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DIARRHOEAL DISEASES CONTROL PROGRAMME

Case-Control Studies of Childhood Diarrhoea:

I. Minimizing Bias

by
S.N. Cousens, R.G. Feachem, B. Kirkwood
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WORLD HEALTH ORGANIZATION

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I. Minimizing Bias

by
S.N. Cousens[1], R.G. Feachem[1], B. Kirkwood[1]
T.E. Mertens[1], P.G. Smith[2]

[1] Department of Tropical Hygiene, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

[2] Tropical Epidemiology Unit, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

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PREFACE

This document is part of a series prepared for the Diarrhoeal Diseases Control Programme of the World Health Organization. The series is a response to an upsurge of interest in the application of the case-control method to the study of childhood diarrhoea. That interest has been stimulated by the realization that, under certain circumstances, the case-control method can be a relatively quick, inexpensive, and reliable method for measuring the impact of diarrhoea control measures or for identifying and quantifying risk factors for diarrhoea.

Case-control studies can be complex in their design and analysis and it is not possible to prepare a manual that can be followed exactly in all circumstances. A considerable amount of epidemiological judgement and skill must be exercised. The aim of this series is to provide the investigator with a clear view of the most important problems in the design, analysis, and interpretation of case-control studies of childhood diarrhoea, and to provide practical suggestions for the resolution of those problems. For the trained and experienced epidemiologist, these documents provide specialized guidance on the application of case-control methods. For others, the series provides an awareness of the methodological issues involved, and a familiarity with the language and concepts of case-control studies.

Diarrhoeal diseases remain one of the leading causes of morbidity and mortality among children in poor communities in all parts of the world. Epidemiological studies have already contributed to an understanding of the risk factors involved and to the design and evaluation of appropriate interventions. Continued work in diarrhoea epidemiology is essential to further refine these interventions and to maximize their impact on severe illness and death. The Diarrhoeal Diseases Control (CDD) Programme of WHO is supporting a range of research projects in this field in many countries. Those seeking financial or technical support for their research, or wishing to contact others undertaking similar investigations, are invited to contact the CDD Programme.

ABSTRACT

Three different ways in which bias may arise in clinic-based case-control studies of risk factors for childhood diarrhoea are discussed: through misclassification of disease or exposure status, through confounding, and through the way in which cases and controls are selected for inclusion in the study. Simple numerical examples are used to illustrate the impact that each of these different types of bias may have on the estimate of the odds ratio obtained from a study, and to show how such bias may cause incorrect conclusions to be drawn from a study. Strategies for avoiding or minimizing each of these types of bias are discussed.

Misclassification of the disease or exposure status of children included in the study as cases or controls will lead to a biased estimate of the odds ratio. If the misclassification is non-differential the estimate will be biased towards 1.0, the null value. Differential misclassification may lead to bias in either direction. In a case-control study of childhood diarrhoea, the child's disease status will generally be ascertained before her/his exposure status. Misclassification of disease status will, therefore, frequently be non-differential. Misclassification of exposure status may often be differential, particularly if the interviewer is aware of the child's status as a case or control.

Confounding is a problem in any type of observational study. Statistical techniques are available for coping with this problem during the analysis, provided that potentially confounding variables are identified at the design stage and that accurate data are collected on them. Matching may also be used to control the effects of some confounding variables.

The choice of control group is, probably, the single most difficult decision facing an investigator designing a case-control study. In this paper discussion is restricted to the choice of controls recruited from among children reporting to health facilities for diseases other than diarrhoea. Two types of selection bias are distinguished; that due to the selection of controls with diseases related to the exposure under investigation and that due to differences in the propensity to report episodes of diarrhoea compared with episodes of the control diseases. Strategies for controlling this latter type of selection bias are discussed.

RESUME

Trois sources de biais pouvant affecter les études cas-témoins sur les facteurs de risque de diarrhée infantile faites dans les dispensaires sont examinées dans ce document: classification erronée de la maladie ou du degré d'exposition, facteurs parasites et, enfin, modalités du choix des cas et des témoins pour l'étude. Des exemples numériques simples illustrent les effets que chacun de ces types de biais peut avoir sur l'estimation du risque relatif résultant d'une étude et montrent comment ces biais peuvent fausser les conclusions tirées d'une étude. Les stratégies permettant d'éviter ou de neutraliser au maximum chacun de ces types de biais sont examinées.

La classification erronée de la maladie ou du degré d'exposition des enfants couverts par l'étude en tant que cas ou témoins biaisera l'estimation du risque relatif. Si l'erreur de classification est non différentielle, le biais de l'estimation tendra vers 1,0, valeur nulle. Une erreur de classification différentielle fera tendre le biais dans l'autre sens. Dans une étude cas-témoins de la diarrhée infantile, on s'assurera généralement de l'état pathologique de l'enfant avant de s'assurer de son degré d'exposition. Aussi l'erreur de classification de l'état pathologique sera-t-elle fréquemment non différentielle. L'erreur de classification du degré d'exposition pourra souvent être différentielle, notamment si l'enquêteur sait si l'enfant est cas ou témoin.

Des erreurs dues à des facteurs parasites peuvent survenir dans toute étude d'observation. Il existe des techniques statistiques permettant de remédier à ce problème pendant l'analyse, à condition que les facteurs parasites potentiels soient identifiés au stade de la conception et que des données précises sur ces facteurs soient recueillies. L'appariement est un autre moyen de neutraliser les effets de certains facteurs parasites.

Le choix du groupe témoin est probablement la décision la plus difficile à prendre pour un chercheur qui conçoit une étude cas-témoin. Dans ce document, la discussion se limite au choix de témoins recrutés parmi les enfants amenés en consultation dans les services de santé pour des maladies autres que la diarrhée. On distingue deux types de biais liés à la sélection: celui qui est dû au choix de témoins atteints de maladies liées à l'exposition étudée et celui qui est dû aux différences concernant la tendance à signaler les épisodes de diarrhée par rapport aux épisodes de maladies témoins. Les stratégies permettant de neutraliser ce dernier type de biais lié à la sélection sont examinées.

1. INTRODUCTION

In 1985 the World Health Organization issued a document entitled "Measuring the impact of water supply and sanitation facilities on diarrhoea morbidity: prospects for case-control methods" (Briscoe et al., 1985). This document was one of the products of two scientific meetings held in Cox's Bazaar, Bangladesh, and Geneva, Switzerland, at which methodologies for measuring the impact of water supply and sanitation projects on health were discussed. In the document, case-control studies were put forward as an alternative to longitudinal studies whose use in this field had been discouraged by a report of an expert panel to the World Bank (IBRD, 1976).

The present series of papers considers the wider application of case-control methods to the study of various risk factors for, and interventions for the control of, childhood diarrhoea caused by enteric infections. This paper, the first of the series, focuses on strategies for minimizing bias in case-control studies of those risk factors and control interventions that have been identified by WHO as priority areas for research (see Table 1 on page 14).

Case-control studies differ from longitudinal studies in that subjects are selected for inclusion in the study according to their disease status rather than exposure status. While the problems of bias due to misclassification and confounding are shared by other types of observational study, selection bias - that is, bias introduced by the way in which cases and controls are selected - is likely to be of special concern in case-control studies.

In this paper we avoid the use of complex algebraic expressions and present instead simple numerical examples wherever possible. Statistical formulae used in the presentation of these examples are cited in Annex 1. We begin by considering a hypothetical case-control study.

2. AN EXAMPLE OF A CASE-CONTROL STUDY

As an example, we consider a fictional case-control study which is designed to assess the association between the presence of domestic animals in the home and the risk of diarrhoea morbidity in children aged less than 5 years. The study is based on patients attending a single health facility. "Cases" are those children reporting to the clinic in whom diarrhoea caused by an enteric infection is diagnosed; "controls" are randomly selected from among children reporting to the clinic who are not suffering from diarrhoea. Information concerning the presence of animals in the households of both cases and controls is collected.

In their simplest form, the results of the study may be presented as a 2 x 2 table:

	Cases	Controls	
Animals present	10	4	14
Animals not present	30	36	66
Total	40	40	80

The measure of association used in the analysis of case-control studies is the odds ratio. For the above table this is calculated as follows:

$$OR = \frac{10 \times 36}{4 \times 30} = 3.0$$

This result suggests that children who live in houses where domestic animals are present are approximately 3 times more likely to suffer an attack of diarrhoea than children in houses without animals.

To assist in interpreting the results, we need to test the statistical significance of the association we have found in our sample. Is there really an underlying association between the presence of animals and risk of diarrhoea or could our result have been obtained by chance? Even when studying a factor that is not associated with diarrhoea (i.e., true odds ratio = 1.0), we are unlikely to obtain from a case-control study an estimate exactly equal to 1.0 due to sampling variations. How likely is it that our estimate of 3.0 has arisen in this way? One method of testing the significance of an association in a 2 x 2 table is to perform a chi-squared (X^2) test (Annex 1). From the table,

$$X^2 = \frac{80 \left(|10x36 - 4x30| - 0.5x80 \right)^2}{40x40 \times 66x14}$$

$$= 2.16$$

Comparing this value against a table of values for a chi-squared distribution with 1 degree of freedom, it may be seen that the probability of obtaining a similar or more extreme result purely by chance, in a situation in which the true odds ratio equals 1, is greater than 0.1. Thus our result is not statistically significant at the 10% level of significance. In this particular example we have not found any strong evidence of an association. There are two possible reasons for this:

(1) no association exists between presence of animals and risk of diarrhoea,

(2) an association does exist, but our study was too small to detect it (i.e., to find a statistically significant association).

