

**Infant antibiotic use and wheeze and asthma risk - A systematic review and meta-analysis**

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Abbreviations: OR - Odds Ratio; RR - Relative Risk; HR - Hazard Ratio; RC - reverse causation; CbI - confounding by indication

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## **ABSTRACT**

### **Aim**

To systematically review and meta-analyse longitudinal studies on antibiotic use and subsequent development of wheeze and/or asthma in the light of study quality, outcome measurement, reverse causation (wheezing or asthmatic symptoms themselves have caused the prescription of antibiotics) and confounding-by-indication (respiratory tract infections that lead to antibiotic use may be the underlying cause triggering later development of asthma symptoms).

### **Methods**

Studies were identified through Pubmed, Medline and Embase searches up to November 1st 2010 and by perusing reference lists. Only English-language papers and studies with a longitudinal observational design were included. Study quality was assessed using the Newcastle-Ottawa scale.

### **Results**

We identified 21 longitudinal studies. The effect of antibiotic use on wheeze/asthma risk varied between studies. Eighteen studies were eligible for meta-analysis, showing a pooled odds ratio of 1.27 (95% confidence interval 1.12-1.43) for wheeze/asthma. When we eliminated studies with possible reverse causation and confounding-by-indication, the pooled risk estimate in the nine remaining studies was attenuated to an odds ratio of 1.12 (0.98-1.26).

Definition of wheeze or asthma and age at follow-up differed widely between studies. Three studies focussed on wheeze/asthma beyond the age of 5-6 years with the presence of active symptoms and/or medication showing a pooled odds ratio of 1.08 (0.93-1.23) which was dominated by one large study.

### **Conclusion**

Heterogeneity of disease definition, reverse causation and confounding-by-indication lead to overestimation of the association between antibiotic use and the subsequent development of wheeze/asthma. The association was weak when fully adjusted for these types of bias.

## INTRODUCTION

Reports on the relationship between antibiotic use in infancy and the subsequent development of wheeze or asthma have been published continuously for over a decade now.

Conflicting results may be attributable to different study designs: cross-sectional or longitudinal, differences in outcome definition and population age at follow-up, and two forms of bias related to causality: reverse causation (RC) and confounding-by-indication (CbI) [1]

Cross-sectional studies may be prone to bias because they do not allow an assessment of the timing of exposure in relation to the occurrence of wheeze or asthma, and therefore longitudinal (cohort) studies are preferred. A recent meta-analysis indeed showed a significant stronger association between infant antibiotic use and asthma in retrospective and cross-sectional studies, as compared to prospective studies [2].

But also in cohort studies caution is still needed before concluding that associations are causal. This paper addresses two issues of causality: reverse causation (RC) and confounding-by-indication (CbI). [1] *Reverse causation* plays a role when the period of antibiotic use and the period that wheezing/asthmatic symptoms had appeared have (partly) coincided. In that situation the symptoms themselves may have caused the prescription of antibiotics.

*Confounding-by-indication* refers to those situations in which the indication for treatment acts as a confounder. In those cases, an association between antibiotic intake and wheeze/asthma is confounded (explained) by a third factor that is *an indication* for antibiotics prescription while at the same time being a risk factor for asthma. This may occur when antibiotics are prescribed for respiratory tract infections which then are the actual risk factor for wheeze/asthma.

Some recent studies [3] and narrative reviews [1] even focussed on the very question whether RC or CbI could explain the reported associations between antibiotic use and respiratory allergy and asthma, without deriving at a conclusive answer.

Heterogeneity in outcome definition might contribute to inconsistency in results. Asthma is a heterogeneous condition and wheezing, its major clinical expression, is a non-specific sign associated with airflow obstruction through narrowed airways.

Infant wheezing is often transient and resolves between the age of 3 to 5 years. Furthermore, early life wheezing is frequently associated with viral respiratory agents, often respiratory syncytial virus (RSV) [4] Therefore, studies focussing on preschool wheeze represent different and more heterogeneous patient populations compared to studies that focus on wheeze-related or asthma-like symptoms that persist into school-age.

In this paper we systematically review the available longitudinal studies and describe how outcome definition, RC and CbI affect the association between antibiotic use in early life and the subsequent development of wheeze or asthma.

