Developmental Anomalies in Farm Animals II. Defining Etiology

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Abstract

Anomalous development is a function of the genetic make-up of the fetus and the environment in which it develops. The cause of anomalous development may occasionally be obvious but more often is obscure, because of its multifactorial nature. Therefore a methodological approach is often required to define the etiology. In this paper we describe a methodological approach that first defines the incident, the genotype of the individuals involved, and the environment in which the fetus has developed. This procedure can be undertaken in a stepwise fashion and need not be followed through to the end if the etiology becomes obvious. This approach highlights the necessity to characterize the defects, to use ancillary tests, and to apply basic epidemiological methods.

Résumé

Anomalies de développement chez les animaux de la ferme
II. Définition de leur étiologie

Un développement anormal dépend du bagage génétique du foetus et de l'environnement dans lequel il se développe; son étiologie peut parfois apparaître évidente, mais elle s'avère plus souvent obscure, à cause de sa nature multifactorielle. Une approche méthodique se révèle par conséquent nécessaire pour en déterminer la cause. Les auteurs décrivent dans leur article une approche méthodique qui définit d'abord l'incident, puis le génotype des individus affectés et l'environnement dans lequel ils se sont développés. On peut aborder le procédé par étapes, sans nécessairement le pousser jusqu'au bout, lorsque l'étiologie du développement anormal devient évidente. Cette approche fait ressortir la nécessité de caractériser les anomalies, d'utiliser des tests auxiliaires et d'appliquer des méthodes épizootiologiques fondamentales.

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Introduction

Development, both normal and abnormal, depends on the genetic background of the fetus and the environment in which it develops (1). Abnormal development occurs when a threshold of genetic and environmental insults is reached and the fetal compensatory mechanisms are overwhelmed (2).

Purely genetic defects can originate from the dam, the sire, or both, and can often be traced using an extended pedigree. However, environmental causes of abnormal development are numerous and often obscure. Environmental teratogens usually have their effect on the embryo during organogenesis at the "critical period", but may interact with defective genes (3). Both purely genetically-determined and environmentally-determined abnormal development can affect more than one individual. For this reason, epidemiology can help define etiologies such as nutritional deficiencies and excesses (4), inhaled chemicals and gases (5), plants (6), chemicals (7), drugs (8,9,10), and biotoxins (11).

The individual anomalous animal may be part of a larger problem or may be an incidental finding. The dam or sire may be responsible for individual anomalies through production of an abnormal karyotype (12). Some anomalous fetuses may arise following a failure to meet temporal-spatial criteria of development.

Often the morphological changes of abnormal development are similar for a number of causes, e.g. arthrogryposis (13,14); hence it is difficult to establish causality using normal morphological and descriptive techniques alone. For this reason, in this paper we aim to give the practitioner a methodological approach to categorize and define the various causes of abnormal development. This approach may require consultation with pathologists, geneticists, cytogeneticists, epidemiologists, and biometricians, if the techniques described are beyond the resources of the practitioner.

Overview

Abnormal development is caused by genotypic and environmental variables, and failure to meet the temporal-spatial requirements of development. Sometimes these variables may interact. The method that will be outlined attempts to examine one variable at a time, while keeping the others constant. The approach starts by defining the developmental anomaly, and in particular, the time at which normal development ceased. If an etiology is not evident at this stage, the question of whether this is a herd or flock problem is approached. Genetic analyses, descriptive epidemiology, natural experiments, and the experimental methods of epidemiology are then used to elucidate the cause of a flock or herd problem. Often, an initial examination of the possible causes of the abnormal development in question generates hypotheses as to probable causes of the defect. The etiology of the anomaly is found when some genetic or environmental factor known to produce the same abnormality is identified or when the defect is reproduced by experiment (Figure 1).

