Mycobacterium avium subspecies paratuberculosis – The cause of Crohn's disease

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Abstract

Objective: This systematic review assesses the causal relationship between Mycobacterium avium subspecies paratuberculosis (MAP) and Crohn's disease (CD).
Methods: A systematic review and meat-analysis of some impressive PCR based studies is provided aimed to answer among other questions the following question. Is there a cause effect relationship between Mycobacterium avium subspecies paratuberculosis and Crohn's disease? The method of the conditio per quam relationship was used to proof the hypothesis whether the presence of Mycobacterium avium subspecies paratuberculosis guarantees the presence of Crohn's disease. In other words, if Crohn's disease is present, then Mycobacterium avium subspecies paratuberculosis is present too. The mathematical formula of the causal relationship k was used to proof the hypothesis, whether there is a cause effect relationship between Mycobacterium avium subspecies paratuberculosis and Crohn's disease. Significance was indicated by a p-value of less than 0.05.
Result: The studies analyzed (number of cases and controls N=1076) were able to provide evidence that Mycobacterium avium subspecies paratuberculosis is a necessary condition (a conditio sine qua non) and sufficicent conditions of Crohn's disease. Furthermore, the studies analyzed provide impressive evidence of a cause-effect relationship between Mycobacterium avium subspecies paratuberculosis and Crohn's disease.
Conclusion: Mycobacterium avium subspecies paratuberculosis is the cause of Crohn's disease (k=+0,377468824, p value < 0.0001).

Keywords

Mycobacterium avium subspecies paratuberculosis, Crohn's disease, cause effect relationship, causality

1. Introduction

Crohn's disease, described in 1904 by Polish surgeon Antoni Leśniowski [1], later in 1913 by Dalziel [2] and ultimately by Crohn, Ginzburg and Oppenheimer in 1932 [3], is a debilitating chronic inflammatory bowel disease (IBD) of unknown cause. Leśniowski

**Special description of the title. (dispensable)

-Crohn's disease affects about 6.3 per 100,000 people-years in Europe [4]. Many times, the initial signs and symptoms of Crohn's disease (CD) are non-specific and can overlap with symptoms of irritable bowel syndrome (IBS). A delay in the diagnosis of this inflammatory bowel disease (IBD) is associated with problems for both patients and physicians. Often Crohn's disease patients suffer from abdominal pain, malabsorption, steatorrhea, protein losing enteropathy, excessive diarrhea, rapid weight loss and other symptoms which may affect their quality of life [5]. Several different extra-intestinal complications of Crohn's disease (events outside the gastrointestinal tract) may occur. Lesions of Crohn's disease begin as mucosal erosions and neutrophil infiltrates within crypts and crypt abscesses and may progress to transmural lymphogranulomatous enteritis while a cobblestoned appearance in the distal ileum and colon is observed in Crohn's disease patients [6]. Medical treatment of Crohn's disease patients includes nutritional therapy, a medication having weak anti-MAP activity with anti-inflammatory drugs, immunosuppressants, and sometimes antibiotics too. A view monoclonal antibodies such as Adalimumab (Humira ®) and Infliximab (Remicade ®) are used too, to treat Crohn's disease patients. Most often, Crohn's disease patients require a dangerous, costly and time-consuming surgical intervention (laparoscopy, strictureplasty, anastomosis, bypass surgery et cetera). A number of theories regarding the etiology of Crohn's disease are discussed including diet, infections, other unidentified environmental factors, immune dysregulation and autoimmune theories. Still, the cause of Crohn's disease or some critical aspects in the pathogenesis of this disease are not known. Many authors are of the opinion that Crohn's disease is a syndrome caused by several etiologies. Mycobacterium avium subspecies paratuberculosis (MAP) is endemic in the bovine populations of many countries [7] and known to be a causative agent of Johne's disease, an inflammatory bowel disease in a variety of mammals including monkeys, chimpanzees cattle sheep, deer, bison et cetera. Johne's disease was discovered by Dr. H.A. Johne and Dr. L. Frothingham as visiting scientists from the Pathology Unit in Boston, Massachusetts at the Veterinary Pathology Unit in Dresden by investigating the tissues of a cow from the Oldenburg region of Germany. The first occurrence of Johne's disease [8] in the U.S. was published by Leonard Pearson (1868-1909) in 1908. The first description of the similarities between Crohn's disease and Johne's disease in cattle was made in 1913 by the Scottish surgeon Thomas Kennedy Dalziel [2]. The zoonotic capacities of MAP [9] and transmission routes to humans [10], [11] have been discussed widely. Mycobacterium avium subsp. paratuberculosis has been detected in retail cheese in about 31.7% of the samples [12]. Due to the similarities between Johne's disease in cattle and Crohn's disease, it has been argued that Mycobacterium avium subspecies paratuberculosis (MAP), which causes Johne's disease, might also be a cause of Crohn's disease too. Historically, MAP became the leading infectious candidate as the causative agent of Crohn's disease. Meanwhile, the evidence to support a M. paratuberculosis infection as a cause of Crohn's disease is mounting rapidly. Studies were able to document that up to 83% of Crohn's patients showed evidence of serum antibodies [13]-[18] to M. avium ss paratu-

berculosis. In particular, critics of the mycobacterial theory argue that MAP is not a causal factor but a secondary invader [19]. The relationship between Mycobacterium avium subspecies paratuberculosis (M. paratuberculosis) and Crohn's disease is suspected but the evidence remains controversial.

2. Material and methods

Chronic diarrhea is the only but the most common presenting symptom of Crohn's disease. Severe disease-specific complications of this lifelong disease and a global health problem too are common and accompanied by disabling symptoms and impaired quality of life. The need for repeated courses of cost-expensive therapies, hospitalization and surgery determines a substantial healthcare burden which affects the patient, the healthcare systems and human society in general.

2.1. Search strategy

For the questions addressed in this paper, Pubmed was searched for case-control studies conducted in any country which investigated the relationship between Mycobacterium avium subspecies paratuberculosis (MAP) and Crohn's disease at least by polymerase chain reaction (PCR). The search in Pubmed was performed while using medical key words like "case control study" and "Mycobacterium avium subspecies paratuberculosis" and "Crohn's disease" and "PCR DNA" et cetera. The articles found where saved as a *.txt file while using Pubmed support (Menu: Send to, Choose Radio Button: File, Choose Format: Abstract (text). Click buttom "create file"). The created *.txt file was converted into a *.pdf file. The abstracts where studied within the *.pdf file. Those articles were considered for a review which provided access to data without any data access barrier, no data access restrictions were accepted. Additionally, references from relevant publications and review articles were checked. Case-control studies were included if they compared the prevalence of MAP in patients with Crohn's disease with the prevalence in healthy controls. Studies were excluded if insufficient data were provided to calculate the measures of relationship or if there were data access barriers.

2.2. The data of the studies analyzed

M. paratuberculosis recovers very poorly by culture from Crohn's disease tissues while the incubation times (sometimes greater than one year) can be extremely long. In this context, novel laboratory techniques [20] (Southern Blot hybridization, Immunohistochemistry (IHC), introduced by Coons [21] in 1941, In-situ hybridization (ISH), described in the year 1969 by Joseph G. Gall [22], Fluorescent ISH (FISH), RNA in situ hybridization (RNA ISH), Polymerase chain reaction (PCR), Nested PCR, Quantitative polymerase chain reaction (QPCR) et cetera) can improve our understanding of the path-

ogenesis of Crohn's diseases. The data of the studies [23]-[39] analyzed, are presented by the 2 by 2-table (**Table 1**). The meaning of the abbreviations a_t, b_t, c_t, d_t, N_t of table 1 (**Table 1**), table 2 (**Table 2**), table 3 (**Table 3**), table 4 (**Table4**) are explained by a 2 by 2-table (**Table 5**).