In our analysis and discussion of the example above, we implicitly assume that the children included in the study constitute an unbiased sample of the whole population. Our assessment of the statistical significance of the observed association is based on this fundamental assumption and may be invalid if bias has occurred to any great degree. We now discuss some ways in which bias may arise in case-control studies of childhood diarrhoea and how it may cause incorrect conclusions to be drawn. Strategies for its minimization are considered.

3. BIAS

In the preceding section, we consider a hypothetical case-control study and note that the estimate of the odds ratio obtained from the study may differ from the true value. This is an inevitable consequence of sampling the population rather than collecting data on everyone. By increasing the sample size this discrepancy can be reduced until, by conducting a census of the whole population, we arrive at the true value of the odds ratio.

The term bias refers to discrepancies between an estimate and the true value of the odds ratio arising from causes other than sampling variation. In general, discrepancies due to bias will not decrease as the sample size is increased. Bias will produce incorrect estimates and may lead investigators to draw the wrong conclusions. The following sections of this paper look in detail at three different ways in which bias may occur: through misclassification of disease or exposure status, through confounding, and through the method of selecting cases and controls.

3.1 Misclassification bias

In any study, it is likely that some individuals will be wrongly classified, as regards either their disease status (case/control) or their exposure status (exposed/unexposed). Bias arising in this way is known as misclassification bias.

3.1.1 Misclassification of disease status

A child's disease status may be misclassified in one of two ways:

(1) A child not suffering from diarrhoea caused by an enteric infection is included as a case: a "false positive",

(2) A child suffering from diarrhoea caused by an enteric infection is included as a control: a "false negative".

The WHO document (Briscoe et al., 1985) discussed the problem of misclassification in some detail. The authors considered some numerical examples of misclassification, and concluded that such bias is likely to be particularly important in studies in which the specificity of the disease measure is poor - i.e., when the false positive rate is high. It was suggested that both the false positive and the false negative rate are likely to be lower in clinic-based case-control studies, in which all recruits can be examined by medically trained staff, than in longitudinal and cross-sectional studies which rely on surveillance and recall. We now consider how each type of misclassification may arise, what its effect might be, and how it could be reduced.

(1) False positives

Some mothers may report that their children have diarrhoea when their stools are normal (Illingworth, 1982). This is likely to occur less frequently in clinic-based case-control studies, in which the mother must bring the child to the clinic, than in community-based studies which rely on surveillance and the mother's recall. A clear case-definition, applied to all potential cases, will help to reduce the number of false positives arising in this way. The case-definition used in a study may however itself lead to classification problems. For example, a commonly used definition of diarrhoea is the passing of 3 or more loose or watery stools in a 24-hour period. For some exclusively breast-fed children 3 or more loose stools per day may be the norm. If such children are recruited as cases (of enteric diarrhoea) they will be "false positives". In such situations the mother's definition of diarrhoea may be more reliable than some external definition.

The use of various antibiotics may lead to diarrhoea (Illingworth, 1982). Children suffering from diarrhoea due to drugs would constitute false positives if included as cases. Mothers should be questioned to ascertain whether the child has recently received any drugs and the child should be recruited as a case if s/he satisfies the requirements of the case-definition. The information on drug use may then be used during the analysis.

Diarrhoea may be associated with measles (Feachem and Koblinsky, 1983), malaria (Jelliffe and Stanfield, 1978), urinary tract infections (Illingworth, 1982), otitis media and respiratory tract infections (Vaughan et al., 1979). There is some evidence to support the hypothesis that measles-associated diarrhoeas (both with- and post-measles) may be due to opportunistic enteric infections (Feachem and Koblinsky, 1983; de Mol et al., 1984). Little is known, however, about the nature of diarrhoeas associated with diseases other than measles. If the association arises through an enteric infection which takes advantage of a lowering of immunity caused by the associated disease, a child presenting with the associated diarrhoea is a true (enteric) case, and is therefore eligible for recruitment as a case if the episode meets the other requirements of the study's case-definition. If, on the other hand, the diarrhoea is a side-effect of the associated disease the child is not a

true case (due to an enteric infection), and is not eligible for recruitment as a case regardless of whether or not the episode satisfies the other selection criteria. Failure to distinguish children reporting with diarrhoeas of this latter type will lead to false positives.

The effect of false positives on the estimate of the odds ratio

Black (1984) has stated that "the recent recognition of bacterial and viral agents of diarrhoea permits the identification of an enteropathogen....in 70-80 percent of the more severe episodes (of diarrhoea) treated at health facilities". Failure to isolate a known enteropathogen during an episode of diarrhoea may occur for a number of reasons. The microbiological techniques used may lack sensitivity. Antimicrobial therapy may have been initiated prior to stool collection. The diarrhoea may result from infection by an unknown (and therefore unsought) enteropathogen or from a cause other than infection by an enteropathogen (a genuine "false positive"). It is likely, therefore, that a substantial proportion of episodes of diarrhoea from which a known enteropathogen cannot be isolated are nevertheless due to an enteric infection. This suggests that in case-control studies of childhood diarrhoea, in which children are recruited when they report to clinics, at least 70-80% of the children seen with diarrhoea are likely to be cases of enteric infections. In studies of diarrhoeas of a particular severity, with perhaps moderate or severe dehydration as a criterion for selection as a case, or of diarrhoeas with certain clinical features, for example, the presence of blood in the faeces (dysentery), the percentage of children seen as potential cases who are genuine enteric cases is likely to be still higher. We shall consider the bias associated with false positive rates of up to 30%, although in practice it is likely that the misclassification rate will be substantially lower than this if the "case" criteria are carefully defined and adhered to in the study.

Example 1 is a case-control study of the association between the presence of animals in the home and risk of diarrhoea. The study recruited 200 cases and 200 controls from a population of which 10% are exposed (have animals in the home), and the true odds ratio is 3.0.

False positive rate		Cases		Controls		
		True	False			
0%	Animals	50	+ 0	20	70	OR = 3.00
	No animals	150	+ 0	180	330	
		200		200	400	X^2 = 14.6, p<0.001
20%	Animals	40	+ 4	20	64	OR = 2.54
	No animals	120	+ 36	180	336	
		200		200	400	X^2 = 9.84, 0.001<p<0.005
30%	Animals	35	+ 6	20	61	OR = 2.32
	No animals	105	+ 54	180	339	
		200		200	400	X^2 = 7.74, 0.005<p<0.01

The numbers of cases in the above example are calculated in the following way. With a false positive rate of 20%, of the 200 children selected as cases, 20% (40 children) are not really cases and have the same exposure as the controls (10% exposed = 4 children). The remaining 160 cases are true cases of whom 25% (40 children) are exposed. The distribution of the cases when the false positive rate is 30% is calculated in a similar way.

Example 2 is a case-control study of the association between improved water supply facilities and risk of diarrhoea. The study recruited 200 cases and 200 controls from a population of which 60% were exposed (i.e., lacked an improved water supply) and the true odds ratio is 1.56.

False positive rate		Cases		Controls		
		True	False			
0%	Unimproved supply	140	+ 0	120	260	OR = 1.56
	Improved supply	60	+ 0	80	140	
		200		200	400	$X^2 = 3.97$, $p<0.05$
10%	Unimproved supply	126	+ 12	120	258	OR = 1.48
	Improved supply	54	+ 8	80	142	
		200		200	400	$X^2 = 3.16$, $0.05<p<0.1$
30%	Unimproved supply	98	+ 36	120	254	OR = 1.35
	Improved supply	42	+ 24	80	146	
		200		200	400	$X^2 = 1.82$, $p>0.10$

It should be noted that, in each of the examples above, the effect of misclassification has been to bias the estimate of the odds ratio towards unity. This will always be true if the misclassification is non-differential: i.e., if the probability of a child's being wrongly classified as a case is independent of whether or not the child is exposed. In the first example, the effect of the bias has been to reduce the estimate of the odds ratio from 3.00 to 2.32 without removing the statistical significance of the association. In the second example, not only is the estimate of the odds ratio reduced, but so is the statistical significance of the association; misclassification has caused the study to "miss" the association. The effect of misclassification of disease is likely to be particularly serious in studies of risk factors like water and sanitation for which the odds ratio may be less than 2.

Strategies for reducing false positives

The selection criteria according to which a child is classified as a case (or as not a case) should be carefully and precisely defined before the study begins, and should be adhered to throughout the course of the study. All cases recruited into a study should be given a thorough clinical examination, including a careful history taking, to ensure that the severity of disease, in particular the level of dehydration, is assessed accurately, that the presence of other clinical features is noted, and that associated diseases such as malaria and otitis media are diagnosed. In some settings in tropical countries the existing health facility staff may be too busy to perform such examinations and it will be necessary to recruit extra well-trained, experienced staff to carry out this work. If possible, the clinical examination should be followed by microscopy on blood (thick films) for all

suspected cases of malaria in areas where malaria and malaria-associated diarrhoea are believed to be common. Since the recruitment of cases takes place at a clinic, where more severe diarrhoea cases present, at least 70-80% of children reporting with diarrhoea are likely to have enteric infections, and it should be possible to increase this figure substantially by careful clinical examination. The recording of detailed data on the signs and symptoms of the episode and any associated diseases will allow some flexibility in case-definition during the analysis. For example, in a study in which malaria is found to be commonly associated with diarrhoea, children satisfying the case-definition, but also suffering from malaria, would be recruited as cases. The data may then be analysed twice, once including those cases also found to be suffering from malaria, and then excluding those cases. If the diarrhoeas associated with malaria are not due to enteric infections, then we would expect to see a change in the estimate of the odds ratio between the first and second analysis as the misclassification is removed (the estimate will increase if the misclassification was non-differential).