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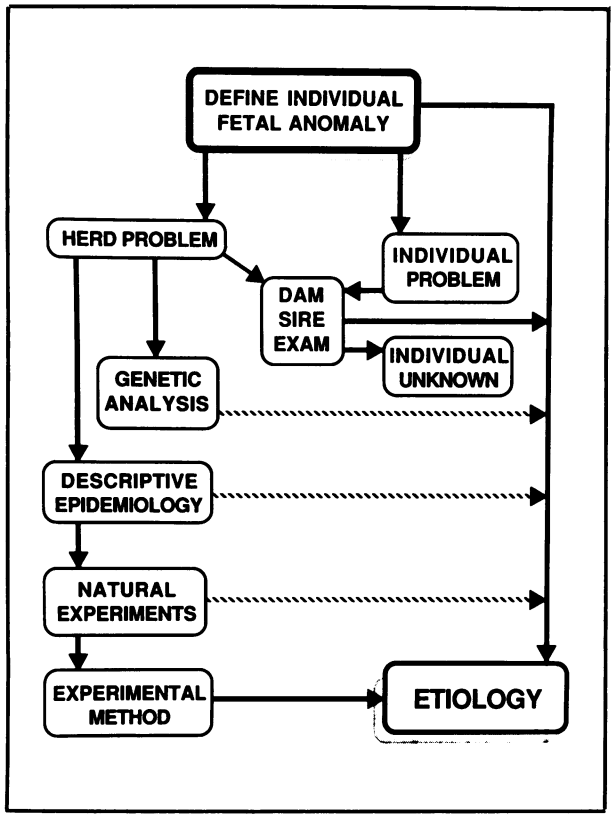

Figure 1. Flow chart of the methodological approach to defining the cause of fetal abnormal development. Solid lines represent concrete definition of etiology, whereas dotted lines represent hypothetical etiology.

Defining the Individual Incident

Clinical Examination and Pathology — Clinical and, if possible, postmortem examination of a defective animal are essential to define the abnormality and to ascertain the time of gestation at which normal development ceased. Often, definition requires the assistance of a pathologist. Postmortem examination enables recording these defects in a thorough standardized fashion, avoiding generalizations such as "crooked calves" and "wobblers", which do not adequately define the problem.

Characterization of the defects sometimes reveals the etiology immediately, as some defects are pathognomonic for a given defective gene or environmental agent. For example, periodic acid Schiff (PAS)-positive accumulations in neurons and other tissues, in conjunction with depression of plasma alpha-mannosidase activity, are characteristic of alpha-mannosidosis, an autosomal recessive trait in Angus cattle (15,16). Similarly, cyclopia in a number of lambs indicates probable prior maternal ingestion of *Veratrum californicum* during the sensitive gestational period of 13.5 days postconception (17). Examples of morphologically abnormal development and associated etiologies can be found in Tables I, II, III, IV.

Estimating the time of cessation of normal development may give important clues to whether the anomaly has a genetic or environmental basis. If a teratogen is suspected, knowledge of the time of exposure may help to elucidate a past infection (e.g. bovine viral diarrhea (BVD)), past exposure to a toxic plant, or some other environmental insult that can be traced back to that time.

Ancillary Tests — Various ancillary tests are available to aid in determining the cause of the anomaly and should be utilized if the etiology is not obvious at this stage. Routine bacteriology, virology, and mycology should be undertaken to eliminate the possibility of infectious agents such as BVD. A summary of some infectious agents shown to cause abnormal development is given in Table V.

Examination of the liver and other organs for trace elements may provide further clues as to etiology. Examples of abnormal development associated with elemental imbalances are: enzootic ataxia (hypomyelinogenesis) in copper deficient lambs (59), and arthrogryposis in manganese deficient calves (116).

Finally, karyotyping of the fetus can be done using lymphocytes from whole blood, if the fetus is alive (117), or cultured fibroblasts taken from pericardial sac or kidney (118). An abnormal karyotype may be suspected at necropsy if multisystem fetal anomalies are noted (119). Multiple systems are often defective because a single chromosome carries genetic information important to several metabolic pathways (120). As such large amounts of genetic information are involved in the production of these morphological abnormalities, a group of defects is not necessarily associated with a specific abnormal karyotype (121). Recently, cytogenetic abnormalities in livestock have been summarized (121,122).

Defining the Prevalence

Genetic disorders occur in families, whereas teratogens usually affect more than one individual in the herd or flock and may have a seasonal occurrence. If more than one abnormal fetus is reported, the crude prevalence of anomalous fetuses, abortions, and stillbirths in the herd or flock should be calculated for the gestational period in question.

