). This probability revealed the sensitivity or ubiquity of the highly conserved MAP HSP70 antigen in the diagnosis of MAP-related MS in comparison to others. The EBV and Helicobacter pylori antigens assessed in some of the included studies attest to the plausibility of the association between MAP and MS observed as an increased/significant OR value as well as differentiation/identification of co-infections of EBV and MAP in MS. It has been reported that the etiopathogenesis of MAP and EBV are similar in MS and that these two organisms can elicit autoantibodies capable of attacking several/common MS-related epitopes (Cossu et al. 2014; Simpson et al., 2015). Among the existing possible infectious risk factors, EBV and MAP are considered the most significant environmental triggers of MS Simpson et al., 2015; Mameli et al., 2014; Cossu et al., 2015). In addition, MAP and EBV can act synergistically via common epitopes in the development of MS in genetically susceptible individuals (A. Cossu et al. 2011).). The reported EDSS scores varied across studies. The EDSS measures disease severity, and an increase in EDSS has been associated with an increase in costs and the overall economic burden of the disease (Naci et al. 2010). The average age of MS patients ranged from 38.0 ± 11.0 – 44.5 ± 11.25. These values are consistent with the literature (Etemadifar, et al. 2020). Similarly, the disease duration and MS onset ranged from 6.0 ± 5.4 to14.1 ± 11.6 years and 29.0 ± 10.5 to 32.7 ± 9.9 years, respectively (Etemadifar et al. 2020; Heine et al., 2020). MS phenotypes distribution decreased in the following: RR > SP > PP > CIS > RIS. Other studies have reported a similar pattern (Dobsona and Giovannoni, 2019; Kobelt et al. 2017; Mohammed, 2016). The study revealed a significant relationship between MAP infection and MS (Figs. 2 and 3). This underscores MAP as a key causative agent/environmental trigger of MS as well as MAP's roles in the onset and progression of MS. Thus, MAP-targeted preventive/curative models may be important in the prevention/management of MS. Also, the prediction intervals of the meta-analytic models of the association between MAP and MS project a strong chance of MS condition following MAP infection in any future events. Therefore, detection of MAP in new cases of MS as well as other infectious agents, should be encouraged to provide effective clinical attention/treatments.

In the present study, the evidence of publication bias with anti-MAP abs meta-analysis could be attributed to study(ies) that reported optical density(ies) of MAP abs in MS patients/Cs without accounting for the corresponding prevalence data. Also, it may not necessarily mean evidence of preferential publishing or non-publishing of studies with positive or negative results, respectively. In other words, negative findings from MAP-related MS studies are not under-represented in the literature. The preceding argument is further supported by the right-skewness test accompanying all the meta-analytic models, which produced a true evidential association between MAP and MS with significant statistical power (Figure S1).

The random-effects-meta-analytic sensitivity analyses reports of anti-MAP abs and MAP DNA data strongly supported that MAP is a contributory environmental trigger for the development of MS (Table 2). In support of the aforementioned, p-curve distribution analysis further showed a proven connection between MAP and a high risk of MS with a significant statistical power ≥ 94%. This suggests that the link between MAP and the risk of MS is not a spurious claim. However, more data are needed to explore its landscape across different countries and continents.

In conclusion, the present meta-analytic synthesis of MAP-related MS data found a considerable and significant association between MAP infection and MS. It strongly supports MAP as an environmental trigger for MS conditions, among other causes. Thus, screening for MAP in new cases of MS could help in/benefit early management/treatment of MS. Future studies on the role of MAP in the progression or severity of MS from one stage to another or phenotypes are highly imperative. In addition, global data gaps, as well as low surveillance status of MAP-related MS, should be addressed.

Author contributions

Conceptualization and coordination, T.C.E. and O.A.I.; Data curation and methodology, T.C.E.; Systematic review of literature and data extraction: C.D.I, B.E.I., A.O.F.; T.A.O.; M.A.A; Software, T.C.E.; Validation, T.C.E.; T.A.O.; Formal analysis, T.C.E.; Resources, O.A.I.; Writing—original draft preparation, T.C.E., T.A.O.; Supervision, O.A.I.; Funding acquisition, O.A.I. All authors contributed to writing—review and editing, and approved the manuscript for publication.

Declaration of Competing Interest

None.

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