## **METHODS**

### **Systematic review**

We included longitudinal observational studies on the association between antibiotic exposure: historical or prospective cohort studies and nested case-control studies within a cohort or case-cohort studies. Cross-sectional and retrospective case-control studies were excluded.

Medline, EMBASE and PubMed were searched up to November 1<sup>st</sup> 2010, using the following keywords: ((antibiotic\* or antibact\*) AND (allergy OR allergic OR hypersens\* OR atop\* OR eczema OR asthma OR wheeze) AND (cohort OR follow-up OR longitudinal OR prospective)). The search was limited to English written papers. Additional studies were found by searching reference lists of pertinent articles.

### **Meta-analyses**

To assess how outcome definition, RC and CbI affect the association between antibiotic use in early life and the subsequent development of wheeze or asthma, we conducted three meta-analyses.

#### *Inclusion criteria*

The first “overall” meta-analysis included all studies from the systematic review that fulfilled the following additional criteria:

#### 1. Assessment of antibiotic exposure

- Information on antibiotic exposure collected prospectively
- Exposure in first year of life through direct oral administration to the infant
- Time lag between antibiotic exposure and assessment of this exposure (e.g. parental report) no more than 1 year (to avoid recall bias); or historically recorded exposure data were available (e.g. from prescription registry).

#### 2. Assessment of outcome(s):

- Wheezing/asthmatic symptoms in first 10 years of life (studies in children over the age of 10, adolescents and adults excluded)
- Wheeze and/or asthma included as a primary outcome measure

#### 3. Measures of association and adjustment for confounding

- Measures of association (Odds ratios (OR), Relative Risk (RR) or Hazard Ratio (HR)) were available along with parameters of precision needed for statistical pooling (e.g. 95% confidence interval (CI), standard error)
- Adjustment for potential confounding factors by stratified or multivariable analysis

#### *Data extraction*

Subsequently, exposure and outcome measures were selected as follows:

## 1. Antibiotic exposure

- Comparisons for any vs. no antibiotic use were selected. If unavailable but associations were presented according to the number of antibiotic prescriptions, the association comparing the highest number of antibiotic courses vs. no antibiotic courses was selected.

## 2. Wheeze and/or asthma outcomes

- When studies presented respiratory outcomes at different ages, the oldest age /longest duration of follow up was selected
- When multiple time points of wheezing/asthmatic symptoms were presented ('ever' versus 'current'), 'current' symptoms were selected to avoid RC
- When associations for multiple respiratory outcomes were presented, the first applicable option from the following list was selected:
  1. doctor's diagnosed asthma
  2. reported asthma
  3. reported wheeze with asthma medication
  4. reported persistent wheeze
  5. reported wheeze
  6. reported transient wheeze

### *Additional meta-analyses*

We then conducted a second meta-analysis restricting the studies of the "overall" meta-analyses to studies that render bias from RC and CbI unlikely:

1. avoidance of *reverse causation*: no overlap between the exposure and development of first wheezing/asthmatic symptoms in statistical analysis
2. avoidance of *confounding-by-indication*: adjusting the analysis for lower respiratory tract infections (or 'chest infections').

If this was not entirely clear from the publications, we contacted the authors for clarification by e-mail.

All authors promptly responded to our queries with satisfactory answers.

Finally, we conducted a third meta-analysis restricting the studies from the "overall" meta-analyses to studies that focused on wheeze/asthma beyond the age of 5-6 years and that included the presence of active symptoms and/or medication use at school-age..

Two of us (JP and IK) independently assessed which studies were eligible for the overall and "strict criteria" meta-analyses, and in case of a conflicting judgment the third author (CT) was consulted. In all cases consensus was reached.

### *Quality assessment of studies in meta-analysis*

Articles eligible for inclusion in the overall meta-analysis were assessed for quality by three reviewers (JP, IK, CT) independently using the Newcastle-Ottawa Quality Assessment Scale (NOS) for cohort studies.[5] The reviewers resolved disagreements by discussion to achieve consensus (for a detailed description of the quality assessment according to the NOS, see online supplement).

### *Statistical analyses*

Measures of association (OR, RR, HR) and their 95% confidence intervals (CIs) were abstracted or derived by using data reported in the publications. When several measures of association were reported, we selected the measure according to criteria mentioned above. When RRs or HRs were presented, we assumed that the OR approximated the RR or HR and treated them as such.