Diagnostic stool microbiology

In some situations the performance of selective stool microbiology, looking for one or two particular enteropathogens, may serve a useful purpose. Different enteropathogens may have different transmission routes and the inclusion, as cases, of children suffering from diarrhoeas due to all etiologies may mask the association between one particular pathogen and a particular transmission route. Selective stool microbiology may help to reveal this association.

Example 3. Suppose that the presence of domestic animals in the household is a strong risk factor for diarrhoea due to Campylobacter (OR = 4.0, say), but is not associated with diarrhoeas due to other etiologies. If 50% of controls are exposed to animals, and 10% of all diarrhoeas are due to Campylobacter, then we might obtain the following results from a case-control study of this association:

	Cases		Controls		
Animals present	16	+ 90	100	206	OR = 1.1
Animals not present	4	+ 90	100	194	
Total	20	+ 180	200	400	$X^2 = 0.25$, $p>0.5$

(Note: 20 of the 200 cases (10%) are due to Campylobacter, the remaining 180 are due to etiologies unassociated with the presence of animals.) On the basis of such results we might conclude that animals do not play an important role in the transmission of diarrhoea. If, however, we are able to exclude those "cases" not due to Campylobacter, we obtain the following results:

	Cases	Controls		
Animals present	16	100	116	OR = 4.0
Animals not present	4	100	104	
Total	20	200	220	$X^2 = 5.42$, $p<0.025$

These results would be regarded as some evidence that animals play an important role in the transmission of diarrhoea due to Campylobacter, evidence which would have been missed had no microbiology been performed.

It may be tempting to suppose that the performance of comprehensive stool microbiology on all cases will solve the problem of false positives. All "cases" in whose stools no enteropathogen is identified may then be excluded from the analysis, leaving only cases that are enteropathogen-positive. It must be remembered, however, that microbiological techniques are not infallible. In fact, the performance of comprehensive stool microbiology may have little influence on the false positive rate and will not reduce it to zero. Some children may be identified as infected by enteric pathogens when they are not, while many others who are so infected will be wrongly excluded from the case series because the pathogen is missed (for reasons outlined earlier). In practice, the performance of comprehensive stool microbiology on all cases will rarely be possible. Such an option is expensive. Sophisticated equipment and techniques are required to test for pathogens such as enterotoxigenic Escherichia coli (ETEC) and these facilities may not be available to many studies carried out in developing countries.

(2) False negatives

The number of false negatives (children suffering from diarrhoea caused by an enteric infection who are recruited as controls) is likely to be low, particularly in a health facility-based study in which all controls undergo a clinical examination. A clinical examination will not, however, remove from the control group persons without diarrhoea but infected with enteric pathogens. Whether one wishes to remove such persons depends on the purposes of the study. In a study of transmission routes (water supply, sanitation, infant feeding mode, domestic hygiene, presence of animals) one might like to compare symptomatic and infected cases with asymptomatic and uninfected controls. On the other hand, in studies of those factors likely to influence the outcome of infection (birth weight, vitamin A deficiency, measles, feeding mode, weaning practices) one would also be interested in comparing symptomatic and infected cases with asymptomatic but also infected controls. Such subtleties will only be possible if appropriate microbiological examinations are conducted on the stools of all controls, as well as cases, and this will seldom be logistically or financially possible.

It is difficult to assess how the use of the more subtle definitions of false negatives described above might affect the estimate of the odds ratio obtained from a study. In case-control studies of water, sanitation and diarrhoea, performed in the Philippines (Baltazar and Briscoe, personal communication) and Malawi (Young and Briscoe, 1986), enteropathogen carriage rates of around 26% were found in controls on whom stool microbiology was performed. Assuming a false negative (asymptomatic carriage) rate of 20% in the earlier examples produces the following results:

Example 1 (cont'd)	Cases	Controls				
		True		False		
False negative rate = 20%	50	16	+	10	76	OR = 2.23
False positive rate = 0%	150	144	+	30	324	
	200	200			400	X^2 = 8.59, p<0.005

Example 2 (cont'd)						
False negative rate = 20%	140	96	+	28	264	OR = 1.43
False positive rate = 0%	60	64	+	12	136	
	200	200			400	X^2 = 2.51, p>0.1

These examples may considerably overestimate the bias associated with a carriage rate of 20%, however, since they assume that asymptomatic carriers will have had the same exposure history as the cases. It is perhaps more likely that the exposure history of asymptomatic carriers lies somewhere between that of the true cases and the non-carriers.

In most studies the only practical way of reducing the false negative rate will be to ensure that children recruited as controls are not suffering from diarrhoea, and the most practical definition of a false negative is that given initially.

Summary

Misclassification of disease status may lead to seriously biased estimates of the odds ratio. For cases recruited in clinics, the isolation rate of enteric pathogens from diarrhoea cases is much higher (70-80%) than for cases recruited in the community (30-50%). This, together with the fact that cases of diarrhoea presenting at a clinic are likely to represent the more severe cases occurring in the community, suggests that the false positive rate in clinic-based case-control studies of diarrhoea may be substantially lower than in studies relying on surveillance and recall of diarrhoea. In order to further reduce the false positive rate, all potential cases should be given a thorough clinical examination and only those "cases" satisfying a clearly specified case-definition should be recruited. In examining "cases" particular care should be taken to record all clinical manifestations of the diarrhoea and to look for signs and symptoms of those diseases with which diarrhoea may be associated. These data may then be used during the analysis to examine the effect on the estimates of the odds ratio of varying the case-definition (e.g., including/ excluding all cases associated with malaria). A further option which may be available in some settings is the performance of stool microbiology on cases. While this is an expensive option which will not usually be available, it may offer certain benefits: it may provide useful information on the relative importance of different enteric pathogens in a particular setting; it may permit an analysis of the association between the various risk factors and individual etiologies; it may help to reduce the false positive rate. Selective stool microbiology will be practical more often than comprehensive stool microbiology.

Since disease status is usually determined before the collection of exposure data, the misclassification will frequently be non-differential with respect to exposure status. In such situations the estimate of the odds ratio will be biased towards 1.0 and the statistical significance of any association will be reduced. It is however possible to conceive of situations where the probability of misclassification of disease status may depend on the child's exposure status. One example of studies in which such misclassification might occur is studies of breast-feeding. Breast-feeding may itself affect the consistency of a child's stools and thus children who are breast-fed may be more likely to be incorrectly classified as cases than children who are not receiving breast milk. A second example is where exposure is associated with distance from the clinic and where increasing distance from the clinic causes only the more severe cases (those more likely to be caused by enteric pathogens) to report. In this situation both exposure and the false positive rate would decrease with increasing distance from the clinic. The possible effects of differential misclassification are illustrated in the following section.

3.1.2 Misclassification of exposure status

Children may be wrongly classified with respect to their exposure to a risk factor of interest. The extent of such misclassification is likely to depend on where the exposure data are collected, at the clinic or at the child's home, and on the nature of the risk factor itself. The presence of disease in the child may also affect a mother's recollection of exposure. Risk factors that concern behaviours are among those on which it will be particularly difficult to collect accurate data. A fieldworker visiting a household will be able to see whether or not the household has a latrine, but a latrine is likely to have little impact on a family's health if it is not used. Simply asking the mother whether the latrine is used will, in many settings, prompt the response that the mother perceives to be

"correct". An alternative to asking about the family's habits is to observe them. Not only will this make heavy demands on fieldworkers' time, but there is also the risk that the presence of the fieldworker will cause the family to change its habits for the period of observation. Stanton et al. (1987) have compared three methods of collecting data on sanitary practices: by 24-hour recall questionnaire, by knowledge-attitude-practice questionnaire and by direct obversation. They found poor agreement between the results of the different methods and concluded that "in urban Bangladesh 24-hour recall and knowledge-attitude-practice questionnaires should not be used as proxies for direct observation of hygiene practices". Stanton et al. did not, however, show that direct observation produced consistent results.

In order to reduce the level of bias introduced into the study by the interviewer, it is highly desirable that the interviewers should be blind to the child's status as a case or control, thus avoiding the possibility that their observations and/or interviewing technique may be influenced by that knowledge. This will be difficult to arrange for interviews conducted at the clinic at the time of recruitment. Blindness of interviewers performing home visits will be easier to maintain if these interviewers do not perform clinic interviews. Interviewers performing home visits should not be told in advance whether the child is a case or a control and should be discouraged from asking the mother (with an explanation). Any questions concerning the outcome of the illness should be asked at the end of the interview.

The following example illustrates what might happen if the fieldworker is not blind to the child's disease status.

Example 4 is a study of domestic hygiene in which the fieldworker is asked to observe the level of cleanliness of the family's cooking area. Suppose for the purposes of this example that there is no association between domestic hygiene and risk of diarrhoea morbidity. The following 2 x 2 table describes the true situation:

	Cases	Controls		
Dirty	80	80	160	OR = 1.0
Clean	120	120	240	
Total	200	200	400	

If the fieldworker believes that the risk of diarrhoea is increased by a low level of cleanliness in the family's cooking area, and s/he is aware of the child's disease status, then s/he may be more likely to classify cases as having dirty kitchens than controls. In such a situation we might obtain the following table:

	Cases	Controls		
Dirty	100	60	160	OR = 2.3
Clean	100	140	240	
Total	200	200	400	$X^2 = 15.84$, $p<0.001$

In previous examples the effect of misclassification has been to reduce the apparent association. This time we observe a different phenomenon: an apparently significant association has appeared where none exists. The reason for this is that we are now dealing with differential misclassification, i.e., misclassification of exposure which is dependent on the child's disease status. Where differential misclassification occurs, the effect may be to increase or decrease the observed magnitude of an association. In general, however, differential misclassification caused by a lack of blindness is perhaps most likely to lead

to an overestimate of the odds ratio. Particular problems associated with the classification of the risk factors/interventions listed in Table 1 are discussed in Annex 2.