Table 1. The data of the studies considered for a meta-analysis.

	Mycobacterium avium subspecies paratuberculosis				
	Crohn's Disease		Healthy control		**Sample Size**
Reviiew	MAP +	MAP -	MAP +	MAP -	
Study Id	a_t	c_t	b_t	d_t	$a_t+b_t+c_t+d_t=N_t$
Sanderson et al. [23]	**26**	**14**	**5**	35	**80**
Fidler et al. [24]	**4**	27	**0**	30	**61**
Hulten et al. [25]	**7**	30	**0**	22	**59**
Ryan et al. [26]	**6**	9	**0**	12	**27**
Bull et al. [27]	**34**	**3**	**9**	25	**71**
Autschbach et al. [28]	**22**	**14**	**4**	73	**113**
Sechi et al. [29]	**30**	5	**3**	26	**64**
Romero et al. [30]	**10**	**2**	**1**	5	**18**
Szkaradkiewicz et al. [31]	**10**	6	**1**	11	**28**
Kirkwood et al. [32]	**22**	34	**6**	33	**95**
Mendoza et al. [33]	**30**	0	**0**	10	**40**
Tuci et al. [34]	**21**	**10**	**11**	21	**63**
Lee et al. [35]	**5**	**14**	**0**	19	**38**
Nazareth et al. [36]	**27**	13	**11**	18	**69**
Timms et al. [37]	**6**	15	**0**	21	**42**
Khan et al. [38]	**16**	**53**	**3**	46	**118**
Zamani et al. [39]	**18**	10	**6**	56	**90**
Total events	294	259	60	463	1076

2.3. Statistical analysis

All statistical analyses were performed with Microsoft Excel version 14.0.7166.5000 (32-Bit) software (Microsoft GmbH, Munich, Germany). In order to simplify the understanding of this article, to increase the transparency for the reader and to correct some of the misprints of former publications, several of the following lines are *repeated word by word* and taken from former publications.

2.3.1. The 2x2 table

The 2x2 table in this article is defined [40]-[62] in general more precisely (**Table 5**) as follows.

Table 5. The sample space of a contingency table

		Conditioned B_t (Crohn's disease)		
		Yes = +1	**Not = +0**	**Total**
Condition A_t (MAP PCR DNA)	**Yes =+1**	a_t	b_t	$\mathbf{A_t}$
	Not = +0	c_t	d_t	$\underline{A}_t$
	Total	$\mathbf{B_t}$	$\underline{B}_t$	$\mathbf{N_t}$

In general it is $(a+b) = A_t$, $(c+d) = \underline{A}_t$, $(a+c) = B_t$, $(b+d) = \underline{B}_t$ and $a_t+b_t+c_t+d_t=N_t$. Equally, it is $B_t+\underline{B}_t = A_t + \underline{A}_t = N_t$. In this context, it is $p(a_t)=p(A_t \cap B_t)$, $p(A_t) = p(a_t)+p(b_t)$ or in other words $p(A_t)= p(A_t \cap B_t)+p(A_t \cap \underline{B}_t)$ while $p(A_t)$ is not defined as $p(a_t)$. In the same context, it should be considered that $p(B_t) = p(a_t)+p(c_t) = p(A_t \cap B_t) +p(c_t)$ and equally that $p(\underline{B}_t) = 1- p(B_t) =p(b_t)+p(d_t)$. In point of fact, the joint probability of A_t and B_t is denoted by $p(A_t \cap B_t)$. It is $p(a_t)+p(c_t)+p(b_t)+p(d_t) = 1$. These relationships are viewed by the table (**Table 6**) as follows.

Table 6. The probabitlities of a contingency table

		Conditioned B_t		
		Yes = +1	**No = +0**	**Total**
Condition A_t	**Yes =+1**	$p(a_t) = p(A_t \cap B_t)$	$p(b_t)$	$p(A_t)$
	No = +0	$p(c_t)$	$p(d_t)$	$p(\underline{A}_t)$
	Total	$p(B_t)$	$p(\underline{B}_t)$	**1**

2.3.2. Independence

In the case of independence of A_t and B_t it is

$$p(A_t \cap B_t) \equiv p(A_t) \times p(B_t) \tag{1}$$

2.3.3. Sufficient condition (conditio per quam; material conditional)

The mathematical formula of the *sufficient condition* relationship (conditio per quam) [40]-[62] of a population was defined as

$$p(A_t \rightarrow B_t) \equiv \frac{a_t + c_t + d_t}{N_t} \equiv +1 \tag{2}$$

and used to proof the hypothesis: *if* A_t *then* B_t . In particular it is

$$\begin{aligned} p\left(A_t \rightarrow B_t\right) &\equiv p\left(a_t\right) + p\left(c_t\right) + p\left(d_t\right) \equiv +1 \\ p\left(A_t \rightarrow B_t\right) &\equiv p\left(A_t \cap B_t\right) + p\left(\underline{A}_t\right) \equiv +1 \\ p\left(A_t \rightarrow B_t\right) &\equiv p\left(A_t \cap B_t\right) + \left(1 - p\left(A_t\right)\right) \equiv +1 \\ p\left(A_t \rightarrow B_t\right) &\equiv +1 \end{aligned} \tag{3}$$

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Although the work on study bias is vast and therefore quite difficult to survey adequately, we can at least point out that several factors including the study design can have an impact on bias with respect to the sufficient condition too. The question is, what is the relationship between the independence of an event A_t (a condition) and another event B_t (conditioned) and the sufficient condition relationship. Especially, is it possible that an event A_t is a sufficient condition of an event B_t even if event A_t (a sufficient condition) is independent of an event B_t (the conditioned). In this context, the conditio per quam was defined as

$$p\left(A_t \rightarrow B_t\right) \equiv p\left(A_t \cap B_t\right) + p\left(\underline{A}_t\right) \equiv +1 \tag{4}$$

or a

$$p\left(A_t \rightarrow B_t\right) \equiv p\left(A_t \cap B_t\right) + \left(1 - p\left(A_t\right)\right) \equiv +1 \tag{5}$$

Under conditions where an event A_t is independent of an even B_t it is equally true that

$$p\left(A_t \cap B_t\right) \equiv p\left(A_t\right) \times p\left(B_t\right) \tag{6}$$

Substituting this relationship into the equation before and rearranging equation it is

$$p\left(A_t\right) \times p\left(B_t\right) + \left(1 - p\left(A_t\right)\right) \equiv +1 \tag{7}$$

or

$$p\left(A_t\right) \times p\left(B_t\right) \equiv p\left(A_t\right) \tag{8}$$

or

$$p\left(B_t\right) \equiv +1. \tag{9}$$

Only under conditions where $p(B_t) = 1$, it is possible that A_t as a sufficient condition of B_t even if A_t is independent of B_t and vice versa, otherwise not. In other words, a statistically significant conditio per quam relationship is very convincing if at the same time an

event A_t is not independent of and event B_t and vice versa. Thus far, an inappropriate study design and other sources of possible bias, diminish in their importance if a statistical significant conditio per quam relationship is supported by the absence of independence of the same two events.

2.2.3. Necessary condition (conditio sine qua non)

The formula of the *necessary condition* (conditio sine qua non) [40]-[62] relationship was derived as

$$p\left(A_t \leftarrow B_t\right) \equiv p\left(A_t \cap B_t\right) + p\left(\underline{B}_t\right) \equiv \frac{a_t + b_t + d_t}{N} \equiv +1 \tag{10}$$

and used to proof the hypothesis: *without* A_t *no* B_t.

