WHAT IS BIAS AND CONFOUNDING AND WHY ARE THEY IMPORTANT?[1,2]

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TUTORIAL ABSTRACT

PRE-PUBLICATION DRAFT: NOT FOR DISTRIBUTION

Well-designed studies differ from clinical experience because explicit constraints are implemented to control for sources of bias. Clinical experience is particularly prone to bias because the same person provides and then evaluates interventions. Biases that occur commonly, and often inadvertently, when relying on clinical experience are typically grouped into categories of *selection bias*, *information bias*, and *confounding*.

Essentially, **selection bias** occurs when animals with certain signalment, history, or physical examination findings are treated differently than animals without those case characteristics. While clinically reasonable, this practice prevents any attempt to accurately compare alternative risks or treatments.

Similarly, **information bias** is very common in clinical case management because we intentionally gather different types and amounts of information about different animals. However, this bias can lead to incorrect associations with either disease-causation or treatment factors.

Confounding can occur when two potential causative factors are correlated but not evenly distributed. Because many risk factors for disease tend to occur clustered together, confounding is a common problem when using our clinical experience to make disease causation or treatment efficacy associations. Controlled, well-designed experiments use a number of tools including random allocation of animals, blinding (aka masking) of observers, and extensive data collection to combat bias and confounding.

Introduction

Determining if the risk of bias and confounding is high for a particular piece of evidence is the foundation for determining the strength of internal validity for a study.[4] Obviously, determining if a study has high internal validity is very important to a critical evidence consumer. Because veterinarians **should** be critical information consumers, a good understanding of sources of bias and confounding is necessary to avoid being fooled by information that does not report the natural world accurately.

[1] From the proceedings of "Because Evidence Matters", the 6th Evidence-Based Veterinary Medicine Association (EBVMA) Symposium, held 14 November 2014.

See symposia.ebvma.org for more information, including the availability of the companion screencast of Dr. Larson's presentation this tutorial is based on.

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[4] Very important for **internal validity** — so you don't get fooled into **wrong** information from research studies due to bias and confounding

Bias

Bias can be defined as systematic error that results in mistaken conclusions regarding the relationship between the exposure (or explanatory factors) and the outcome. Please notice that random error ? *the distribution of measurements of factors found in the natural world* - are not considered to be bias. These errors are randomly (and in a non-biased manner) distributed amongst individuals and groups. A study that is considered to have high internal validity is said to **lack bias**.

Confounding

Some people consider confounding to be just another form of bias, while others consider it to be a special form of bias and still others consider it to be in a separate category from bias. We will consider confounding to be a special form of bias that can be defined as *the mixing of the effects of one risk factor with another*. If confounding exists in a observation or study, the important error that has been committed is that an apparent relationship between a risk factor and a health outcome is not true.

When consuming research information, being able to identify potential sources of bias and confounding is essential to understand what research studies mean (and what they don't mean). When practicing clinical medicine, bias and confounding can distort observations about potential causes and contributors to disease and can cause one to have erroneous interpretations about the effectiveness of interventions. For researchers, a firm understanding of bias and confounding is necessary to appropriately design, conduct, and interpret research studies.[5]

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Please recognize that bias and confounding can (and do) completely distort study results and can lead to interpretations that are completely wrong![6]

That is why there is such a great risk of observations and studies being biased or confounded. A great deal of the risk comes from the complexity of biology and the multifactorial nature of disease. If biology were simple and disease causation and cure very straightforward, bias and confounding would be minor problems.

In reality, reports, even reports in respected journals, can be invalidated by bias and confounding because the content experts who

[5] The tools that researchers use to control for bias and confounding include:

1. Appropriate **study design**
2. Appropriate **statistical tests**
3. An understanding of the commonness and importance of bias and confounding so that study results are interpreted in light of these risks.

[6] Beware! When you read results from a veterinary study, an apparent link between a risk factor and a disease may be real, or just an anomaly of how the study was done

wrote them and the peer reviewers who critiqued them did not have a firm understanding of the sources of bias and confounding inherent in every observation of the natural world.

Given the complexity of biology, health, and disease, the challenge for researchers and veterinary practitioners is to obtain valid study results that represent the true nature of the relationship between exposures and disease. This requires consideration of all possible errors due to bias and confounding.

Bias: in-depth

Selection Bias

Selection bias occurs at the time that study subjects are selected for a study. Remember, a study is comparing one group of subjects (those with the risk factor, outcome, or treatment of interest) to another group of subjects (the controls — those without the risk factor, outcome, or treatment of interest). If those two groups differ in ways in addition to the risk factor, outcome, or treatment, then ?selection bias? invalidates the study results. In other words, selection bias distorts what we are interest in learning — the estimate of relationship between a particular exposure or treatment and disease.[7]

In practice, selection bias can be a problem for any study that does not utilize random allocation of subjects to treatments — such as in observational studies. Therefore, for *case-control*, *cohort*, and *cross-sectional studies*, finding appropriate control groups is often problematic.