TABLE 1. List of risk factors and their related interventions, identified by the World Health Organization as priority areas for research

Risk factor	Related intervention
Poor weaning practices	Promotion of improved weaning practices
Poor domestic and personal hygiene	Promotion of improved hygiene practices
Lack of adequate water supply and sanitation facilities	Installation and use of water supply and sanitation facilities
Vitamin A deficiency	Vitamin A supplementation
Low birth weight	Prevention or management of low birth weight
Lack of breast-feeding (or low prevalence and/or short duration of breast-feeding)	Promotion of breast-feeding
Measles	Measles immunization
Presence of domestic animals in the home	Promotion of improved segregation of animals and humans

Summary

Some "exposures" (low birth weight, vitamin A deficiency, measles non-vaccination/history, non-breast-feeding, some weaning practices) may be assessed with reasonable reliability, in many settings, by inspection of medical cards, clinical examination or suitable questioning of mothers, and such data may be elicited at the health facility. Other exposures (water supply and sanitation, some weaning practices, domestic and personal hygiene, presence of animals in the household) will require careful definition and home visits to at least a sample of households to confirm the validity of data collected at the clinic.

Interviewers should remain unaware, if possible, of the hypotheses under test in the study and also of the case/control status of the children in the study. Mothers should also be kept unaware of the hypotheses under test to avoid any increase in recall bias. Failure to maintain such blindness may lead to differential misclassification of exposure which will often result in an overestimate of the underlying association.

3.2 Confounding

A second way in which bias may arise in case-control studies is through confounding. Confounding is bias that appears in the estimate of the (crude) odds ratio when the

exposure-disease relationship under study is distorted by the effects of extraneous variables. This will occur if there exist variables which are both risk factors for the disease under study (diarrhoea) and also associated with the exposure of interest. Such variables are called confounders or confounding variables. Confounding is likely to occur in studies of diarrhoea, a disease for which there are multiple risk factors which are often associated with each other in populations under study. Failure to take account of these associations between risk factors will lead to biased odds ratio estimates. We illustrate the effects of confounding with three numerical examples.

3.2.1 Examples of confounding

Example 5 is a study of the association between infant feeding mode and risk of diarrhoea morbidity in children aged less than 1 year. The results, when presented in a single 2 x 2 table, show little evidence of an association.

	Cases	Controls		
Non-breast-fed	22	20	42	OR = 1.11
Breast-fed	178	180	358	
	200	200	400	X^2 = 0.027, p>0.75

The study also collected data on the socioeconomic status of each child. Dividing the children into two groups on the basis of their socioeconomic status we obtain two 2 x 2 tables:

	Socioeconomic status: Low			Socioeconomic status: High		
	Case	Control		Case	Control	
Non-breast-fed	8	1	9	14	19	33
Breast-fed	142	49	191	36	131	167
	150	50	200	50	150	200
	OR = 2.72			OR = 2.68		

In both the low and high socioeconomic groups the estimated odds ratio (stratum-specific odds ratio) is greater than 2.5, suggesting that there may be an association between infant feeding mode and increased diarrhoea morbidity (we still need to test the statistical significance of the association). The reason for this discrepancy between the first, crude, analysis and the second, stratified, analysis may be explained as follows. In our sample of children, those who are not breast-fed are more likely to suffer from diarrhoea than those who are breast-fed, but they are also more likely to come from homes with high socioeconomic status, a factor which will help to protect them against diarrhoea. This association between breast-feeding and socioeconomic status is a common situation in developing countries. In the crude 2 x 2 table, the effect of non-breast-feeding is hidden (confounded) by the (unseen, opposite) effect of high socioeconomic status.

Example 6 is a study of the association between vitamin A deficiency and risk of diarrhoea morbidity. Again we present a simple analysis of the data in a single 2 x 2 table.

	Case	Control		
Vitamin A deficient	60	20	80	OR = 3.86
Non-deficient	140	180	320	X^2 = 23.76, p<0.001
	200	200	400	

These results suggest that there is a statistically significant association between vitamin A deficiency and risk of diarrhoea, and that the magnitude of this association, as measured by the odds ratio, is about 4. Stratifying again on socioeconomic status produces the following two tables:

Low SES

	Case	Control	
Vit. A deficient	56	14	70
Non-deficient	106	63	169
	162	77	239

OR = 2.38

High SES

	Case	Control	
Vit. A deficient	4	6	10
Non-deficient	34	117	151
	38	123	161

OR = 2.29

We see that both the stratum-specific odds ratios (2.38, 2.29) are lower than the crude estimate of 3.86. In this example, taking account of (controlling for) a confounder (socioeconomic status) has reduced the apparent magnitude of the association. This may be explained as follows. Vitamin A deficiency increases a child's risk of diarrhoea. At the same time, a child with vitamin A deficiency is likely to come from a household with low socioeconomic status, a factor which will also increase the child's risk of diarrhoea. Failure to take account of this association between vitamin A deficiency and socioeconomic status (as happens in the simple [crude] analysis) will add the unseen effect of socioeconomic status to that of vitamin A deficiency, increasing the apparent association between vitamin A deficiency and risk of diarrhoea.

Example 7 is a study of the association between water supply and risk of diarrhoea morbidity.

	Case	Control		
Poor supply	126	97	223	OR = 1.81
Good supply	74	103	177	X^2 = 7.95, p<0.005
	200	200	400	

In this example we consider sanitation as a confounding variable.

Sanitation

No

Case	Control	
72	42	114
57	53	110
129	95	224

OR = 1.59

Yes

Case	Control	
54	55	109
17	50	67
71	105	176

OR = 2.89

Notice that the two stratum-specific estimates are different. This suggests that there is an interaction between water supply and sanitation facilities, i.e., the effectiveness of the improved water supply in reducing the risk of diarrhoea morbidity depends on whether or not adequate sanitation facilities are available (and used). One interpretation of these findings might be that the introduction of a water supply in an area where sanitation facilities already exist may reduce the incidence of diarrhoea by over 60% (from 1 episode in the exposed [poor supply] group to 0.35 episodes [1 divided by 2.89] in the unexposed group), while the introduction of the same supply in an area where no sanitation facilities are available will only reduce diarrhoea morbidity by about 30% (from 1 episode to 0.63 [1/1.59] episodes). Such results may have important policy implications but must be interpreted with care. Suppose, for example, that the following policy decision has to be taken. Some money is available for water supply improvements, but the amount available is insufficient to pay for improvements covering the whole region. Should the money be spent in those areas where sanitation facilities already exist, or in areas in which there are no sanitation facilities? Given the results above it is tempting to conclude that it will be more cost/effective to implement the water supply improvements in areas with sanitation facilities. This may not always be so. If the incidence rate of diarrhoea in areas with sanitation facilities is 2 episodes per child per year, then improving the water supply may be expected to reduce this rate to about 0.7 episodes per child per year (2 x 0.35), a reduction of 1.3 episodes per child per year. If the incidence rate of diarrhoea in areas without sanitation facilities is 6 episodes per child per year, then improving the water supply may be expected to reduce this rate to about 3.8 episodes per child per year (6 x 0.63), a reduction of 2.2 episodes per child per year. Thus, although in this example water supply improvements lead to a greater percentage reduction in the incidence rate of diarrhoea in areas with sanitation facilities, more episodes of diarrhoea per child are averted by improvements in areas without sanitation facilities.

Summary

The three examples above illustrate the three different ways in which failure to control for a confounding variable may bias the estimate of the odds ratio:

(1) it leads to an underestimate of the true association,

(2) it leads to an overestimate of the true association,

(3) it causes an interaction effect to be overlooked.

A variable which, when included in the analysis, does not lead to a difference between the crude odds ratio and the stratum-specific odds ratio is not a confounder.