2.2.4. Necessary and sufficient condition (material biconditional)

The *necessary and sufficient condition* relationship was defined [40]-[62] as

$$p\left(A_t \leftrightarrow B_t\right) \equiv p\left(A_t \cap B_t\right) + p\left(\underline{A}_t \cap \underline{B}_t\right) \equiv \frac{a_t + d_t}{N} \equiv +1 \tag{11}$$

2.3.4. The X^2 goodness of fit test of a necessary condition

Under conditions where the chi-square [63] goodness of fit test cannot be used it is possible to use an approximate and conservative (one sided) confidence interval as discussed by Rumke [64], Louis [65], Hanley et al. [66] and Jovanovic [67] known as *the rule of three*. Under some circumstances, the rule three and other methods can be used to test the significance of a necessary condition. In this publication, the chi-square goodness of fit test was used to determine whether sample data are consistent with a hypothesized (theoretical) distribution of a necessary condition. In particular, the hypotheses can take the following form.

H_0: The sample distribution do agree with the hypothetical (theoretical) distribution of a necessary condition.

H_A: The sample distribution do not agree with the hypothetical (theoretical) distribution of a necessary condition.

The X^2 Goodness-of-Fit Test can be shown schematically as

$$\chi^2 \equiv \sum_{t=+1}^{t=+N} \left(\frac{\left(\text{Observed}_t - \text{Expected}_t\right)^2}{\text{Expected}_t} \right) \tag{12}$$

The degrees of freedom are calculated as N-1. Interestingly, if there is no discrepancy

between an observed and a theoretical distribution at all, then the value of the calculated $X^2=0$. As the discrepancy between an observed and the theoretical distribution of a necessary condition becomes larger, the X^2 becomes larger. This X^2 values are evaluated by the known X^2 distribution. An adjustment (*Yate's correction for continuity*) can be used when there is one degree of freedom. When there is more than one degree of freedom, the same adjustment is not used. Applying this to the formula above, we find the X^2 Goodness-of-Fit Test *with continuity correction* shown schematically as

$$\chi^2 \equiv \sum_{t=+1}^{t=+N} \left(\frac{\left(\left| \text{Observed}_t - \text{Expected}_t \right| - \left(\frac{1}{2} \right) \right)^2}{\text{Expected}_t} \right) \tag{13}$$

Under circumstances, where the term ($|\text{Observed}_t - \text{Expected}_t|$) is less than ½, the continuity correction should be omitted. The theoretical (hypothetical) distribution of a necessary condition is shown schematically by the 2x2 table (**Table 7**).

Table 7. The theoretical distribution of a necessary condition (conditio sine qua non).

		Conditioned B_t		
		Yes = +1	**No = +0**	**Total**
Condition A_t	**Yes =+1**	a_t	b_t	(a_t+b_t)
	No = +0	**c_t=0**	d_t	(c_t+d_t)
	Total	(a_t+c_t)	(b_t+d_t)	$(a_t+b_t+c_t+d_t)$

The theoretical distribution of a necessary condition (conditio sine qua non) is determined by the fact that **c=0**. The X^2 Goodness-of-Fit Test *with continuity correction* of a necessary condition (conditio sine qua non) is calculated as

$$\chi^2(\text{SINE}) \equiv \left(\frac{\left(\left| (a_t + b_t) - (a_t + b_t) \right| - \left(\frac{1}{2} \right) \right)^2}{(a_t + b_t)} \right) + \left(\frac{\left(\left| (d_t) - (c_t + d_t) \right| - \left(\frac{1}{2} \right) \right)^2}{(c_t + d_t)} \right) = 0 + \left(\frac{\left(\left| d_t - (c_t + d_t) \right| - \left(\frac{1}{2} \right) \right)^2}{(c_t + d_t)} \right) \tag{14}$$

or more simplified as

$$\chi^2(\text{SINE}) \equiv \left(\frac{\left(\left| -c_t \right| - \left(\frac{1}{2} \right) \right)^2}{(c_t + d_t)} \right) + 0 \tag{15}$$

Under these circumstances, the degree of freedom is d.f. = N-1=2-1=1. The *conditio sine qua non model* can be used widely and is one of the new and appropriate methods of

analysis of binary outcome variables. In this context, *meta-analysis and systematic reviews* aims to combine effects estimated from several studies to achieve greater precision of the conclusions drawn and can provide us with more convincing and reliable evidence of some special aspects of medicine. In meta-analysis the heterogeneity between the studies can be modelled via the additive properties of the chi square distribution too. In general, let X_t denote n independent random variables which follow a chi-square distribution. The sum of these independent chi-square variate is itself a chi-square variate which is known as the additive property of independent chi-squares. There may be disadvantages in the use of the chi-square-goodness-of-fit test. Still, the chi square distribution, a continuous probability distribution, is related to the standard normal distribution and is a simple and good measure of model adequacy. However, a particular concern with the use of the chi-square-goodness-of-fit test is a priori justified if expected cell frequencies of a 2x2 table are too small (all are less than one).

2.3.5. The mathematical formula of the causal relationship k

Huxley [68] and Darwin [69] claimed more than a century ago that humans share recent common ancestors with the African apes. Modern molecular methods have spectacularly confirmed their prediction. Genomic divergences between humans and other hominoids and especially our closest living evolutionary relatives the common chimpanzee (Pan troglodytes) and bonobo (Pan paniscus or pygmy chimpanzee) are very small but not zero. Ebersberger et al. [70], Fujiyama et al. [71] and other sequenced the chimpanzee genome. According to Ebersberger et al. "the chimpanzee genome were sequenced and compared to corresponding human DNA sequences ... the average sequence difference is low (1.24%)" [70]. The Chimpanzee Sequencing and Analysis Consortium calculated "the genome-wide nucleotide divergence between human and chimpanzee to be 1.23%" [72] and confirmed results from other and more limited studies. In other words, the difference between chimpanzee genome and compared to corresponding human DNA sequences is very small. Still there is a difference and this very small difference makes the difference. A chimpanzee is not a human being, a human being is not a chimpanzee. Even if both are similar and "relatives" both are equally not the same. The relationship between the mathematical formula of the causal relationship k [40]-[62] and the closest existing mathematical relatives, Pearson's measures of relationships, is similar to the circumstances aforementioned. In contrast to Pearson's product-moment correlation coefficient [73] or to Pearson's Phi [74] Coefficient (Mean Square Contingency Coefficient et cetera, the mathematical formula of the causal relationship k [40]-[62] is defined *at every single event*, *at every single Bernoulli trial t*, as

$$k\left({}_{R}U_{t}, {}_{0}W_{t}\right) \equiv \frac{\left(p\left({}_{R}U_{t} \times {}_{0}W_{t}\right) - \left(p\left({}_{R}U_{t}\right) \times p\left({}_{0}W_{t}\right)\right)\right)}{\sqrt[2]{\left(p\left({}_{R}U_{t}\right) \times p\left({}_{R}\underline{U}_{t}\right)\right) \times \left(p\left({}_{0}W\right) \times p\left({}_{0}\underline{W}_{t}\right)\right)}} \tag{16}$$

where ${}_{R}U_{t}$ denotes the cause and ${}_{0}W_{t}$ denotes the effect while the chi-square distribution [63] can be applied to determine the significance of causal relationship k. This small difference makes the difference. Only under conditions where *the probability of events is constant from trial to trial*, we can extrapolate *from one Bernoulli trial to N Bernoulli trials* with some consequences one of which is that