For a *cohort study*, we want the control group to differ from the group with the risk factor of interest **only** by that factor of interest and to be the same in all other factors.

For a *case-control study*, we want the control group to differ from the group with the outcome of interest **only** by that outcome and to be the same in all other outcomes. But, as you can easily recognize, by excluding animals that don?t have the exposure or outcome of interest, we are at risk of creating control groups that have other important factors that differ from the *treatment* group.

[7] The **distortion** due to selection bias occurs if:

1. The procedures used to select study subjects results in differences between study groups other than the factor being investigated
2. Factors that influence study participation (or study continuation) differ between treatment groups

Diagnostic Bias

Diagnostic bias — as a form of selection bias — also occurs before subjects are identified for inclusion in one of the study groups. Problems can occur when some potential study subjects are more likely to undergo diagnostic testing or more thorough diagnostic testing than other potential study subjects. This is usually because something about the animal or its history influences the diagnostic strategy.

Please recognize that what we are describing is perfectly expected and appropriate for high-quality clinical practice — using the signalment and patient history to guide diagnostic work-ups. But, also recognize that this clinically-appropriate approach is the definition of *bias* —treating some groups systematically different than other groups because of the different goals of clinical practice and clinical research. Medical records and databases are very prone to several sources of bias and are less valuable for research than one would like to think.

Because it is critical for researchers to avoid selection bias as they design studies, and it is equally important for clinicians to recognize selection bias as they critically evaluate whether to read the results from a study, it is important to know how studies should be designed to prevent selection bias.

To start with, the population that is identified as the source for study subjects should be one that has similar characteristics for all factors other than the factors being investigated. Then, animals (or groups of animals) that meet the definition for the exposure or outcome being investigated (and the appropriate controls) should be randomly chosen from the *pool* of potential subjects. Unfortunately, if subjects were selected in a way that resulted in biased study groups, it is impossible to correct the fatal flaw once the study has started.

In an ideal world, investigators would never publish results from such a study and critical information consumers would ignore the results if they were published. But unfortunately, the information will probably be published and consumed and used for clinical decision-making.

Information Bias

The second broad category of bias types is *information bias*[8]. Some sub-types of information bias include: **misclassification bias** and **observer or interviewer bias** which are further divided into the following:

[8] Information bias occurs when any information used to evaluate risk factors or outcomes is gathered in a way that is not perfectly identical between study groups.

Forms of observer or interview bias:

1. Recall bias
2. Reporting (or wish) bias
3. Surveillance bias
4. Observer bias

Another potential form of information bias is **measurement error**— but only if that error is more likely to occur in one study group than another. Similarly, loss to follow-up that is more common in one

study group is also a source of information bias — as more information is lost in one study group than another. Controlling information bias is critical for researchers and recognizing information bias is critical for research consumers. Appropriate study design that ensures careful collection of data so that the type, quality, and quantity of information from all study groups is as alike as possible is absolutely essential in order to obtain valid study results. If any information is collected in a way that differs between study groups — the study is considered to be fatally flawed — and the results are meaningless.[9]

[9] Again, beware! When you read results from a veterinary study — an apparent link between a risk factor and a disease may be real, or just an anomaly of how the study was done.

Confounding: in-depth

One reason that confounding often is treated differently than selection bias or information bias is because, unlike other types of bias — it can be mathematically controlled[10] at the time of analysis — if the confounding factor was identified before the study started and data that accurately describes the confounding factor is recorded. The definition of confounding is *a third factor which is related to **both** exposure and outcome, and the confounding factor accounts for some or all of the observed relationship between the exposure and outcome*.

[10] Confounding is not like other forms of bias because it can be mathematically controlled at the time of analysis if we know it exists and we collected data on the confounding factor. Selection and information biases cannot be mathematically controlled.

It is important to recognize that a confounder is not the result of the exposure. I often find that a useful teaching example is the investigation of the apparent link between birth rank and Down Syndrome in humans. In this famous example, the mother's age acts as a classic confounder.

Birth Order	1st	2nd	3rd	4th	5th+
Number/10,000 life births	6	7	8	11	17

Table 1: **Birth order & Down Syndrome risk:** Raw data indicate that risk of Down syndrome is higher in higher birth-order children

Maternal age	<20	20-24	25-29	30-34	35-39	40+
Number/10,000 life births	4	4	5	9	27	84

Table 2: **Maternal age & Down Syndrome risk:** However, this is because the risk of Down syndrome is also higher for older mothers

Birth order	1st	2nd	3rd	4th	5th+
Average maternal age	23	26	29	32	35

Table 3: **Birth order & Down Syndrome risk:** Because the mother's age increases with birth order, there is a potential confounding factor of maternal age.