To derive a pooled odds ratio from individual studies, we used a random-effects meta-analysis model.

Heterogeneity was quantified with the I-square index, which describes the proportion of total variation in study estimates due to heterogeneity.[6] To assess the potential presence of publication bias, the size of the estimated effect of the included studies was plotted against the standard error (funnel-plot). Begg's rank correlation [7] and Egger's linear regression tests [8] were used to detect asymmetry in funnel plots.

Statistical analyses were conducted with Stata version 10.1 (STATA Corp, College Station, Texas, USA).

## **RESULTS**

### **Study selection and characteristics**

We identified 634 papers of which 35 were original studies on the association between antibiotic exposure and the risk of allergic diseases and/or wheeze and/or asthma (Figure 1).

Fifteen studies were excluded for the following reasons:

- antibiotic exposure was collected retrospectively (4 studies) [9-12]
- no report on wheeze and/or asthma, but other allergic outcomes only (6 studies) [13-18]
- no association measure for wheeze or asthma separately but an overall outcome called "atopy" that also included eczema and/or hay fever (1 study) [19]
- prenatal antibiotic use reported only (3 studies) [20-22]
- adult population (1 study) [23]

Searching reference lists resulted in the identification of eight additional studies, of which one ecological study [24], six cross-sectional studies [25-30] and one that met our inclusion criteria.[31] Altogether this resulted in 21 studies fulfilling our selection criteria (Online supplement Table E1).

**Table 1.** Assessment of methodological quality of studies according to the Newcastle-Ottawa Scale for cohort studies (sorted by total quality score).

Study	Selection <sup>†</sup>	Comparability <sup>‡</sup>	Outcome <sup>¶</sup>	Included in “strict-criteria” analysis*
Marra 2009 [36], Canada	****	**	***	yes
Kozyrskyj 2007[43], Canada	****	**	***	yes
Martel 2009 [37], Canada	****	**	**	yes
Harris 2007[44], UK	****	**	**	yes
Celedon 2004 [45], USA	****	**	**	yes
Mai 2010 [32], Sweden	***	**	**	yes
Wickens 2008 [3], New-Zealand	****	**	*	yes
Kusel 2008[38], Australia	****	**	*	yes
Ponsonby 1999 [48] USA	****	*	**	no
Su 2010 [34], USA	****	*	*	no
Schmitt 2009 [35], Germany	***	*	**	no
McKeever 2002 [47], UK	****	*	*	no
Alm 2008[40], Sweden	***	*	*	no
Kummeling 2007 [42], Netherlands	***	*	*	no
Celedon 2002 [46], USA	***	*	*	no
Dom 2010 [33], Belgium	**	**		yes
Simon 2008[39], USA	**	*		no
Verhulst 2008[41], Belgium	**	*		no

<sup>†</sup>maximum of four stars; <sup>‡</sup>maximum of two stars; <sup>¶</sup>maximum of three stars; \*included in 1<sup>st</sup> restricted meta-analysis (RC and Cbl unlikely)

## **Systematic review**

In seven of these 21 studies wheeze or asthma were not associated with antibiotic use [31, 33, 35, 45, 46, 48, 49], so that bias deriving from reverse causation and confounding-by-indication is not of concern. Below we will discuss the remaining 14 studies in the light of reverse causation and confounding-by-indication.

### *Reverse causation (RC)*

In a couple of studies, exposure and outcome partly coincided and RC could not be excluded [39,50] Four other studies [3, 41, 42, 44] corrected for RC by means of excluding from the analyses children who had already developed symptoms at the time of antibiotic use. In three of them [3, 41, 44] initially positive associations between antibiotic use and wheeze or asthma disappeared after correction, whereas in the fourth study [42] the association between antibiotic use and wheeze remained after correcting for RC. RC as an explanation was unlikely in six studies that had ensured that exposure (antibiotic use) and follow-up periods took place subsequently and were not overlapping. [34, 36-38, 43, 47] Furthermore, Alm *et al.* [40] found an increased risk of wheeze in the first year of life after antibiotic use in the first days after birth. This is such a short period of overlap that also here we deemed RC unlikely.