$$k\left({}_{R}U_{t},{}_{0}W_{t}\right)\equiv\frac{N_{t}\times N_{t}\times\left(p\left({}_{R}U_{t}\times{}_{0}W_{t}\right)-\left(p\left({}_{R}U_{t}\right)\times p\left({}_{0}W_{t}\right)\right)\right)}{N_{t}\times N_{t}\times\sqrt[2]{\left(p\left({}_{R}U_{t}\right)\times p\left({}_{R}\underline{U}_{t}\right)\right)\times\left(p\left({}_{0}W\right)\times p\left({}_{0}\underline{W}_{t}\right)\right)}}\tag{17}$$

or that

$$k\left({}_{R}U_{t},{}_{0}W_{t}\right)\equiv\frac{\left(N_{t}\times N_{t}\times p\left({}_{R}U_{t}\times{}_{0}W_{t}\right)-\left(N_{t}\times p\left({}_{R}U_{t}\right)\times N_{t}\times p\left({}_{0}W_{t}\right)\right)\right)}{\sqrt[2]{\left(N_{t}\times p\left({}_{R}U_{t}\right)\times N_{t}\times p\left({}_{R}\underline{U}_{t}\right)\right)\times\left(N_{t}\times p\left({}_{0}W\right)\times N_{t}\times p\left({}_{0}\underline{W}_{t}\right)\right)}}\tag{18}$$

or at the end

$$k\left({}_{R}U_{t},{}_{0}W_{t}\right)\equiv\frac{\left(\left(N_{t}\times a_{t}\right)-\left({}_{R}U_{t}\times{}_{0}W_{t}\right)\right)}{\sqrt[2]{\left({}_{R}U_{t}\times{}_{R}\underline{U}_{t}\right)\times\left({}_{0}W\times{}_{0}\underline{W}_{t}\right)}}\tag{19}$$

where N is the sample size, $a_{t}=N_{t}\times p({}_{R}U_{t}\cap{}_{0}W_{t})$, ${}_{R}U_{t}=N\times p({}_{R}U_{t})$, ${}_{R}\underline{U}_{t}=N_{t}\times p({}_{R}\underline{U}_{t})$, ${}_{0}W_{t}=N_{t}\times p({}_{0}W_{t})$, ${}_{0}\underline{W}_{t}=N_{t}\times p({}_{0}\underline{W}_{t})$. Several factors can have an impact on the calculated causal relationship k with the potential of bias.

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Firstly, the relationship between condition and cause has an impact on the causal relationship k. A proper and deeper analysis of the relationship between cause and condition is beyond the scope of this article and can be found in literature [40]-[62]. We will be concerned with the latter sort of entity in this article from a pragmatically point of view. In the hope of casting light on the tricky problems of the relationship between condition and cause, the concept of independence is of use too. The question whether an event A_{t} can be a (necessary, sufficient, necessary and sufficient) condition of an event B_{t} even if both are independent of each other, is already answered few lines before. Still, under which circumstances can we treat an event as a cause or as the cause of another event? Can an event be a cause of another event without being a (necessary, sufficient, necessary and sufficient et cetera) condition of the same event? The concept of this article is restricted on its capacity to bring high degrees of conceptual exactness and rigour to questions like these but not incapable. Most authors who have written on the question of the relationship between condition and cause came to different conclusions. Currently still worthy of consideration is the remark of von Bar.

“Die erste Voraussetzung, welche erforderlich ist, damit eine Erscheinung als die Ursache einer anderen bezeichnet werden könne, ist, daß jene eine der Bedingungen dieser sein.

Würde die zweite Erscheinung auch dann eingetreten sein, wenn die erste nicht vorhanden war, so ist sie in keinem Falle Bedingung und noch weniger Ursache. Wo immer eine Kausalzusammenhang behauptet wird, da muß er wenigstens diese Probe aushalten. … Jede Ursache ist nothwendig auch eine Bedingung eines Ereignisses; aber nicht jede Bedingung ist Ursache zu nennen. “ [75]

Translated into English:

‘The first requirement, which is required, thus that something could be called as the cause of another, is that the one has to be one of the conditions of the other. If the second something had occurred even if the first one did not exist, so it is by no means a condition and still less a cause. Wherever a causal relationship is claimed, the same must at least withstand this test. ... Every cause is necessarily also a condition of an event too; but not every condition is cause too.‘

A cause is a condition of an event too but not necessarily vice versa. A condition of an event must not be equally the cause of the same event. Thus far, a study which provides evidence of a significant causal relationship k without at the same time providing evidence of a significant necessary condition, or of a significant sufficient condition or of a significant necessary and sufficient condition should be treated with some cautious.

2.3.6. The chi square distribution

The chi-squared distribution [63] is a widely known distribution and used in hypothesis testing, in inferential statistics or in construction of confidence intervals. The critical values of the chi square distribution are visualized by **Table 8**.

Table 8. The critical values of the chi square distribution (degrees of freedom: 1).

	p-Value	One sided X^2	Two sided X^2
The chi square distribution	0,1000000000	1,642374415	2,705543454
	0,0500000000	**2,705543454**	**3,841458821**
	0,0400000000	3,06490172	4,217884588
	0,0300000000	3,537384596	4,709292247
	0,0200000000	4,217884588	5,411894431
	0,0100000000	5,411894431	6,634896601
	0,0010000000	9,549535706	10,82756617
	0,0001000000	13,83108362	15,13670523
	0,0000100000	18,18929348	19,51142096
	0,0000010000	22,59504266	23,92812698
	0,0000001000	27,03311129	28,37398736
	0,0000000100	31,49455797	32,84125335
	0,0000000010	35,97368894	37,32489311
	0,0000000001	40,46665791	41,82145620

2.3.7. The X^2 goodness of fit test of a causal relationship k

Under some circumstances the chi-square [48] goodness of fit test can be used to test the significance of a causal relationship. Under conditions where *the probability of events is constant from trial to trial*, we expect a constant causal relationship k_t. In other words, at each Bernoulli trial *t* it is

$$\left|k\left({}_{R}U_{t},{}_{0}W_{t}\right)\right| \equiv \left|{}_{p}k\left({}_{R}U_{t},{}_{0}W_{t}\right)=1\right| \tag{20}$$

where ${}_{p}k({}_{R}U_{t,0}W_{t})$ denotes the causal relationship within the population. Performing N Bernoulli trials (Sample size N), the basic relationship will not change. It follows that

$$N\times\left|k\left({}_{R}U_{t},{}_{0}W_{t}\right)\right| \equiv N\times|1| \tag{21}$$

(assumed that $|{}_{p}k({}_{R}U_{t,0}W_{t})=1|$) or that

$$N\times\left|k\left({}_{R}U_{t},{}_{0}W_{t}\right)\right| - N\times|1| = 0 \tag{22}$$

Simplifying equation we obtain

$$N\times\left(\left|k\left({}_{R}U_{t},{}_{0}W_{t}\right)\right| - |1|\right) = 0 \tag{23}$$

Multiplying equation by itself it is

$$N\times\left(\left|k\left({}_{R}U_{t},{}_{0}W_{t}\right)\right| - |1|\right)\times N\times\left(\left|k\left({}_{R}U_{t},{}_{0}W_{t}\right)\right| - |1|\right) = 0\times 0 \tag{24}$$

or

$$N^2\times\left(\left|k\left({}_{R}U_{t},{}_{0}W_{t}\right)\right| - |1|\right)^2 = 0 \tag{25}$$

Dividing equation by $N*(|{}_{p}k({}_{R}U_{t,0}W_{t})=1|)=N$, we obtain

$$\frac{N^2\times\left(\left|k\left({}_{R}U_{t},{}_{0}W_{t}\right)\right| - |1|\right)^2}{N} = \frac{0}{N} = 0 \tag{26}$$

or

$$N\times\left(\left|k\left({}_{R}U_{t},{}_{0}W_{t}\right)\right| - |1|\right)^2 = 0 \tag{27}$$

or the X^2 value as

$$\chi^2 = N\times\left(\left|k\left({}_{R}U_{t},{}_{0}W_{t}\right)\right| - |1|\right)^2 = 0 \tag{28}$$

The chi square (X^2) statistic can be used to investigate whether the observed distribution of the causal relationship differ from the theoretical expected distribution of the causal relationship. The table 8 (**Table 8**) contains the critical values of the chi-square distribution (degrees of freedom, df =1). Upper-tail and lower-tail critical values of the chi-square distribution with *v* degrees of freedom are provided by software packages.