3.2.2 Confounding in case-control studies of childhood diarrhoea

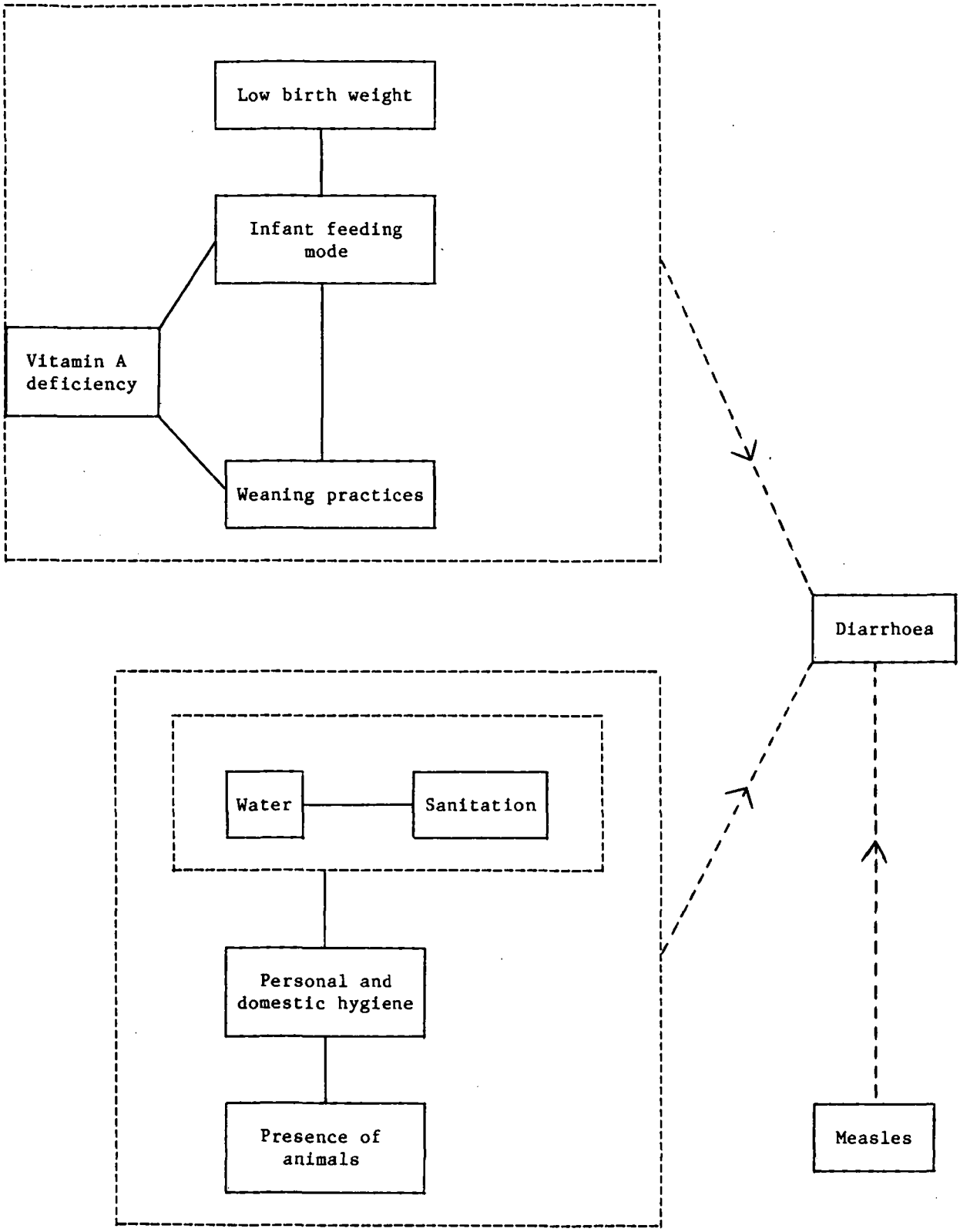
A confounding variable is one that satisfies both of two conditions:

(1) it is a risk factor for the disease under study,

(2) it is associated with the risk factor of interest, but is not a consequence of it.

Thus, any risk factor is a potential confounder for any other risk factor. Whether it is in practice a confounder will depend on whether or not the two factors are associated in the study population. To be able to control the effect of confounding as demonstrated in the examples above, it is necessary to collect data on the confounding variable(s). It is essential, therefore, that all potential confounders be identified at the design stage.

FIGURE 1. Likely associations between risk factors



_____ indicates two variables likely to be mutual confounders

There are, however, many potential risk factors for diarrhoea and therefore many potential confounders. To collect comprehensive data on all these potential confounders would require extremely long questionnaires which would almost certainly be completed badly. This will increase the rate of misclassification, particularly of exposure and confounding variables. As we have already seen, misclassification of disease or exposure status leads to biased estimates of the odds ratio. Misclassification of a confounding variable will reduce our ability to control the confounding and thus will also lead to a biased estimate of the odds ratio.

Therefore, when designing a study, it will usually be necessary to consider carefully which risk factors are most likely to be associated with each other. Figure 1 represents our suggestions of the ways in which the risk factors included in Table 1 are most likely to be associated with each other. Thus, in a study of personal and domestic hygiene we would suggest that information be collected not only on the family's personal and domestic hygiene practices, but also on its water supply (quality and quantity), whether or not there are sanitation facilities, and on the presence of animals. It should be emphasised, however, that these are suggestions and that Figure 1 does not purport to show exhaustively all possible associations, but only those that might be expected to occur most commonly. In addition to the risk factors listed in Table 1 there are several other variables, which are not in themselves of much interest as risk factors since no intervention is available or feasible, but which may nevertheless act as confounders. In Table 2 we list a number of these variables, on which we recommend that data should always be collected. As with Figure 1, this list is illustrative and should not be treated as definitive.

TABLE 2. **Potential confounders: Variables that should be recorded by all case-control studies of childhood diarrhoea. During the analysis, the effect of each on the crude estimate of the odds ratio should be examined to determine whether or not confounding has occurred.**

Age of child	
Sex of child	
Socioeconomic status of family - as measured by:	family income father's occupation mother's occupation family possessions
Distance from home to clinic	
Time taken to travel from home to clinic	
Clinic of recruitment (if more than 1 clinic used)	
Size of household	
Birth order of the child	
Anthropometric status of the child	
Maternal education	
Time period of recruitment into study	

3.2.3 Dealing with confounding

In illustrating the possible effects of confounding (Examples 5, 6 and 7), we have also demonstrated one way of dealing with the problem, namely stratification. Stratification is effective because within each stratum all children are (approximately) equal with regard to the confounder. Thus, within each stratum the confounder can have (little or) no effect on the estimate of the odds ratio.

In situations like examples 5 and 6, in which both (all) of the stratum-specific odds ratios are roughly equal, it is useful to have a single, summary estimate of the odds ratio of the association between exposure and disease. There is less point in calculating a single, summary estimate in a situation like example 7, in which there is a strong interaction present, since it is difficult to interpret the summary estimate. Mantel and Haenszel (1959) proposed a summary estimate of the odds ratio (called the Mantel-Haenszel estimator) and a test for the overall significance of the association (Annex 1).

Example 5 (cont'd)

	Socioeconomic status					
	Low			High		
	Case	Control		Case	Control	
Non-breast-fed	8	1	9	14	19	33
Breast-fed	142	49	191	36	131	167
	150	50	200	50	150	200

$$OR = \frac{8 \times 49}{1 \times 142} = 2.72 \qquad OR = \frac{14 \times 131}{19 \times 36} = 2.68$$

The Mantel-Haenszel estimate of the odds ratio is calculated in the following way:

$$\text{Mantel-Haenszel OR} = \frac{\frac{(8 \times 49)}{200} + \frac{(14 \times 131)}{200}}{\frac{(1 \times 142)}{200} + \frac{(19 \times 36)}{200}}$$

$$= 2.69$$

Note that the estimate lies between the 2 stratum-specific estimates. This will always be so since the estimate is a weighted average of the separate, stratum-specific estimates. To test the statistical significance of the overall association we perform the following calculations:

$$N = \left[\left|\frac{(8 \times 49 - 1 \times 142)}{200} + \frac{(14 \times 131 - 19 \times 36)}{200}\right| - 0.5\right]^2$$

$$= [6.5]^2$$

Note: the 0.5 is a continuity correction.

$$D = \frac{(150 \times 50 \times 9 \times 191)}{200 \times 200 \times 199} + \frac{(50 \times 150 \times 3 \times 167)}{200 \times 200 \times 199}$$

$$= 6.812$$

Then, under the null hypothesis of no association,

$$X^2 = \frac{N}{D} = 6.20$$

is distributed as a chi-squared variable with 1 degree of freedom. Thus, the association between infant feeding mode and risk of diarrhoea morbidity is statistically significant at the 5% level.

Example 6 (cont'd)

	Low SES			High SES		
	Case	Control		Case	Control	
Deficient	56	14	70	4	6	10
Non-deficient	106	63	169	34	117	151
	162	77	239	38	123	161
	OR = 2.38			OR = 2.29		

Mantel-Haenszel $$OR = \frac{\frac{(56 \times 63)}{239} + \frac{(4 \times 117)}{161}}{\frac{(14 \times 106)}{239} + \frac{(6 \times 34)}{161}} = 2.36$$

$$N = \left[\left| \frac{(56 \times 63 - 14 \times 106)}{239} + \frac{(4 \times 117 - 6 \times 34)}{161} \right| - 0.5 \right]^2$$

$$= [9.69]^2$$

$$D = \frac{(162 \times 77 \times 70 \times 169)}{(239 \times 239 \times 238)} + \frac{(38 \times 123 \times 10 \times 151)}{(161 \times 161 \times 160)}$$

$$= 12.556$$

Then $$X^2 = \frac{N}{D} = 7.48, \; p<0.01$$

The association between vitamin A deficiency and diarrhoea morbidity is statistically significant at the 1% level.

The technique of stratification may be used to control for the effect of a confounding variable with more than two levels and/or to control simultaneously several confounding variables. However, in situations in which there are a number of confounders, and therefore a large number of strata, another statistical technique, logistic regression, is frequently used to control confounding. Logistic regression will not be discussed in this paper. Interested readers are referred instead to Breslow and Day (1980) or Schlesselman (1982).

Another strategy for coping with confounding is matching. This involves selecting controls who are similar to cases with regard to the variable(s) being matched upon. For example, in a study matching on age the recruitment of a case aged 3 years will lead to the recruitment of a control aged 3 years. Clearly, the decision on whether or not to match in a study must be taken at the design stage and will have implications for the day-to-day administration of the study. Less obviously, matching also has implications for the analysis of the data. Matching will be discussed in detail in the third paper in this series.