Taken together, this results in two studies with doubtful results [39, 50], three studies in which the association disappeared after correcting for RC [3, 41, 44], and eight that found a positive association that could not be explained by RC. [34, 36-38, 40, 42, 43, 47].

Finally, the study by Mai and colleagues [32] first eliminated CbI before tackling the influence of reverse causation. Therefore, this study will only be discussed in the next section, together with the remaining eight studies in which the positive association could not be explained by RC.

### *Confounding-by-indication (CbI)*

Several of the eight studies mentioned above (reverse causation excluded) did not collect relevant information about the indication for antibiotic prescription or episodes of infections at the time of antibiotic exposure [34, 40, 42, 47], whereas the other studies did. [36-38, 43] The study by Kusel *et al.* [38] illustrates why CbI should be taken into account. In this study children with lower tract respiratory infection (LTRI) and wheeze had used antibiotics twice as often as compared with children with LTRI without wheeze or a higher tract respiratory infection (HTRI). The children with LTRI and wheeze had a threefold higher risk of developing asthma until the age of five years. After adjustments for the indication for antibiotic use (LTRI with wheeze, without wheeze, HTRI, or other symptoms such as fever) the association between antibiotic use and asthma diagnosis almost entirely disappeared. This clearly illustrates CbI and indicates that it may not be a direct causal effect of antibiotics. It is more likely that patients with an underlying susceptibility to react to infections with wheeze are more prone to receive antibiotics.

In the study by Mai and colleagues [32] associations between antibiotic use in the first year of life and wheeze/asthma at the ages of 4 and 8 years became weaker after adjustment for respiratory infections, and



only remained significant for the outcomes at age 4 years. Following exclusion of children with wheeze, asthma and eczema symptoms in their first year of life, thus eliminating reverse causation, completely eliminated the associations between antibiotic use and wheeze and asthma at both the ages of 4 and 8 years [32].

Two studies adjusted for consultation behaviour or number of illness visits [34, 47], and one of them expressly stated that this was to resolve CbI [34] however we contend that this method is not specific enough for the exact indication for antibiotics.

Finally, in three other studies, the associations between antibiotic use and asthma remained after adequate adjustment for infections. [36, 37, 43]

Taken together, this results in four studies that did not collect data on the indication for antibiotic prescription or episodes of lower respiratory tract infections [34, 40, 42, 47], two studies in which the association disappeared after adjusting for CbI [32, 38] and three studies in which a positive association remained after controlling for both RC as well as CbI [36, 37, 43].

### *Outcome definition*

Studies differed widely regarding the outcome definition and age at follow-up.

Three studies examined the development of (recurrent) wheeze in the first or two years of life only [40-42]. Noteworthy, these are the studies that reported by far the strongest associations with antibiotic use (Fig 2A). Six studies had follow-up periods until the age of 4-5 years [3, 33, 34, 38, 45, 46] of which one focused on recurrent wheeze [33] and the remaining five were based on the outcome “asthma” using various definitions [3, 34, 38, 45, 46]. It has to be kept in mind that a diagnosis of asthma in preschool children is difficult to make establish, as it can often not be distinguished from infection-related wheeze. As a consequence this may result in serious misclassification of transient wheezers as asthma cases.

One study focused on transient wheeze, excluding those children with persisted wheezing up to the age of 6 years [39].

Although many studies had a follow up beyond preschool age [32, 36, 37, 43, 44, 47, 48], several of them did not distinguish between age periods of diagnosis, thus still resulting in many cases being diagnosed already at preschool ages. [36, 37, 47, 48]. The studies by Martel et al. and Ponsonby et al. did conduct subgroup analysis based on age of asthma onset, but with a cutoff as early as 3 and 4 years respectively [37, 48]. The studies by McKeever et al. and Marra et. al with a follow-up of 9-11 years, used survival analysis [36, 47] but the vast majority of asthma cases were diagnosed before the age of 5 years, and therefore did not agree to our criterion for the third meta-analysis.

This leaves only three studies that did explicitly focus on the persistence of wheeze or asthma into school-age. These studies with a follow-up until the age of 7-8 years all included current (past 12 months) wheezing/asthmatic symptoms and/or current medication use into their outcome definition [32, 43, 44].