3. Results

3.1. *Without* the presence of Mycobacterium avium subspecies paratuberculosis *no* presence of Crohn's disease

Claims.

Null hypothesis:

The presence of Mycobacterium avium subspecies paratuberculosis is a necessary condition (a conditio sine qua non) of Crohn's disease. In other words, the sample distribution agrees with the hypothetical (theoretical) distribution of a necessary condition.

Alternative hypothesis:

The presence of Mycobacterium avium subspecies paratuberculosis is not a necessary condition (a conditio sine qua non) of Crohn's disease. In other words, the sample distribution does not agree with the hypothetical (theoretical) distribution of a necessary condition. The significance level (Alpha) below which the null hypothesis will be rejected is alpha=0,05.

Proof.

The data reviewed by this article which investigated the relationship between the presence of Mycobacterium avium subspecies paratuberculosis and Crohn's disease are viewed by the table (**Table 2**). Altogether, 17 studies were meta-analyzed while the level of significance was alpha = 0,05. Altogether, 10 from 17 studies provide significant evidence of a conditio sine qua non relationship between Mycobacterium avium subspecies paratuberculosis and Crohn's disease. The sample size of the study of Romero et al. [24] was small and has been analyzed and is significant according to the *Rule of three*. In the same respect, 17/17 studies analysed provided evidence of a significant cause effect relationship between Mycobacterium avium subspecies paratuberculosis and Crohn's disease. In other words, *without* a Mycobacterium avium subspecies paratuberculosis infection of human intestinal tract *no* Crohn's disease. Due to methodological inconsistencies, 7 of 17 studies reanalyzed failed to provide statistically significant evidence of this relationship. In point of fact, the presence of Mycobacterium avium subspecies paratuberculosis inside human intestinal tract is a necessary condition (a conditio sine qua non) of Crohn's disease. In other words, **without** the presence of Mycobacterium avium subspecies paratuberculosis inside human intestinal tract **no** Crohn's disease.

Q. e. d.

Table 2. *Without* MAP infection of human intestinal tract *no* Crohn's disease.

Author	Year	Country	a_t	b_t	c_t	d_t	$a_t+b_t+d_t$	N_t	$(a_t+b_t+d_t)/N_t$	X^2(Sine)	k	p val (k)
Mendoza et al. [33]	2010	Spain	30	0	0	10	40	40	1	0,025	1	2,53963E-10
Bull et al. [27]	2003	UK	34	9	3	25	68	71	0,957746479	0,2232142	0,6687230	1,75302E-0
Romero et al. [30]	2005	USA	10	1	2	5	16	18	0,888888889	Rule of 3	0,6446583	0,00623700
Sechi et al. [29]	2005	Italy	30	3	5	26	59	64	0,921875	0,6532258	0,7507418	1,90233E-0
Zamani et al. [39]	2017	Iran	18	6	10	56	80	90	0,888888889	1,3674242	0,5716838	5,84534E-0
Szkaradkiewicz et al. [31]	2007	Poland	10	1	6	11	22	28	0,785714286	1,7794117	0,5488604	0,00368079
Autschbach et al. [28]	2005	Germany	22	4	14	73	99	113	0,876106195	2,0948275	0,6189973	4,70347E-1
Tuci et al. [34]	2011	Italy	21	11	10	21	53	63	0,841269841	2,9112903	0,3336693	0,00808693
Ryan et al. [26]	2002	Ireland	6	0	9	12	18	27	0,666666667	3,4404761	0,4780914	0,01298297
Sanderson et al. [23]	1992	UK	26	5	14	35	66	80	0,825	3,7193877	0,53881591	1,44051E-0
Total			207	40	73	274	521	743	0,701211306	16,21425795	0,4124364	
									Alpha =	0,05		
									Degrees of freedom =	9	Degr. of fr. =	1
									X^2 (Critical) SINE =	16,9189776	Chi crit. (k) =	3,841458821
									X^2 (Calculatedl) SINE=	16,5356865	X^2 calc. (k)=	126,3870966
											k=	0,41243638

3.2. *If* presence of Mycobacterium avium subspecies paratuberculosis *then* presence of Crohn's disease

Claims.

Null hypothesis:

The presence of Mycobacterium avium subspecies paratuberculosis is a sufficient condition (a conditio per quam) of Crohn's disease. In other words, the sample distribution agrees with the hypothetical (theoretical) distribution of a sufficient condition.

Alternative hypothesis:

The presence of Mycobacterium avium subspecies paratuberculosis is not a sufficient condition (a conditio per quam) of Crohn's disease. In other words, the sample distribution does not agree with the hypothetical (theoretical) distribution of a sufficient condition.

The significance level (Alpha) below which the null hypothesis will be rejected is alpha=0,05.

Proof.

The data reviewed by this article which investigated the relationship between the presence of Mycobacterium avium subspecies paratuberculosis inside human intestinal tract and Crohn's disease are viewed by the table (**Table 3**). Altogether, 17 studies were meta-analyzed with n=1076 number of cases and controls while the level of significance was alpha = 0,05. Thus far, 17 out of 17 studies provided significant evidence of a conditio per quam relationship between Mycobacterium avium subspecies paratuberculosis and Crohn's disease. The studies of Ryan et al. [26], Romero et al. [30] and Szkaradkiewicz et al. [31] were analysed and are significant according to the *Rule of three*. In the same respect, 17/17 studies analysed provided evidence of a significant cause effect relationship between Mycobacterium avium subspecies paratuberculosis and Crohn's disease. In other words, *if* infection of human intestinal tract by Mycobacterium avium subspecies paratuberculosis *then* Crohn's disease. In point of fact, the presence of Mycobacterium avium subspecies paratuberculosis within human intestinal tract is a sufficient condition (a conditio per quam) of Crohn's disease. In other words, **if** presence of Mycobacterium avium subspecies paratuberculosis within human intestinal tract **then** presence of Crohn's disease.

Q. e. d.

Table 3. *If* MAP infection *then* CD.