The prevalence is equal to the number of anomalies, or stillbirths observed at birth divided by the total number of births (123,124,125). Multiplication of this number by a common radix (usually percentage) makes comparison of prevalences among herds and families easier.

Prevalence measures anomalies at birth, but does not include defective fetuses that are not carried to term (127). Therefore, the prevalence at birth will be less than the actual incidence of the defect, if some defective fetuses were aborted before term, as they would not be included in the calculation (133). For this reason, it is important to make a separate calculation of the prevalence of occurrence of abortion and early embryonic deaths (return to service after diagnosed pregnant) in the herd (127), since an increase in these losses may be part of the defective development problem. These calculations of prevalence should be combined with the prevalence of anomalies if the prevalence of abortions and stillbirths is greater than normal (128).

TABLE I
Examples of Specific Defective Development with Known Etiology in Cattle

Organ System	Defect	Etiology (Ref)	Other Associated Problems
Skin	Epitheliogenesis imperfecta	- Simple autosomal recessive gene (18)	- Brachygnathia inferior
	Parakeratosis	- (a) Simple autosomal lethal factor (19) - (b) Chromosomal anomalies (20)	- Atresia ani - Erosions of gastrointestinal tract, exudative dermatitis
	Protoporphyria	- Simple autosomal (21)	- Photosensitization, hepatic cirrhosis, central nervous signs
Skeletal	Chondrodysplasia	- Multilocus complex defective genes (22,23,24)	- Dwarfism (Telemark Dexter, and others)
	Osteopetrosis	- Simple autosomal recessive (25)	- Small body size, impacted teeth, open fontanelle, lack of marrow cavities
	Arthrogryposis	- Lupine feeding between day 40 and 70 of gestation (16) - Simple autosomal recessive (17) - Complex genetic etiology (26,27) - Akabane virus (28)	- Kyphosis, torticollis, scoliosis and (genetic etiology) - Cleft palate
Muscular	Muscular hyperplasia	- Pair of incompletely recessive genes (29)	- Hypoplastic genital tracts, dystocia, retarded sexual maturity, joint and bone problems
Central Nervous System	Hydranencephaly	- Akabane virus (30)	- Arthrogryposis
	Internal hydrocephalus	- Simple autosomal recessive (31,32) - Bovine viral diarrhea (33)	
	Cerebellar hypoplasia	- Autosomal recessive gene (34,35) - Bovine virus diarrhea virus (36)	
	Hypomyelinogenesis	- Recessive gene (37) - Bovine virus diarrhea (38)	- Hydranencephaly
	Storage diseases - Gangliosidosis - Glyconeogenesis - Mannosidosis - Neurofilament	- Autosomal recessive genes (39) (40) (41) - Suspect autosomal recessive gene (42)	- Accumulation of oligosaccharides in other tissues
Cardio-vascular	Ventricular septal defects	- Autosomal dominant (43)	- Hydrocephalus
Lymphatics	Dysplasia	- Autosomal recessive (44)	- Dystocia with anasarca
Digestive	Atresia and Atresia coli	- Autosomal (45) recessive? - Pregancy diagnosis trauma (46)	
Repro-ductive tract	Multiple defects of male reproductive tract	- Chromosomal anomalies (47)	
	Freemartinism	- Fused placentation (48) Chimerism, H-Y antigen (49)	

TABLE II
Examples of Specific Defective Development with Known Etiology in Sheep and Goats