	Birth Order					
Maternal age	**1st**	**2nd**	**3rd**	**4th**	**5th+**	**All**
< 20	5	4	-	-	-	4
20-24	4	5	4	4	2	4
25-29	5	5	5	5	5	5
30-34	10	10	8	9	8	9
35-39	27	30	24	30	25	27
40+	85	75	86	94	85	84
All	6	7	8	11	17	9
Average age	23	26	29	32	35	

Table 4: **Birth order & Down Syndrome risk:** In this chart of raw data from a large database of births, we can look at birth order and maternal age separately for their effect on the risk of giving birth to an infant with Down Syndrome

	Birth Order					
Maternal age	**1st**	**2nd**	**3rd**	**4th**	**5th+**	**All**
< 20	5	4	-	-	-	4
20-24	4	5	4	4	2	4
25-29	5	5	5	5	5	5
30-34	10	10	8	9	8	9
35-39	27	30	24	30	25	27
40+	85	75	86	94	85	84
All	6	7	8	11	17	9
Average age	23	26	29	32	35	

Table 5: **Birth order & Down Syndrome risk:** If birth order is held constant (by considering one column at a time), then you can see that higher risk occurs with older maternal age.

	Birth Order					
Maternal age	**1st**	**2nd**	**3rd**	**4th**	**5th+**	**All**
< 20	5	4	-	-	-	4
20-24	4	5	4	4	2	4
25-29	5	5	5	5	5	5
30-34	10	10	8	9	8	9
35-39	27	30	24	30	25	27
40+	85	75	86	94	85	84
All	6	7	8	11	17	9
Average age	23	26	29	32	35	

Table 6: **Birth order & Down Syndrome risk:** By looking at the data another way: If maternal age is held constant (by considering one row at a time), then the **risk does not change** with birth order

	Birth Order					
Maternal age	**1st**	**2nd**	**3rd**	**4th**	**5th+**	**All**
< 20	5	4	-	-	-	4
20-24	4	5	4	4	2	4
25-29	5	5	5	5	5	5
30-34	10	10	8	9	8	9
35-39	27	30	24	30	25	27
40+	85	75	86	94	85	84
All	6	7	8	11	17	9
Average age	23	26	29	32	35	

Table 7: **Birth order & Down Syndrome risk:** By looking at the table carefully, one can see that Down syndrome risk increases with maternal age (down the columns), but not with birth order (across the rows).

Holding maternal age constant (looking at each row), Down syndrome and birth order are (nearly) independent. Holding birth order constant (looking at each column), the rate of Down syndrome increases with (depends on) maternal age.

Therefore, we conclude that Down syndrome is unrelated to (or independent of) birth order when adjusted for maternal age.

Confounding variables

To determine if a factor is a potential confounder whenever we want to know whether there is a true association between a given exposure and a given disease, we want to make sure that it is not because of the presence of a third variable — a **confounding variable**.

Confounding variables represent another pathway to get to the disease under study, a pathway we are not interested in studying... a nuisance pathway. That is why confounding is often called a **mixing of effects**.

Confounding can lead to:

1. An *overestimate* or *underestimate* of a true association
2. Can mask a true association completely
3. Can produce an apparent association when there is none

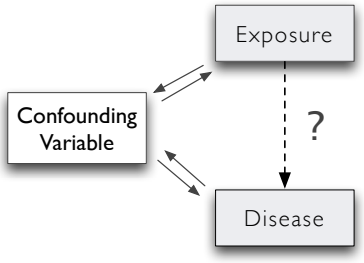

Figure 1: A confounding variable is another pathway to get to the disease, or a *mixing of effects*

In order for a variable to be a **confounder**, it must be distributed differently (unequally) between exposed and unexposed populations. In addition, for a variable to be a confounder, it must be associated with the disease — independent of the exposure of interest.

Therefore, to determine if a variable is a potential confounder, you must ask *two* questions:

1. Is the variable distributed unequally between exposed and unexposed populations?
2. Is the variable associated with the disease, independent of the exposure of interest?

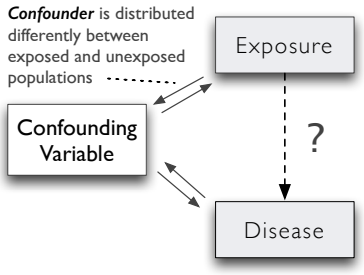

Figure 2: A confounder is distributed differently between exposed and unexposed populations

Using the Down Syndrome example; if we are interested in investigating the link between birth order and risk of Down Syndrome, but we are concerned that *mother's age* may be a potential confounder, we would need to answer our two questions:

1. Is mother's age distributed unequally between different birth orders? The answer is *yes*, and
2. Is mother's age associated with Down Syndrome? Again, the answer is *yes*.