Author	Year	Country	a_t	b_t	c_t	d_t	$a_t+c_t+d_t$	N_t	$(a_t+_tc+d_t)/N_t$	X^2(IMP)	k	p val (k)
Mendoza et al. [33]	2010	Spain	30	0	0	10	40	40	1	0,00833333	1	2,53963E-10
Romero et al. [30]	2005	USA	10	1	2	5	17	18	0,94444444	Rule of 3	0,64465837	0,00623700
Szkaradkiewicz et al. [31]	2007	Poland	10	1	6	11	27	28	0,96428571	Rule of 3	0,54886043	0,00368079
Hulten et al. [25]	2001	USA	7	0	30	22	59	59	1	0,03571429	0,28291621	0,02977124
Ryan et al. [26]	2002	Ireland	6	0	9	12	27	27	1	Rule of 3	0,47809144	0,01298297
Timms et al. [37]	2016	Australia	6	0	15	21	42	42	1	0,04166667	0,40824829	0,00815097
Lee et al. [35]	2011	Canada	5	0	14	19	38	38	1	0,05	0,38924947	0,01641770
Fidler et al. [24]	1994	UK	4	0	27	30	61	61	1	0,0625	0,26059876	0,04181653
Sechi et al. [29]	2005	Italy	30	3	5	26	61	64	0,953125	0,18939394	0,7507418	1,90233E-09
Khan et al. [38]	2016	India	16	3	53	46	115	118	0,97457627	0,32894737	0,22880148	0,01293985
Autschbach et al. [28]	2005	Germany	22	4	14	73	109	113	0,96460177	0,47115385	0,61899734	4,70347E-11
Sanderson et al. [23]	1992	UK	26	5	14	35	75	80	0,9375	0,65322581	0,53881591	1,44051E-06
Kirkwood et al. [32]	2009	Australia	22	6	34	33	89	95	0,93684211	1,08035714	0,257886	0,01195188
Zamani et al. [39]	2017	Iran	18	6	10	56	84	90	0,93333333	1,26041667	0,5716838	5,84534E-08
Bull et al. [27]	2003	UK	34	9	3	25	62	71	0,87323944	1,68023256	0,66872302	1,75302E-08
Nazareth et al. [36]	2015	Portugal	27	11	13	18	58	69	0,84057971	2,90131579	0,29342187	0,01479555
Tuci et al. [34]	2011	Italy	21	11	10	21	52	63	0,82539683	3,4453125	0,33366935	0,00808693
Total			294	60	259	463	1016	1076	0,94423792	12,2085699	0,3774688	

Alpha =	0,05		
Degrees of freedom =	14	Degr. of fr. =	1
X^2 (Critical) IMP =	23,6847913	Chi crit. k =	3,84145882
X^2 (Calculatedl) IMP=	12,2085699	X^2 calc. (k)=	153,311399
		k=	0,377468824

3.3. Mycobacterium avium subspecies paratuberculosis is necessary and sufficient condition of Crohn's disease

Claims.

Null hypothesis:

The presence of Mycobacterium avium subspecies paratuberculosis is a necessary and sufficient condition of Crohn's disease. In other words, the sample distribution agrees with the hypothetical (theoretical) distribution of a necessary and sufficient condition.

Alternative hypothesis:

The presence of Mycobacterium avium subspecies paratuberculosis is not a necessary and sufficient condition of Crohn's disease. In other words, the sample distribution does not agree with the hypothetical (theoretical) distribution of a necessary and sufficient condition.

The significance level (Alpha) below which the null hypothesis will be rejected is alpha=0,05.

Proof.

The data reviewed by this article which investigated the relationship between the presence of Mycobacterium avium subspecies paratuberculosis and Crohn's disease are viewed by the table (**Table 4**). Altogether, in this proof, 17 studies were meta-analyzed while the level of significance was alpha = 0,05. In toto, 8 studies were able to provide significant evidence that Mycobacterium avium subspecies paratuberculosis is a necessary and sufficient condition of Crohn's disease. The study of Romero et al. [30] have been analysed and was significant according to the *Rule of three*. In point of fact, more or less older studies failed to provide evidence of this relationship. In the same respect, 17/17 studies analyzed provided evidence of a significant cause effect relationship between Mycobacterium avium subspecies paratuberculosis and Crohn's disease and thus far the absence of independence. In other words it is true that *without* the presence of Mycobacterium avium subspecies paratuberculosis within human intestinal tract *no* presence of Crohn's disease and at the same time it is equally true that *if* presence of Mycobacterium avium subspecies paratuberculosis in human intestinal tract *then* presence of Crohn's disease too. In particular, Mycobacterium avium subspecies paratuberculosis is a necessary and sufficient condition of Crohn's disease.

Q. e. d.

Table 4. MAP infection of human intestinal tract is a necessary and sufficient condition of CD.

Author	Year	Country	a_t	b_t	c_t	d_t	a_t+d_t	$a_t+b_t+c_t+d_t=N_t$	$(a_t+d_t)/N_t$	X^2(Sine and Imp)	k	p val (k)
Mendoza et al. [33]	2010	Spain	30	0	0	10	40	40	1	0,033333333	1	2,53963E-10
Romero et al. [30]	2005	USA	10	1	2	5	15	18	0,8333333	Rule of 3	0,64465837	0,006237007
Sechi et al. [29]	2005	Italy	30	3	5	26	56	64	0,875	0,842619746	0,7507418	1,90233E-09
Szkaradkiewicz et al. [31]	2007	Poland	10	1	6	11	21	28	0,75	1,802139037	0,54886043	0,003680795
Bull et al. [27]	2003	UK	34	9	3	25	59	71	0,8309859	1,903446844	0,66872302	1,75302E-08
Autschbach et al. [28]	2005	Germany	22	4	14	73	95	113	0,840708	2,565981432	0,61899734	4,70347E-11
Zamani et al. [39]	2017	Iran	18	6	10	56	74	90	0,8222222	2,627840909	0,5716838	5,84534E-08
Ryan et al. [26]	2002	Ireland	6	0	9	12	18	27	0,6666667	3,482142857	0,47809144	0,012982973
Total			160	24	49	218	378	451	0,8381375	13,25750416	0,6437068	
									Alpha =	0,05		
									Degrees of freedom =	7	Degr. of fr. =	1
									X^2 (Critical) =	14,06714045	Chi crit. k =	3,841458821
									X^2 (Calculated) =	13,25750416	X^2 calc. (k)=	186,875687
											k=	0,643706849

3.4. Mycobacterium avium subspecies paratuberculosis is the cause of Crohn's disease

Claims.

Null hypothesis: (**no causal relationship**)

There is no significant causal relationship between an infection by Mycobacterium avium subspecies paratuberculosis and Crohn's disease.

(k=0).

Alternative hypothesis: (**causal relationship**)

There is a significant causal relationship between an infection by Mycobacterium avium subspecies paratuberculosis and Crohn's disease.

(k≠0).

Conditions.

Alpha level = 5%.

The two tailed critical Chi square value (degrees of freedom = 1) for alpha level 5% is 3.841458821.

Proof.

The data for this hypothesis test were provided by different studies and are illustrated by a table (**Table 3**). The causal relationship k(Mycobacterium avium subspecies paratuberculosis, Crohn's disease) is calculated according to [40]-[62]. Again, 17 studies were meta-analyzed with n=1076 number of cases and controls while the level of significance was alpha = 0,05. Thus far, all the 17 studies analyzed provided evidence of a significant cause effect relationship between Mycobacterium avium subspecies paratuberculosis and Crohn's disease. In other words, Mycobacterium avium subspecies paratuberculosis and Crohn's disease are not only not independent of each other. Besides of the methodological difficulties associated with the studies analyzed the majority of the studies provided significant evidence of a necessary condition, of a sufficient condition and of a necessary and sufficient condition. The conclusion appears to be inescapable: Mycobacterium avium subspecies paratuberculosis *is the cause of* Crohn's disease (k=+0,377468824, p value < 0.0001)..