## Meta-analysis

Eighteen studies on wheeze and asthma were included in the overall meta-analysis. Figure 1 shows the quality grading according to the Newcastle-Ottawa scale (NOS). The pooled risk estimate (odds ratio; OR, see Figure 2A) was 1.27 with a 95% confidence interval (95% CI) of 1.12-1.43, indicating a slight increase in the risk of developing asthma and/or wheeze after antibiotic use in the first year of life. There is no indication of publication bias, as both the Begg's ( $p = 0.85$ ) and Egger's test ( $p = 0.14$ ) do not indicate asymmetry of the funnel plot (Figure 3). It has to be noted that publication bias cannot be ruled out completely because of low sensitivity of both tests for fewer than 20 studies.[51]

The results were greatly variable between studies, and the test for heterogeneity was statistically significant (I-square index 75.7%, test for heterogeneity  $P < 0.001$ ).

Nine studies fulfilled the criteria for inclusion in the first restricted meta-analysis (RC and CbI unlikely) (Figure 2B).

With the exception of the study by Dom et al. [33], these studies were also the studies that scored highest on the NOS. The pooled OR was 1.12; 95% CI 0.98-1.26; and the results showed less heterogeneity (I-square statistic decreased to 46.3%, test for heterogeneity  $P = 0.06$ ). This pooled OR is substantially lower than the OR in the overall meta-analysis, but because the OR is still somewhat above 1.0, it indicates that potential bias from RC and CbI does not entirely explain the increased risk. However, the lower 95% confidence limit of 0.98 indicates that an OR of 1.0 (no effect) cannot be excluded with certainty.

Only three studies fulfilled the inclusion criteria, persistence of wheeze/asthma into school age, for the third meta-analysis. Since the study by Harris et. al contributed over 80% to the weight, the pooled OR (1.08; 0.93-1.23) was dominated and closely resembled the association from this study (OR = 1.08; 0.94-1.25, see Online Supplement FigureE1).

## DISCUSSION

With this paper we sought to investigate how two methodological concepts, namely reverse causation (RC) and confounding-by-indication (CbI) affect the potential association between antibiotic use in early life and the subsequent development of asthma/wheezing. To this end, we compared the pooled overall effect size of a “strict criteria” meta-analysis (RC and CbI unlikely) with that of the overall meta-analysis that included well-designed studies but where RC and CbI could not be excluded. Furthermore, we studied the effect of heterogeneity in the disease definition on the outcome in a third meta-analysis.

In the overall meta-analysis the overall pooled estimate indicated a small but statistically significantly increased risk (pooled OR 1.27). When this was restricted to the nine studies that met the strict criteria for exclusion of RC and CbI, the pooled estimate shrunk to 1.12, which lost statistical significance.

Heterogeneity in the definition of asthma may affect the results. A reliable diagnosis of asthma can only be established from age 5-6 onward. Four studies focused specifically on wheeze in early childhood [39-42] and none of them were included in the strict meta-analysis. Three studies focused on active symptoms/medication use at school age [32, 43, 44] and all three were included in the “strict criteria” meta-analysis. The other studies did not follow-up until school-age [3, 33, 38, 45] or did follow-up until school-age but did not (profoundly) distinguish between those cases with only symptoms at preschool age and those who persisted to have symptoms into school-age [2, 37]. Thus, in the strict meta-analysis the definition of asthma heterogeneous and one should be cautious drawing any firm conclusion on the association between antibiotic use and true asthma.

To further narrow down to true asthma, we did conduct a third meta-analysis only including those studies that focussed on active wheeze/asthma at school age. Unfortunately, only three studies could be included in this meta-analysis resulting in an unstable effect size largely influenced by only one study .

More studies are therefore needed with a follow-up long enough in order to reliably define asthma and with a minimal risk for bias due to reverse causation and confounding-by-indication.

The negative impact of antibiotics on the gastrointestinal microbiota is often referred to as the underlying biological mechanism explaining the potential causal effect of antibiotic use on asthma. Administration of antimicrobial agents causes disturbances in the ecological balance between the host and the normal intestinal microbiota and also leads to selective overgrowth of opportunistic pathogens such as *Clostridium difficile* or *Candida albicans*. [52] The “microflora hypothesis” states that the association between antibiotic use and asthma is caused by the negative impact on the gastrointestinal microbiota. [53].