Summary

Confounding is something that occurs in populations, through no fault of the investigator, and may be dealt with satisfactorily provided the appropriate data are collected. Failure to account for confounding will produce biased estimates, and it is essential that careful consideration is given to the problem at the design stage.

Confounders are variables that are associated with both the disease under study (diarrhoea) and the exposure of interest. The investigator should attempt to identify all such variables when designing the study. This will enable decisions to be taken on matching and the data to be collected during the study. We have put forward in Figure 1 and Table 2 a possible starting point for investigators, but emphasize that these are suggestions rather than definitive answers to these problems. Each investigator must decide for his/her own setting which of these suggestions to retain and whether there are other potentially confounding variables that have not been considered here.

3.3 Selection bias

The third type of bias considered in this paper is selection bias. This refers to any bias introduced by the investigator, by the way in which s/he selects cases and controls for inclusion in the study.

If we take as our cases those children reporting to a particular health facility during a given time period, in whom diarrhoea caused by an enteric infection is diagnosed, then, as Miettinen (1985) has pointed out, we have implicitly defined our population of controls. The controls we select for inclusion in the study should form a random sample from the population of children who, had they suffered a bout of infectious diarrhoea of similar severity during the study period, would have reported to the health facility, i.e., would have been recruited as cases. Thus the problem of avoiding selection bias is that of correctly selecting controls which is, as Spitzer (1985) states, "the most difficult judgement in 'case-control' research". In this paper we consider only selection bias arising from the recruitment as controls of children suffering from a disease other than infectious diarrhoea, reporting to the same clinic as the cases. Other ways of selecting controls will be discussed in the fourth paper in this series.

Briscoe et al. (1985) considered in detail the problems of selection bias that arise through the use of diseased controls in case-control studies of childhood diarrhoea. We may distinguish two different ways in which selection bias can occur in such studies.

(1) through different reporting rates for diarrhoea and the control diseases,

(2) through the choice of a control disease(s) associated with a risk factor of interest.

3.3.1 The problem of differential reporting rates

Example 8 is a clinic-based case-control study of the association between water supply/sanitation and risk of diarrhoea morbidity in children aged less than 5 years. The table below describes the target population:

	Cases	Controls	
Poor facilities	700	6300	7000
Good facilities	400	7600	8000
	1100	13900	15000

$$\text{Population OR} = \frac{700 \times 7600}{6300 \times 400} = 2.11$$

Suppose that a household's distance from the clinic is associated with whether or not it possesses good water supply/sanitation facilities, but not with the risk of diarrhoea, i.e., distance is not a confounder.

Distance from clinic

< 2 km

	Cases	Controls	
Poor facilities	100	900	1000
Good facilities	200	3800	4000
	300	4700	5000

OR = 2.11

> 2 km

	Cases	Controls	
Poor facilities	600	5400	6000
Good facilities	200	3800	4000
	800	9200	10000

OR = 2.11

Notice that families living close to the health centre are more likely to possess good facilities and that both of the stratum-specific odds ratios are identical to the overall odds ratio, confirming that distance is not a confounder. Let us now consider the effect of distance on reporting rates. Suppose that all cases of diarrhoea report to clinics, regardless of distance, and that children living less than 2 km from the clinic also report for the control disease(s). Suppose, however, that children living more than 2 km from the health centre report for the control disease(s) only 10% of the time, perhaps because the control disease is not considered sufficiently serious to merit walking more than 2 km for treatment. Then our case-control study is effectively sampling from the following population:

	Cases			Controls			
	< 2 km		> 2 km	< 2 km		> 2 km	
Poor facilities	100	+	600	900	+	540	2140
Good facilities	200	+	200	3800	+	380	4580
	1100			5620			6720

$$\text{OR} = \frac{700 \times 4180}{1140 \times 400} = 5.08$$

In this example, children living near the health facility with better water/sanitation facilities are over-represented in the control series, leading to an overestimate of the true association. Similarly, an example could be constructed in which the effect of the selection bias would be to reduce the apparent association.

For selection bias to arise in this way a variable (e.g., distance) must satisfy two conditions simultaneously:

(1) it is associated with the risk factor of interest,

(2) it is associated with different reporting rates for the control diseases compared with those for diarrhoea.

Briscoe et al. (1985) identified 3 factors that might act in this way: distance from household to clinic, socioeconomic status and the presence of a village healthworker. Two strategies were proposed to control these sources of bias. The first strategy, preventive in nature, consists of choosing controls from among children suffering from diseases considered to be of a similar severity to diarrhoea. Then, it is suggested, the effect of the above factors on the propensity to report the control diseases will be similar to their effect on the propensity to report diarrhoea, and the association in criteria 2 above will be weak. The list of control diseases proposed by Briscoe et al. (1985) is reproduced in Table 3 and it is recommended that this strategy be used for choosing the control diseases. However, it should be borne in mind that mothers' perceptions of the severity of different diseases may vary between cultures and therefore different control diseases may be appropriate in different settings. Furthermore, the list of control diseases presented in Table 3 was proposed for studies of water supply and sanitation facilities and may be inappropriate for studies of other risk factors. This issue is discussed in detail in the next section.

TABLE 3. Potential control diseases for case-control studies of the relationship between water supply and sanitation and childhood diarrhoea (Briscoe et al., 1985)

chicken pox
whooping cough
measles
mumps
malaria
otitis
deafness
other ear diseases
sore throat
influenza
tonsilitis
pneumonia
bronchitis
other respiratory illnesses
fever

The second strategy proposed by Briscoe et al. is corrective in nature. Even after choosing one's control diseases carefully in order to minimize the bias described above, it is likely that some residual bias will remain. It was therefore recommended that estimates of 4 parameters should be used to estimate the magnitude and direction of any remaining bias. This approach presents two problems: first, the estimation of the parameters and second, the lack of any confidence limits for the adjusted estimate of the odds ratio. We propose here an alternative approach to this problem, namely stratification of the data:

Example 8 (cont'd)

	Cases		Controls		
	< 2 km	> 2 km	< 2 km	> 2 km	
Poor facilities	100	600	900	540	2140
Good facilities	200	200	3800	380	4580
	1100		5620		6720

Estimated OR = 5.08

Stratifying on distance produces the following 2 tables:

	Distance					
	< 2 km			> 2 km		
	Cases	Controls		Cases	Controls	
Poor facilities	100	900	1000	600	540	1140
Good facilities	200	3800	4000	200	380	580
	300	4700	5000	800	920	1720
	OR = 2.11			OR = 2.11		

The two stratum-specific estimates of the odds ratio are both identical to the (true) population odds ratio calculated at the beginning of this example. Thus, the Mantel-Haenszel estimator, which is a weighted average of the stratum-specific estimates, is also equal to the true odds ratio (2.11), i.e., our estimate is now correct. Stratification, the strategy used for controlling confounding, has also succeeded in controlling (removing) the effect of this type of selection bias. In effect, the selection bias arising from different reporting rates for diarrhoea and the control diseases has changed distance, which is not a confounder in the whole population, into a confounder in our sample.

That selection bias arising in this way can be controlled in a straight-forward fashion does not mean that we should pay it no attention in designing our study. We shall have more confidence in the results of a study in which bias has not occurred than in a study in which bias is known to have occurred and has later been "corrected" in the analysis. A study should always be designed with the aim of minimizing bias. We now consider how the choice of control diseases may introduce bias into a study.

3.3.2 The choice of control diseases

The choice of the control disease(s) to be used in a case-control study of childhood diarrhoea should be guided by three considerations:

(1) the disease(s) should be perceived to be of a similar severity to reported diarrhoea,

(2) the disease(s) must not be associated with the risk factor(s) under investigation,

(3) the disease(s) should cover a reasonable proportion (>20%, say) of the children reporting to the health facility.

The reasons underlying the first requirement are discussed above and in the document by Briscoe et al. (1985). The bias that may arise through the use of a control disease that is associated with the risk factor/intervention of interest is illustrated in Example 9 below:

Example 9 is a case-control study of the association between low birth weight and risk of diarrhoea morbidity during infancy. Suppose for the purposes of this example that 10% of all children are born with low birth weight and that low birth weight increases risk of diarrhoea morbidity. Suppose further that controls are selected from children reporting with respiratory infections and that low birth weight also increases the risk of respiratory infections in infants. Then we might obtain results similar to those below:

	Cases	Controls (Respiratory cases)	
Low birth weight	60	70	130
High birth weight	140	130	270
	200	200	400

OR = 0.80

Our results appear to suggest that low birth weight may reduce the risk of diarrhoea during infancy. This contradicts our earlier assumption that, in this example, low birth weight increases risk of diarrhoea morbidity. We have obtained this biased estimate of the odds ratio because the controls we selected were not representative of the whole population with regard to the exposure of interest (low birth weight). If the controls had been representative of the whole population, we would have expected 20 (10% of 200) to have had low birth weights and the estimate of the odds ratio would have been 3.86.

In contrast to the selection bias arising from differential reporting rates, the bias introduced by an association between a control disease and the risk factor/intervention of interest cannot be controlled in the analysis, except by excluding those controls from the analysis. For this reason, it is recommended that controls should be selected from children suffering from a variety of complaints. If it is later realized that one of the control diseases is associated with a risk factor of interest, the controls suffering from that disease may be excluded from the analysis without losing the whole control group.