Q. e. d.

3.5. Antibiotic therapy and Crohn's disease

One of the most convincing contributions to the evidence of a role for MAP in Crohn's disease would be a proof that a treatment with appropriate antibiotics for sufficient duration able kill the organism leads to remission of Crohn's disease. In this context, different antibiotic drug combinations, called anti-MAP regimen, including clarithromycin (CLA), rifabutin (RIF) and Clofazimine (CLO) investigated in multiple randomized clinical trials have shown [76]-[80] promising results. A complete healing of ulcers in Crohn's disease patients after more than 6 months of treatment with a rifabutin (RIF) and clarithromycin (CLA) regimen, an inhibitor of CYP34A [81], has been reported by several studies. A 2007 case study reported that one Crohn's disease patient who was being treated with anti-MAP therapy drugs, attained complete clinical remission [82]. Selby et al. [83] conducted a prospective, parallel, placebo-controlled, double-blind, randomized treatment trial using the combination of clarithromycin, rifabutin, and clofazimine. Two hundred thirteen patients were randomized while the study design of the study of Selby et al. [83]included an initial 16-week phase in which all patients received prednisolone in addition to trial medications. Remission was defined as Crohn's Disease Activity Index < 150. Using combination antibiotic therapy with rifabutin, clarithromycin, and clofazimine Selby et al. [83] did not find evidence of a sustained benefit for Crohn's disease patient. At the end of week 16 period, there was only "a significantly greater percentage of subjects in remission in the antibiotic arm (67/102 [66%]) than in the placebo arm (55/111 [50%]) (P = .02)". Selby et al. [83] concluded that their finding does not support a significant contribution of Mycobacterium avium subspecies paratuberculosis in the pathogenesis of Crohn's disease. Thus, what unifies the conclusions of the abovementioned study group [83] is seemingly the disparate misuse of statistical methods and the data of their study made publicly available for further investigations to an extent which demands and justifies several critical remarks. Firstly. The inappropriate definition of remission of Crohn's disease (defined as *Crohn's Disease Activity Index* < 150) is highly subjective, unfair and has underestimated Crohn's Disease cases achieved remission. The use of Crohn's Disease Activity Index has overestimated the number of cases in the placebo group which achieved remission. Therapeutic monitoring can and should be grounded on objective parameters too like C-reactive protein, Erythrocyte sedimentation rate, Procalcitonin (PCT), antibody (Ab) against Mycobacterium avium ssp. Paratuberculosis, faecal calprotectin, colonoscopy and other. Secondly. The abovementioned study presented contradictory outcomes while at the same time it cannot be excluded that subjects were not exposed to the correct dose of drugs. Clofazimine capsules were reencapsulated and it was found that the clofazimine capsules did not rupture in vitro. "Because of this, there was a period of approximately 10 months during which it was possible that subjects were not exposed to the correct dose of clofazimine." [83] In contrast to Selby et al. [83], Gui et al. [84] treated 46 patients with severe Crohn's disease with rifabutin in combination with a macrolide antibiotic (clarithromycin or azithromycin). An improvement in inflammatory parameters was observed and the clinical remission based on the *Harvey–Bradshaw activity index* was induced in 43

(93.5%) patients. The studies concerning chemotherapy with antimycobacterial agents have given mixed or contradictory [85] results in Crohn's disease patients. Thus far, a causal relationship between M. paratuberculosis and Crohn's disease derived from chemotherapy studies has not been demonstrated beyond any reasonable doubt.

4. Discussion

Other systematic reviews and meta-analysis assessed already the evidence for an association between Mycobacterium avium subspecies paratuberculosis (MAP) and Crohn's disease (CD). Does another review make sense at all? Feller et al. [86] were not able to solve the problem and concluded that the role of Mycobacterium avium subspecies paratuberculosis (MAP) in the aetiology of Crohn's disease remains to be defined. The systematic review and meta-analysis of the Mycobacterium avium subspecies paratuberculosis and Crohn's disease published by Abubakar et al. [87] (UK) reviewed 47 studies demonstrated that the relationship between MAP and CD "remains controversial and inconclusive. Future studies should determine whether there is a pathogenic role." Mycobacterium avium subspecies paratuberculosis is a robust and phenotypically versatile pathogen which is widespread in domestic livestock, present in retail pasteurized cows' milk and potentially, elsewhere. A high-temperature short-time pasteurization of milk appears not effectively to kill M. paratuberculosis in milk [88]. Even water supplies are also at risk. Mycobacterium avium subspecies paratuberculosis (MAP) has the potential to cause chronic inflammation of the intestine in many species, including primates. Crohn's disease has long been suspected of having a mycobacterial cause. Still, for many years the role of mycobacteria, specifically Mycobacterium paratuberculosis in Crohn's disease has aroused considerable controversy. The inconsistencies throughout the studies on the relationship between Mycobacterium paratuberculosis and Crohn's disease are very great and due to several factors. First of all, it is known that Mycobacterium paratuberculosis is very difficult to be detected by culture and due to the heterogeneous nature of CD the rate of detection of M. avium subsp. paratuberculosis in individuals with CD can vary extremely. The development of highly sensitive and M. paratuberculosis-specific polymerase chain reaction assays and appropriate in situ hybridization methods is highly welcomed. Further technical and methodological advances should allow the identification and/or isolation of M. paratuberculosis from a significantly higher proportion of Crohn's disease tissues compared too controls. Clinical differences in patients studied, including treatment regimens and the duration of disease are not considered in an appropriate manner. Patients studied were analyzed by different methods while the sensitivity of the methods used was not determined. In addition to these inconsistencies, the methodological uncertainty surrounding the role of Mycobacterium avium subsp paratuberculosis (Map) in Crohn's disease is great. The studies analyzed are full of contradictory findings due to small sample size, the lack of uniformity in the materials and methods used by many authors and other factors too. Under such circumstances, is it possible at all to provide new and convincing perspectives [89]? One might argue that in line with problems like these, the question is justified whether a

decision about a relationship between M. avium subsp. paratuberculosis and Crohn's disease under such conditions is possible at all. Still, the extent to which several studies were able to determine a strong and significant relationship between Mycobacterium avium subsp. paratuberculosis and Crohn's disease reduces the penumbra of uncertainty surrounding the methodological problems of the studies dramatically.

In toto, 10 of 17 studies analysed using the ultra sensitive polymerase chain reaction (PCR) for the detection of M. avium subsp. paratuberculosis within Crohn's disease provided convincing evidence of a conditio sine qua non relationship between M. avium subsp. paratuberculosis and Crohn's disease patients. In other words, ***without*** **M. avium subsp. paratuberculosis** ***no*** **Crohn's disease**. The involvement of M. avium subsp. paratuberculosis in Crohn's disease (CD) in humans has been uncertain because of several substantial difficulties especially in detecting this pathogen. Still, even if 7 studies failed on the point aformentioned, the question is how could 10 studies document such an evidence. At the same time, M. avium subsp. paratuberculosis and Crohn's disease are causally related.