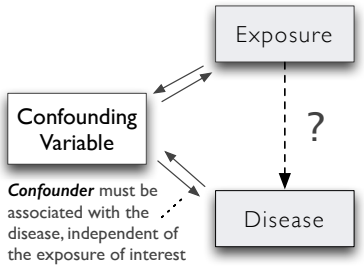

Figure 3: For a variable to be a **confounder**, it must be associated with the disease — independent of the exposure of interest

Anytime the answer to both questions is *yes*, you have identified a potential confounder that must be measured during the study. It must be included in the statistical analysis of your original question about the link between the exposure of interest (birth order) and the outcome of interest (Down Syndrome).

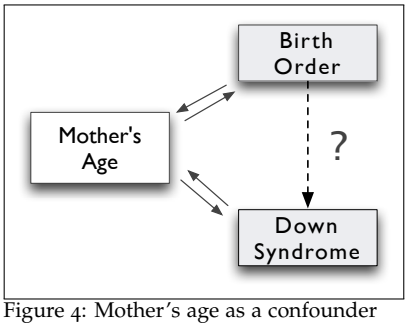

Figure 4: Mother's age as a confounder in evaluating the relation of birth order to Down Syndrome

Strategies to control bias

There are a number of strategies that should be used to control bias and confounding.

One strategy is to have **explicit enrollment criteria** for study subjects. This acts to limit selection bias and clearly describes the type of population to which the study results can be appropriately applied.

Another important strategy to control for bias and confounding is to use **random allocation of study subjects** whenever possible (that is use randomized controlled studies). For observational studies where random allocation is not possible, randomly selecting study animals from an appropriate *pool* of candidates should be utilized. This will limit allocation bias (a form of selection bias) and is our best control for known and unknown confounders

A third strategy is to **blind all the people involved in the study** so that they are unaware which animals (or groups of animals) are in each study group. This limits information bias. In other words all the study subjects should be evaluated in exactly the same way and with the same intensity

And finally, clearly **stating the null hypothesis**, **justifying the sample size used**, **reporting the number of withdrawals from each study group**, and **limiting the number of outcomes measured**. This allows the reader to evaluate the report for potential sources of bias and confounding, because the presence or absence of bias and confounding determine the internal validity of studies. A thorough understanding of these important concepts is critical.

Fatal flaws

I often use the term ***fatal flaw*** when describing reports that fail to have satisfactory internal validity.

Selection bias risk

One *fatal flaw* is when a study has significant risk of selection bias I am alerted to the risk of selection bias if animals are not appropriately assigned or identified to treatment group. In other words, if a study is intended to be a *randomized controlled trial*, animals should be assigned to treatment group using an appropriate random method. If a study is an *observational study*, the study subjects should be randomly selected from a pool of animals that meet the inclusion criteria for specific study groups.

Unequal numbers between study groups is a strong indication of potential selection bias, unless the unequal numbers are specifically explained in the materials and methods section. And finally, if there

is any indication that anyone influenced which animals ended up in each treatment group, then selection bias is confirmed.

Another indication that selection bias is a concern is when a study lacks a clear and consistent case-definition for inclusion in the study. Or, if *match*animals in case-control and cohort studies appear inappropriate because of evidence of inconsistent case-definitions between treatments. Or, there is inconsistent length or intensity of follow-up between study groups.

To allow the reader to assess the potential for selection bias, reports should include a table that lists important characteristics of the various treatment populations at the start of the study so that the study groups can be assessed for their similarities.

Information bias risk

Another *fatal flaw* is when a report has concerning potential for information bias. Anytime people making subjective evaluations are not blinded to each animal's treatment group, I am very concerned about the likelihood of information bias. The lack of blinding for individuals reading objective measurements is only slightly less concerning. Lack of blinding can lead to the lack of a clear and consistent case definition for outcomes; meaning I am very concerned that different criteria were used to identify treatment success (or other outcome) between treatment groups.

Confounding risk

Troubling confounding risk is another *fatal flaw*.

Complete confounding occurs if all the animals is each treatment group differs from all the animals in the other treatments in any way other than the treatment of interest.

Uncontrolled partial confounding occurs when animals with a factor that impacts the outcome are unevenly distributed between treatment groups. Partial confounding can be controlled during the experimental design phase with blocking, and in the data analysis phase by including the confounding factor as a covariate, but only if the confounder is identified before the study starts and all necessary information about the confounder is recorded. ■