Table 4 lists, for each risk factor in Table 1, the diseases from Table 3 that may be associated with that risk factor and which are therefore not recommended for use in studies of that risk factor. In practice, exclusion of all the diseases possibly associated with the risk factors of interest here (Table 1) will lead to a wide list of exclusion criteria which, taken to an extreme, would exclude all conditions related to poverty and the environmental deprivation which prevails in many tropical countries. For example, it might

be hypothesized that Vitamin A deficiency is associated with many of the diseases listed in Table 3. If this is so, the use of controls with these diseases in studies of the association between Vitamin A deficiency and childhood diarrhoea will always introduce uncontrollable selection bias. In such a situation the selection of controls by some other method may be advisable. This will be discussed in a later paper in this series.

TABLE 4. Some control diseases which should not be selected in studies of various risk factors

Risk factor/intervention of interest	Unsuitable control disease
Weaning practices and breast-feeding	Respiratory infections
Personal/domestic hygiene	Skin infections
Water and sanitation facilities	Typhoid fever, hepatitis A, skin infections, nematode infections
Vitamin A deficiency	Infectious diseases
Low birth weight	Infectious diseases
Measles/measles immunization	Measles, other vaccine-preventable diseases

4. DISCUSSION

This document examines three potential sources of bias in case-control studies of risk factors for childhood diarrhoea: misclassification of disease or exposure status, confounding, and the method of selecting cases and controls. Any of the above sources may lead to seriously biased estimates of the odds ratio and may even affect the broad conclusions drawn from the study.

In clinic-based case-control studies of diarrhoea, misclassification of disease status will often be non-differential leading to underestimates of the odds ratio, tending to mask any existing associations. To reduce the rate of misclassification of disease status, all cases and controls should be given a thorough clinical examination at the time of recruitment, and details of the signs and symptoms of the episode of diarrhoea and any associated illnesses should be recorded for all cases. This will allow some flexibility in the case-definition during the analysis.

Misclassification of exposure status may be differential, leading to over- as well as underestimates of the odds ratio. Apparently significant associations may be observed where none exists. To reduce misclassification of exposure status, mothers and fieldworkers should be kept "blind", if possible, to the hypotheses under test in the study, and fieldworkers should be kept blind, if possible, to the child's status as case or control. The most appropriate method of data collection will vary from risk factor to risk factor.

Confounding is likely to occur and must be controlled. Failure to do so may result in under- or overestimates of the odds ratio. All potential confounders must be identified at the design stage and data collected on them for use during the analysis. Matching may also be used to control confounding and will be discussed in detail in the third paper in this series.

The most difficult decision the investigator is likely to face when designing a case-control study concerns the choice of control group. The choice of inappropriate controls will introduce selection bias which will lead to under- or overestimates of the odds ratio. In this paper we have discussed only the selection of controls from among children reporting to health facilities with diseases other than diarrhoea. These should be selected from children reporting with diseases that have a perceived severity similar to that of diarrhoea which is not associated with any of the risk factors of interest. Alternative sources of controls will be discussed in the fourth paper in this series.

At this point readers may feel that the use of the case-control approach in the study of childhood diarrhoea is fraught with difficulties and is therefore best avoided. This is not so. We have outlined some of the problems that may confront the investigator in this field, but it should be remembered that most of these are not specific to the case-control design. Confounding, if it exists, will be a problem for any type of observational design, as will misclassification. Indeed, misclassification of disease status is likely to be considerably reduced in case-control studies compared with longitudinal and cross-sectional studies which rely on surveillance and recall. Only selection bias is peculiar to the case-control method. In this paper we have discussed ways of reducing and controlling selection bias when diseased controls are recruited. In a later paper we shall consider the choice of other control groups.

None of the problems of bias likely to arise in a case-control study of diarrhoea is insurmountable. They can be reduced or avoided by careful design and appropriate analysis. Given some of the other advantages that they offer (small sample-size, short study period, relatively low cost) case-control methods are appropriate for the study of childhood diarrhoea in a variety of situations.

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ANNEX 1

STATISTICAL FORMULAE

1. Analysis of a single 2 x 2 table

	Case	Control	
Exposed	a	b	r1
Unexposed	c	d	r2
	m1	m2	n

$$\text{Odds ratio} = \frac{a \times d}{b \times c}$$

$$X^2 = \frac{n \times [\,|axd - bxc| - 0.5xn\,]^2}{m1 \times m2 \times r1 \times r2}$$

The statistical significance of the observed association is found by comparing the value of X^2 with the percentage points of the chi-squared distribution with 1 degree of freedom. If X^2 is greater than 3.84, then the association is significant at the 5% level; if X^2 is greater than 6.63, then the association is significant at the 1% level.

2. Stratified analysis

The data have been divided into several strata, each of which may be represented in the form of a 2 x 2 table. The (i) indicates that this table represents the ith strata.

	Case	Control	
Exposed	a(i)	b(i)	r1(i)
Unexposed	c(i)	d(i)	r2(i)
	m1(i)	m2(i)	n(i)

$$\text{Mantel-Haenszel OR} = \frac{\frac{a(1)xd(1)}{n(1)} + \frac{a(2)xd(2)}{n(2)} + \ldots\ldots}{\frac{b(1)xc(1)}{n(1)} + \frac{b(2)xc(2)}{n(2)} + \ldots\ldots} \ldots$$

$$\text{Mantel-Haenszel } X^2 = \frac{N}{D}$$

where

$$N = \left[\left|\frac{a(1)xd(1) - b(1)xc(1)}{n(1)} + \frac{a(2)xd(2) - b(2)xc(2)}{n(2)} + \ldots\ldots\right| - 0.5\right]^2$$

and

$$D = \frac{m1(1) \times m2(1) \times r1(1) \times r2(1)}{n(1) \times n(1) \times [n(1)-1]} + \frac{m1(2) \times m2(2) \times r1(2) \times r2(2)}{n(2) \times n(2) \times [n(2)-1]} + \ldots\ldots$$

The statistical significance of the observed overall association, as estimated by the Mantel-Haenszel odds ratio, is found by comparing the value of the Mantel-Haenszel X^2 statistic with the percentage points of the chi-squared distribution with 1 degree of freedom. If X^2 is greater than 3.84, then the association is significant at the 5% level; if X^2 is greater than 6.63, then the association is significant at the 1% level.

ANNEX 2

MISCLASSIFICATION OF EXPOSURE

Water supply and sanitation facilities

Various methodological problems associated with studies of the health impact of water and sanitation projects have been discussed by Blum and Feachem (1983). This recognition of the problems with other types of study led to the suggestion that the case-control method might be a suitable alternative for use in health impact evaluations (HIE) (Briscoe et al., 1986) and a considerable effort has been devoted to developing the application of the case-control approach to evaluate the impact of water and sanitation projects (Briscoe et al., 1985).

Two studies have been completed recently in the Philippines (Briscoe et al., 1988) and Malawi (Young and Briscoe, 1986). Data from the Malawi study provide useful information on the reliability of water source data collected at clinics. The mothers of the recruits into the study were asked, at the clinic, from what type of source they drew their drinking water. During a follow-up interview in the home this response was checked and the source visited. Assuming that the information obtained in the household was correct, the misclassification rates at the clinics were as set out below:

Misclassification rates for water source data collected at clinics in Malawi

Source (household data)	% incorrect at the clinic
Improved (piped) source	9% said they did not use a piped source
Unimproved source	11% said they did use a piped source

No indication is given as to whether the misclassification rates differed between cases and controls. Assuming that they did not, we can consider the possible effects of such misclassification rates.

Our example is a case-control study of the association between water quality and diarrhoea. A true odds ratio of 1.5 is assumed.

	Cases	Controls	
Unimproved quality	300	250	550
Improved quality	200	250	450
	500	500	1000

OR = 1.50

If misclassification occurs at the same rate as at the clinics in Malawi we shall obtain the following table:

Annex 2

	Cases	Controls	
Unimproved quality	300-33+18	250-27.5+22.5	530
Improved quality	200-18+33	250-22.5+27.5	470
	500	500	1000

$$OR = \frac{285 \times 255}{245 \times 215} = 1.38$$

To illustrate how the above figures were arrived at, we consider the distribution of the cases. Out of the 500 cases, 300 use unimproved sources. Of these 300, 11% (= 33) are misclassified as using an improved source. Of the 200 cases genuinely using an improved source, 9% (= 18) are incorrectly classified as using an unimproved source. Thus we arrive at 300 - 33 + 18 = 285 cases classified as using unimproved sources, and 200 - 18 + 33 = 215 cases classified as using improved sources.

The misclassification rates found in the Malawi study cannot be assumed to hold in other settings; it is quite likely that they will vary considerably from study to study. To reduce misclassification bias introduced by the use of clinic data, all homes might be visited to check on clinic responses; but this will greatly increase the resources required for the study. An alternative approach is to estimate the level of bias, as was done above, using data collected in the homes of a subsample of the recruits. This avoids the need to visit all households. It is not recommended that studies should rely wholly on data collected at clinics.

A further problem encountered in the Malawi study concerned the measurement (classification) of the quantities of water used in the household for various purposes. It was found that there were large differences in the quantities recorded by different fieldworkers. One approach to this problem is not to estimate the quantity of water itself, but instead to collect data on the distance of the household from the source and the time taken to fetch water (which are likely to be correlated with quantity). One argument in favour of this is that in implementing a water supply a planner can design the supply (subject to cost) to achieve certain targets in terms of the population's distance from the supply. Less easy to design for is a target expressed in terms such as "70% of the population should use more than 20 litres of water per capita per day". To assess accurately a household's distance from its water supply, it will usually be necessary to visit the home.

As noted in the main discussion, the possession of, or access to, a latrine will have no impact on a family's health status if the facilities are not used. Information on the utilization of latrines may be obtained in three ways:

(1) questioning the mother,

(2) inspecting the latrine,

(3) observing the family.