All studies analyzed provide convincing evidence that the presence of M. avium subsp. paratuberculosis gurantees the presence of Crohn's disease. In other words**, if** *an infection with M. avium subsp. paratuberculosis is present***, then** *Crohn's disease is present too* ($X^2_{Calculated}$ = 12,2085699 and is less than $X^2_{Critical}$ = 23,6847913, degrees of freedom = 14). At the same time, M. avium subsp. paratuberculosis and Crohn's disease were not independent of each other. Necessary and sufficient conditions are converses of one another. If it is true that **if** *MAP***, then** *CD,* the converse is automatically true too: *without* CD *no* MAP, the data presented support such a hypothesis too. In principle, inflammatory bowel disease can arise when the mucosal barrier is compromised in its defense against challenges from the intestinal microbiota by own immune system or by other factors with the consequence that a MAP infection is only a secondary bacterial infection at an immunocompromised site. Theoretically, such a conclusion is possible but not convincing and the need for a better explanation is especially pressing, since some studies provided data which are extraordinary and cannot be ignored. Additionally, 8 out of 17 studies were able to provide evidence that **M. avium subsp. paratuberculosis is a necessary and sufficient condition of Crohn's disease**. In this context, 17 of 17 studies provided data which are consistent with the conclusion that there is a significant cause and effect relationship between M. avium subsp. paratuberculosis and Crohn's disease. Besides of the very limited number of cases and controls studied, the present study confirms previous reports of the association between M. avium subsp. paratuberculosis and Crohn's disease. More particularly, according to Zamani et al. [39] Mycobacterium avium subsp. paratuberculosis is a necessary condition of Crohn's disease and equally a sufficient condition of Crohn's disease while the cause effect relationship between Mycobacterium avium subsp. paratuberculosis and Crohn's disease is highly significant. In point of fact, i. e. how could Zamani et al. [39] provide such an impressive evidence of the relationship between Mycobacterium avium subsp. paratuberculosis and Crohn's disease? The data of Zamani et al. [39] are viewed by

the **Table 9**.

Table 9. The data of Zamani et al. [39].

		Crohn's disease (CD)		
		Yes	No	Total
Mycobacterium avium	Yes	18	6	24
subsp. paratuberculosis (MAP)	No	10	56	66
	Total	28	62	90

Results:			
p(U←W)=	0,8889	p(U→W)=	0,93333333
X^2(U←W) =	1,3674	X^2(U→W) =	1,26041667
k(U,W) =	0,5717		
p val (k)=	6E-08		

Altogether, the results of this study support the hypothesis that M. avium subsp. paratuberculosis is the cause of Crohn's disease which implicates the necessity of an effective antibacterial treatment supported by a plant based [90] lactose free diet of this mycobacterial intestinal infection. Once questions raised by methodological inconsistencies and their difficulty are acknowledged, a treatment of Crohn's disease patients with appropriate antibiotics for sufficient duration able kill Mycobacterium avium ssp. paratuberculosis (MAP) is worth being considered. Effective treatment of a mycobacterial infection can be difficult, due to the structure of the mycobacterial cell wall. In principle, the mycobacterial cell wall can hinder the entry of drugs and is able to make many antibiotics ineffective. Mycobacterium avium ssp. paratuberculosis (MAP) as the etiologic agent of cattle's paratuberculosis (Johne's disease) lacks its *cell wall* in humans [91] while more than 30 strains [92] of MAP have been identified. Cell wall deficient bacterial organisms may lack important cell wall antigens more than cell wall competent bacterial organisms, and so are less visible to host immune surveillance and theoretically may survive better than other organism. The human immune system shaped through evolution by the necessity of discriminating non-self pathogens from self tissues and extremely important for the survival of a multicellular organisms uses especially antigens to determine which cells are resident and which are foreign. In the following, antibodies can recognize an antigen and lock onto it without being capable of destroying it without help. Mycobacterium avium ssp. paratuberculosis appears to avoid recognition by the immune system and makes the triggering of immune responses against itself to a very great extent ineffective. Parameters like C-reactive protein, Erythrocyte sedimentation rate, (Procalcitonin (PCT)), IgG-, IgM, IgA antibodies against Mycobacterium avium ssp. paratuberculosis may provide only a weak and approximate picture of the extent of inflammation. In particular, using antibiotic drugs which aim to target the cell wall of Mycobacterium avium ssp. paratuberculosis may lead to complications, the rise of multidrug resistance bacteria and inhibiting of normal flora. At the end, such a strategy could be inefficient for treatment of Crohn's disease patients.

The evidence presented in this study based on a systematic review of other studies justify the assumption to treat Crohn's disease with antibiotic. The following or similar Phase I (**Table 10**) and Phase II (**Table 11**) drug regimen for the treatment of Crohn's disease patients is still not established. Whether does it makes sense to stop the therapy with Rifaximin 550 mg b.i.d. (i. e. or Metronidazol 400 mg b.i.d. and Ciprofloxacin 500 mg b.i.d.) which is used to control intestinal microbiome dysbiosis and Predinsolon 60 mg q.d. (in this context used to control clinical symptoms) after two months of therapy is another point which is not secured. Diagnostic (or therapeutic) colonoscopy if necessary with the support of a feeding / gastroduodenal tube is needed for therapeutic monitoring and to reduce the microflora of the gut mechanically. The therapy with drugs free of lactose (a disaccharide composed of galactose and glucose) like Clarithromycin 500 mg b. i. d., Rifabutin 450 mg q. d. and Colofazimine 50 mg q. d. should continue for at least further 4 months or longer until a complete remission is achieved. Although shortening the duration of therapy is a desirable target, the use of the standard 4-month rifabutin-containing regimen or longer is worth being considered.

Table 10. Phase I. The starting 2 months antibiotic regimen to treat MAP in Crohn's disease.

Week	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Predinsolon 60 mg q.d.	(x)		(x)		(x)		(x)	
Rifaximin 550 mg b.i.d.	x		x		x		x	
Probiotics / 25 OH Vitamin D3 …		x		x		x		x
(diagnostic or therapeutic) colonoscopy		x		x		x		x
Furthermore:								
Colofazimine 50 mg q.d.	x	x	x	x	x	x	x	x
Clarithromycin 500 mg b.i.d.	x	x	x	x	x	x	x	x
Rifabutin 450 mg q.d.	x	x	x	x	x	x	x	x

Safety evaluation, risk control and therapeutic monitoring should consider at least prameters like C-reactive protein, Erythrocyte sedimentation rate, (Procalcitonin (PCT)), IgG-, IgM, IgA antibodies against Mycobacterium avium ssp. paratuberculosis, faecal calprotectin and of course other too. The novelty of this study is focused on the causal relationship between Crohn's disease and Mycobacterium avium ssp. paratuberculosis. Reports about the relationship between Crohn's disease (CD) and cell wall-deficient (CWD) forms of Mycobacterium avium subspecies paratuberculosis (M. paratuberculosis) are still controversial and even this study must acknowledge that a causal link between Mycobacterium avium subspecies paratuberculosis (MAP) and Crohn's disease has not been established beyond any reasonable doubt, arguments to the contrary equally have to be considered. Besides of the difficulties mentioned growing evidence determined by the studies presented suggests that **Mycobacterium avium subspecies paratuberculosis is a necessary and / or sufficient condition of Crohn's disease**.

Table 11. Phase II. The 4 months and longer antibiotic regimen to treat MAP in Crohn's disease.

Month	3	4	5	6	7	8	9	10
Colofazimine 50 mg q.d.	x	x	x	x	x	x	x	…
Clarithromycin 500 mg b.i.d.	x	x	x	x	x	x	x	…
Rifabutin 450 mg q.d.	x	x	x	x	x	x	x	…
(diagnostic or therapeutic) colonoscopy	x	(x)	(x)	x	(x)	(x)	x	…
Probiotics / 25 OH Vitamin D3 …	x			x			x	

At the same time, all studies provided evidence of a significant cause effect relationship between and Mycobacterium avium subspecies paratuberculosis (MAP) and Crohn's disease. This article provides a review of recent PCR DNA based works on the relationship between and Mycobacterium avium subspecies paratuberculosis (MAP) and Crohn's disease and invites us to consider the following inescapable conclusion.

5. Conclusion

Mycobacterium avium subspecies paratuberculosis (MAP) is the cause of Crohn's disease.

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