As the infant intestinal microbiota plays a crucial role in the development of immunoregulation and oral tolerance induction [54], disruption of the microbiota might also disrupt oral tolerance, potentially by interfering with the dendritic cells that promote antigen-specific regulatory T cell responses [55].

Epidemiological studies have indeed shown differences in gut microbiota composition of allergic and non-allergic children [56].

If the microbiota is the causal link in the association between antibiotic use and asthma/wheezing then timing, dosage and type of antibiotics would all influence this association, because: i. especially in early life, when the immune system is still immature, the gut microbiota is thought to play an important role in immunoregulation [54]; ii factors influencing the microbiota in early life may have a profound effect on the mature microbial composition [57]; iii the gut microbiota (and maybe also the airway microbiota) is more heavily disturbed by broad-spectrum than small-spectrum antibiotics.

Regarding the timing of exposure to antibiotics we restricted our analysis to antibiotic prescriptions in the 1<sup>st</sup> year of life. Some studies looked at very early age, with one study reporting an increased risk of wheezing in children exposed to antibiotics in the neonatal period [40], while the other studies determining antibiotic use in the first weeks or months did not find an association [3, 41, 48, 49].

Several studies included the number of antibiotic courses in their analysis [32, 34, 36, 38, 40, 43, 45-47]. In three studies the highest risk for asthma/wheezing was found for children exposed to more than 4 courses of antibiotics in the first year of life [36, 43, 47], while in the remaining studies no (dose-response) association was found [32, 34, 38, 40, 45, 46].

Information on the classes and spectrum of antibiotics was available in five studies. [35, 36, 43, 47, 50] One study found a positive association between broad-spectrum antibiotic use and atopic wheeze [50] and in another study the broader-spectrum antibiotics produced a stronger increased risk for asthma than the narrow-spectrum antibiotics. [47] The influence of type of antibiotics was difficult to interpret in the remaining three studies for various reasons such as very small numbers, circumstantial evidence or very small effects sizes [35, 36, 43]

Of note, the lack of association between antibiotic use and allergic sensitization [3, 23, 31, 38, 42, 44, 46] as reported in the majority of the studies is not in favor of a causal effect of antibiotics on asthma development.

In conclusion, wheeze and asthma were related to antibiotic use in about half of the identified longitudinal studies. Reverse causation and confounding-by-indication by respiratory tract infections could explain these associations in many but not all of these studies.

A causal effect cannot be dismissed completely, and the disturbing influence of antibiotics on gut or airway microbiota may explain the effect. Alternatively, the observed association in the “strict” meta-analysis could still be due to confounding by consultation behavior or residual confounding by other factors. The opportunities that currently exist in clinical trials should be used well, by inclusion of asthma as adverse outcomes, for instance in trials after the long-term effects of prophylactic antibiotic use such as after premature rupture of amniotic membranes (ORACLE study [58]). Other opportunities exist in studies on the prophylactic effects of probiotics on antibiotic-associated diarrhea in children. These have shown promising but not yet proved effectiveness [59], and have not yet investigated the long-term effects on asthma.

We recommend that new prospective observational studies on the association between antibiotic exposure in early life and subsequent wheeze and asthma should be designed in such a way that bias due to reverse

causation, confounding-by-indication and confounding by consultation behavior is minimized. Collecting detailed information on the number of antibiotic courses, the indication for prescription of the antibiotics and the type of antibiotics prescribed is crucial. It may also be worthwhile to narrow the exposure to the first 6 months of life, as the window of opportunity for modulating the infant's immune system may not span the entire first year of life.

Regarding the outcome assessment follow-up until the age of at least 5-6 years is recommended; since only then a distinction can be made between children who only suffered from transient wheeze and those who went on to develop persistent wheeze and asthma. We especially recommend to extend the follow up of existing well designed cohort studies reviewed here.