Option 1 may lead to misclassification, as discussed earlier, while option 3 will be costly in resources and still may not avoid misclassification. Option 2 should provide information on whether or not the latrine is used but will not reveal by whom or how regularly. It is therefore recommended that information should always be obtained by both questioning and inspection of the latrine and, if feasible, by observation as well.

Annex 2

Weaning practices

Ashworth and Feachem (1985) listed the following faulty weaning practices which might increase a young child's risk of diarrhoea morbidity: (1) using foods of low energy and nutrient concentration, (2) selecting single foods of low nutritional value, (3) using contaminated foods, (4) feeding at infrequent intervals, (5) introducing weaning foods too early or too late, (6) weaning abruptly, and (7) giving a disproportionately small share of the family food.

Not all of these practices will be found in all settings. The particular data that need to be collected may vary, therefore, from study to study. Practices (5) and (6) require information on the ages at which weaning began and ended. These data may be obtained at the clinic and no household visit is necessary. Information regarding practices (1), (2) and (4) may also be collected by questioning the mothers at the clinic. However, misclassification of children who are in the process of being weaned is likely to be reduced by a home visit, inspection of the foods given to the child, and, in the case of practice (4), observation. It will be difficult to collect accurate data on practices (3) and (7) without making a home visit. Information on practice (7) will be best collected by observation in the home, while studies of practice (3) would benefit from the performance of environmental microbiology on samples of weaning foods.

Breast-feeding

When studying risk factors for developing diarrhoea it is important to ascertain the child's exposure status prior to the onset of the disease. An example of one of the problems that might arise if this is not done is illustrated for Vitamin A deficiency (see below). In some cultures, mothers stop giving breast milk to children during an episode of diarrhoea. If the investigator is unaware of this and obtains information on whether the child is currently breast-fed, then s/he may end up with a gross overestimate of the role of breast-feeding in reducing risk of diarrhoea. Data on feeding mode may be collected at the clinic and are perhaps best collected in a series of yes/no questions, for example:

Prior to the episode of illness was the child receiving:

(a)	breast milk	yes/no
(b)	bottle milk	yes/no
(c)	other milk	yes/no
(d)	other fluids	yes/no
(e)	solid food	yes/no

From the answers to these questions it is possible to categorize children in a variety of different ways, e.g., wholly breast-fed, partially breast-fed, non-breast-fed. Categorizations may be varied during the analysis.

Domestic and personal hygiene

Feachem (1984) has reviewed the evidence linking domestic and personal hygiene with diarrhoea morbidity. Data on domestic and personal hygiene should, whenever possible, be collected in the home rather than at the clinic, and by observation rather than questioning. Furthermore, questions/observations should be as precise as possible, each focusing on one specific hygiene-related behaviour, and requiring minimal subjective judgement on the part of the fieldworker. For example, the question "Are there human faeces visible in the yard?" requires less judgement on the part of the fieldworker than the question "Is the yard clean and tidy?". Studies of behaviours such as hand-washing will require careful training of the fieldworkers to standardize observation techniques and the recording of observations as well as a considerable investment of fieldworkers' time.

Annex 2

Vitamin A deficiency

In studying Vitamin A deficiency as a risk factor for childhood diarrhoea it must be remembered that the relationship between diarrhoea and a child's Vitamin A status is likely to be a complex, two-way affair (Feachem, 1987). In order to study whether Vitamin A deficiency increases a child's risk of diarrhoea, as opposed to whether diarrhoea leads to Vitamin A deficiency, it will be necessary to assess what a child's Vitamin A status was before the onset of diarrhoea. As an illustration of the importance of this point consider the following example of a case-control study of Vitamin A deficiency as a risk factor for diarrhoea. Suppose that Vitamin A deficiency does not increase a child's risk of diarrhoea (true OR = 1.0) but that diarrhoea leads to Vitamin A deficiency in 10% of cases, and that the prevalence of Vitamin A deficiency in the community is 5%. Then, if a child's Vitamin A status is measured at the time of reporting (i.e., during the diarrhoeal episode for cases), we shall obtain the following results:

	Cases	Controls		
Deficient	20+38	20	78	OR = 3.22
Non-deficient	380-38	380	722	
	400	400	800	X^2 = 19.45, p<0.001

The table shows that 38 (10% of 380) of the cases have become Vitamin A deficient as a result of the episode of diarrhoea for which they are recruited into the study. We might be tempted to treat these results as strong evidence that Vitamin A deficiency is a risk factor for diarrhoea. Clearly this would be wrong, since we stated at the beginning of the example that Vitamin A deficiency does not increase a child's risk of diarrhoea. The table is evidence of an association between diarrhoea and Vitamin A deficiency but, because Vitamin A status was determined during the diarrhoeal episode rather than before it began, we cannot deduce from the table which is the cause and which the effect.

Approaches to assessing (classifying) a child's Vitamin A status

1. Examination for clinical (eye) signs:

   The diagnosis of clinical signs (xerophthalmia) requires well-trained staff. Some of the signs (conjunctival xerosis, Bitot's spots) may appear and disappear in a short time interval and their presence may be due to the episode of diarrhoea for which the child is presenting. (If this is so, it may suggest a low Vitamin A status prior to the onset of diarrhoea.) Other signs (e.g., corneal xerosis) are less transient and are likely to indicate Vitamin A deficiency at some time (not necessarily immediately) prior to the onset of diarrhoea. Mothers should also be questioned to ascertain whether the child suffered from night blindness in the period preceeding the onset of diarrhoea.

2. Measurement of serum Vitamin A levels:

   This approach is not recommended. It is expensive and invasive. Furthermore, since serum levels of Vitamin A can fluctuate considerably over short periods of time, it will give no reliable indication of the child's status prior to onset.

3. Conjunctival impression cytology (Wittpenn et al., 1986):

   The results of this new technique may reflect the child's Vitamin A status a week prior to the test, i.e., prior to onset for children with a disease duration of less than 7 days. The use of conjunctival impression cytology may help to avoid the problem of distinguishing cause from effect. The technique remains uncertain with respect to application and interpretation and several trials are under way.

Annex 2

Low birth weight

In their review of the literature, Ashworth and Feachem (1985) were unable to find any satisfactory data on the association between low birth weight and diarrhoea. However, data from Nigeria (Ayeni and Oduntan, 1978), India (Rao and Inbaraj, 1973) and Guatemala (Mata, 1976) suggest that low birth weight is strongly associated with infant mortality (from all causes combined). More recently, Victora et al. (1987) have reported finding an association between low birth weight and risk of infant mortality in Brazil. In particular, they found that risk of death due to diarrhoea increased as birth weight decreased. Preliminary evidence from Sri Lanka suggesting that there may also be a strong link between low birth weight and diarrhoea morbidity has been reported by Mertens et al. (1987).

The retrospective nature of the case-control method may disqualify it for use in the study of the relationship between diarrhoea and low birth weight in some developing countries where birth weight is rarely recorded. Only in settings where a high proportion of births take place in hospitals, or are attended by a midwife who records the birth weight, will it be feasible to use a case-control study for such a purpose. In areas where birth weight is rarely recorded it is likely that those children for whom it is done will not form a representative cross-section of the community, and thus any results that are obtained from them will be difficult to interpret.

If a case-control study is considered feasible, data may be collected at the recruiting clinic, from a medical record card. Follow-up visits to some households may be necessary if the mother has not brought the child's card. Information should be checked against central records if possible.

Measles

In case-control studies of measles as a risk factor for diarrhoea children reporting with measles must be excluded from the control series. Mothers of all children (cases and controls) should be asked to recall whether the child has suffered from measles in the last 6 months and whether s/he was brought to a health facility. If so, the record of that visit should be sought in order to confirm the diagnosis of measles. It is inevitable that misclassification will occur if no medical record is available and it is necessary to rely on both the mother's diagnosis and recall. Some of this misclassification may be reduced by asking the mother to describe measles. If the child is said to have suffered from measles she should be asked to describe the symptoms. Information on immunization against measles should be taken from the child's medical card whenever possible. All the above information may be collected at the clinic.

Presence of animals in the household

Domestic animals may be carriers of a number of different entero-pathogens. In studying the presence of domestic animals in the household as a risk factor for diarrhoea, data collected in the household, by observation, will be more reliable than those collected at the clinic. The investigator should aim to collect information on the number of animals kept, the species kept, and the level of human-animal contact or segregation. Are the animals kept in a separate enclosure or free to wander round the yard? Are they allowed access to the house and/or cooking area?

Annex 2

SUMMARY OF RECOMMENDATIONS FOR COLLECTION OF EXPOSURE DATA

Risk factor/intervention	Recommendations
Water supply and sanitation	Information on water supply may be obtained at clinics. Visits should be made to a sample of households to check the reliability of data obtained in this way. Studies of water quantity should collect data on distance to source and time taken (usually home visit). Information on sanitation should be collected by questioning and, if possible, by inspection (home visit).
Weaning practices	Data on some practices may be collected at the clinic. For others home visits will be needed. Observation and environmental microbiology may be necessary.
Breast-feeding	Data may be collected at the clinic. Questions should refer to feeding prior to the episode of illness.
Domestic hygiene	Data are best collected in the home, by inspection and observation.
Vitamin A deficiency	Data are best collected at the clinic, using conjunctival impression cytology or by clinical examination and questioning of the mother.
Low birth weight	Data may be collected at the clinic, from the child's medical card, checked centrally if possible.
Presence of animals in the household	Data are best collected during household visits, by inspection and observation.

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