### **Competing Interests**

None to declare

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Figure 2A

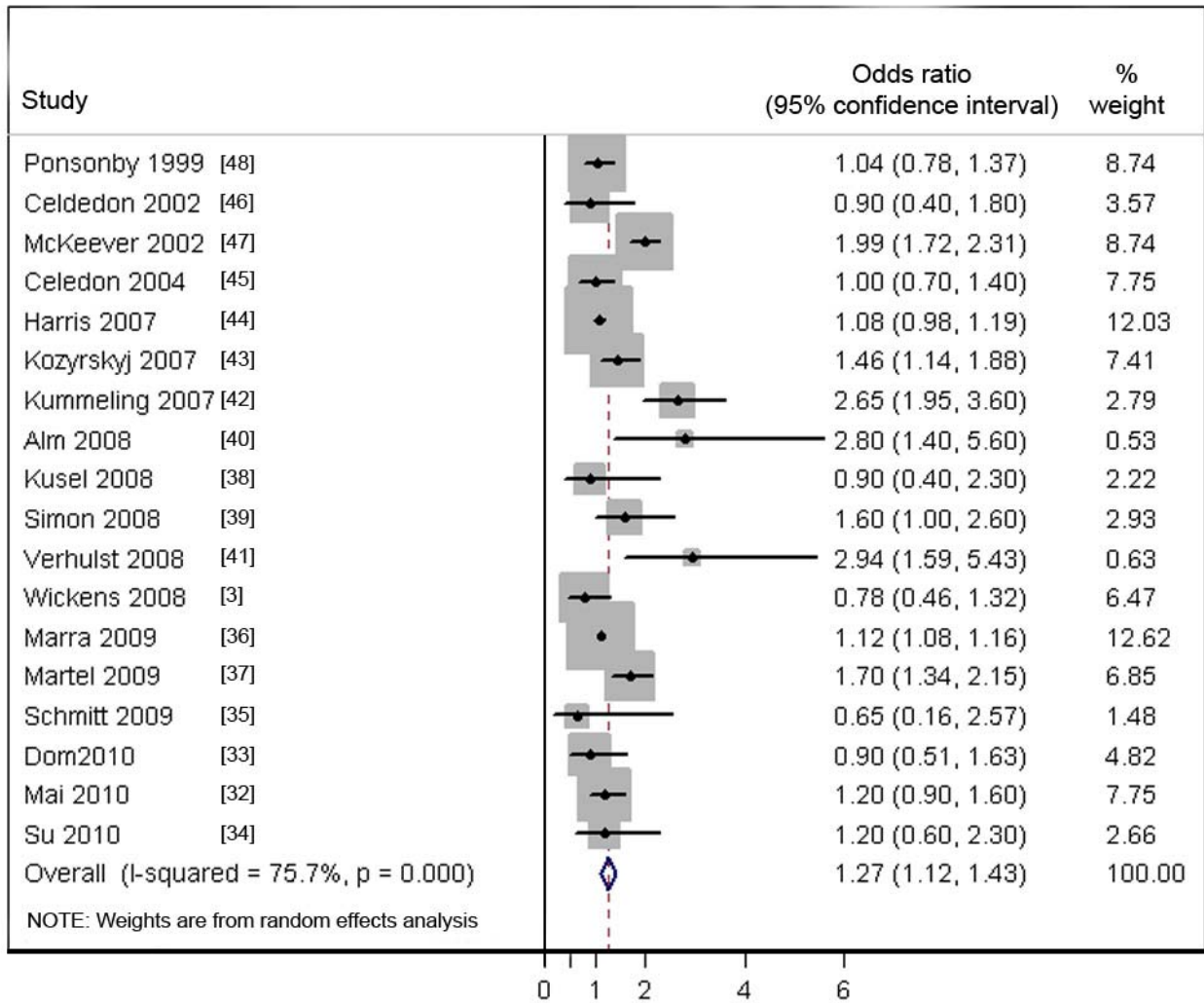
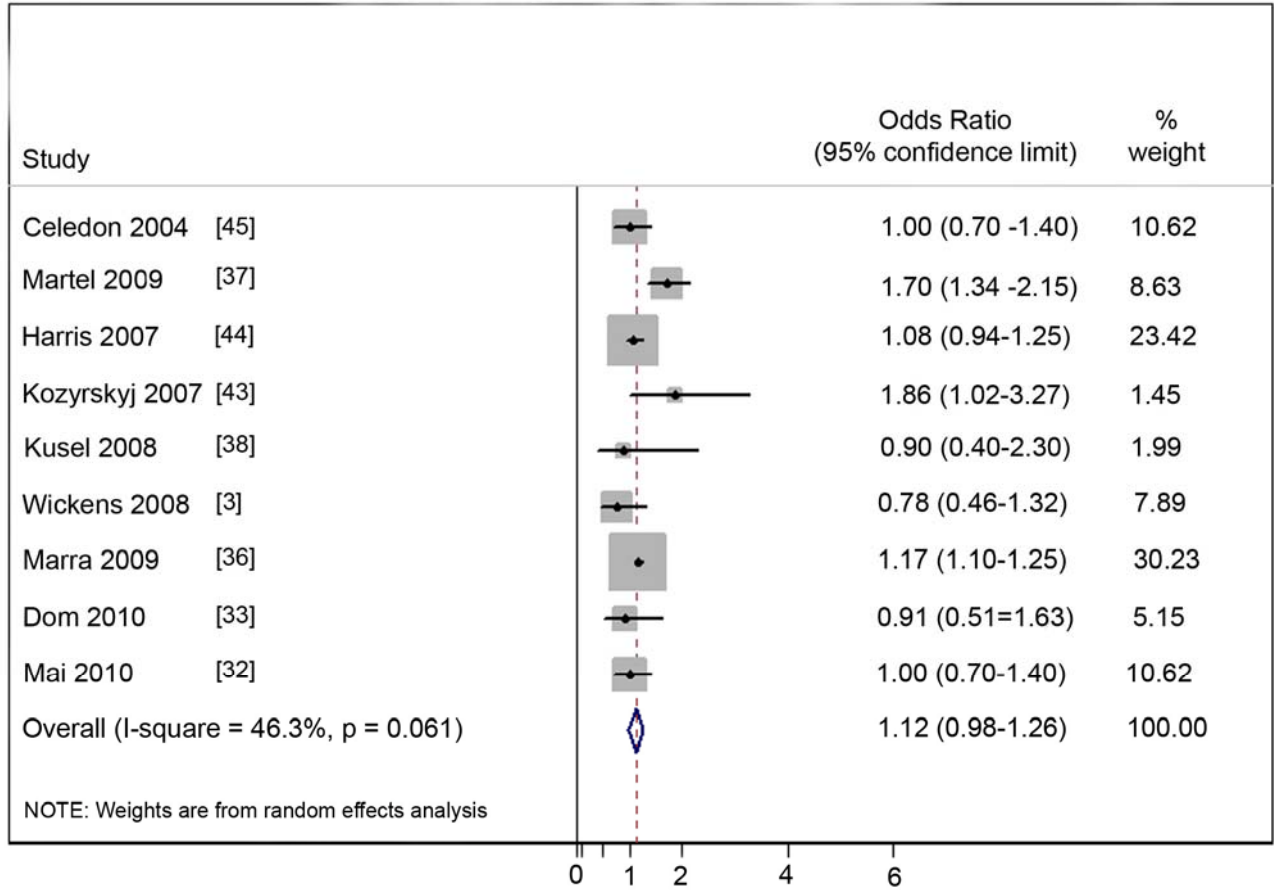
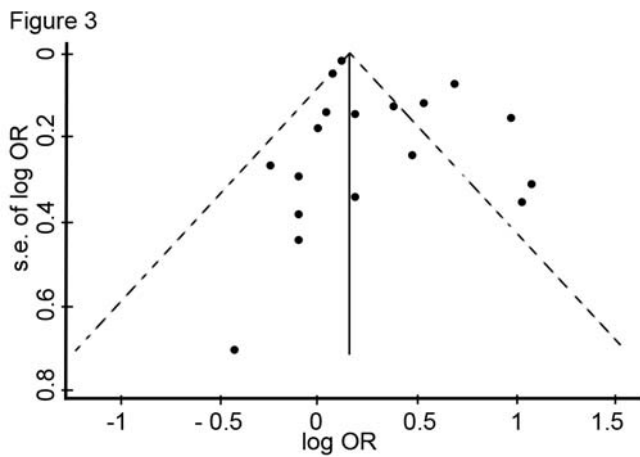


Figure 2B



**Figure 3.** Begg’s funnel plot with pseudo 95% confidence limits of all 18 studies included in the overall meta-analysis. (s.e.: standard error)





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