Organ System	Defect	Etiology (Ref)	Other Associated Problems
Skin	Epitheliogenesis imperfecta	- Simple autosomal recessive gene (50)	
	Skin fragility	- Simple autosomal recessive gene (51,52)	- Joint laxity and delayed wound healing
Skeletal	Cyclopia	- *Veratrum californicum* (15)	- Limb defects
	Bowed forelegs	- *Oxytropis* and *Astragalus* (53) - *Tachymene* (54) - Parbendazole (55)	
	Chondrodysplasia	- Autosomal recessive? (56)	
	Arthrogryposis	- Bluetongue virus (57) - Recessive gene? (58)	
Central Nervous System	Hydranencephaly	- Akabane virus (30) - Copper deficiency (59) - Bluetongue virus (60)	
	Hypomyelinogenesis	- Copper deficiency (59) - Border disease virus (61)	- Hairy coat
	Storage disease Beta-mannosidosis	- Autosomal recessive (62,63)	- Accumulations of ß-mannoside in other tissues
Cardio-vascular	Ventricular septal defect	- Autosomal recessive (64)	
Digestive tract	Atresia ani	- Possibly sex linked (65)	Urogenital and skeletal defects
Body cavity	Inguinal hernia	- Autosomal recessive (66)	
Repro-ductive tract	Freemartinism	- Fused placenta Chimerism (67)	
	Mammary hypoplasia	- Autosomal dominant (68)	

Knowledge of the crude prevalence of anomalies, abortions, and stillbirths not only documents how severe the problem is, but also gives an idea as to whether the anomalies are only part of a bigger problem involving early abortions/resorptions and, later, stillbirths. Prevalence must be used in conjunction with definition of the anomaly as described above as prevalence does not indicate when during gestation normal development failed.

So far, the morphology of the defect, time of gestation at which the onset of abnormal development began, and the crude prevalence of the problem have been established. An appraisal of these findings may reveal the etiology at this stage. If not, the investigation should proceed to a detailed maternal examination, genetic analysis, and environmental analysis.

Defining the Maternal Environment

There should be a thorough general clinical examination (129) of the dam that has produced abnormal offspring. Often, an animal that appears clinically normal may have been ill during gestation. A thorough clinical examination may elucidate this underlying problem. Particular attention should be paid to external genitalia, mammary glands, and the palpation of the reproductive tract.

Clinical evaluation of the individual may indicate overt disease during pregnancy, alterations in normal homeostatic functions, or chronic disease. Clinical examination of representatives of the herd/flock may reveal overt, or covert, disseminated disease. Examination of herd records and production figures may give further clues about subclinical disease in the herd.

Reproductive history of the individual that produced the anomalous fetus is particularly important. Such a history should include information on previous abortions, failure to conceive, early abortions, previous malformations, and the sire that produced the deformed offspring (127).

Ancillary testing of the dam is somewhat limited. Serological examination of paired serum samples taken at two week intervals from members of the herd may reveal previous or current infection with a teratogenic infectious agent. Karyotyping of the dam to determine the presence of Robertsonian translocations and other abnormal chromosomal structures, can be done using whole blood (117). Urine and blood enzyme levels are sometimes useful in making a diagnosis in some storage diseases, e.g. alpha-mannosidosis in Angus cattle (16). Serum hormonal levels and trace elements may only be of limited use.

TABLE III
Examples of Specific Defective Development with Known Etiology in Pigs

Organ System	Defect	Etiology (Ref)	Other Associated Problems
Skin	Hypotrichosis	Lack of iodine (69)	- Enlarged thyroids
Skeletal	Arthrogryposis	- *Nicotiana tobacum* (70,71,72) - *Datura stramonium* (73) - *Conium maculatum* (74) - *Prunus serotina* (75)	
		- Autosomal recessive (76)	- Brachygnathia, cleft palate, kyphosis, atresia ani and skull deformities
		- Methallibure (77)	- Diaphragmatic hernia
	Splaylegs (Myofibrillar hypoplasia)	- Multiple causes, possibly interaction of genes and environment (75)	
Muscular	Porcine stress syndrome	- Genetic, unknown (78)	
Central Nervous System	Myoclonia congenita	Sex linked recessive (79)	- Splayleg
	Hypomyelinogenesis	Aujeszky's disease (79) Hog cholera (80)	
	Storage disease – GM_2 gangliosidosis	Autosomal recessive (81)	
Cardio-vascular	Hemophilia (porcine von Willebrand's disease)	Autosomal recessive (82)	
Digestive	Atresia ani	- Autosomal recessive ? (83) - *Salmonella typhimurium* (84)	
Repro-ductive system	Inverted nipples	Autosomal recessive (85)	- Piglets fail to thrive
Respira-tory	Respiratory distress syndrome	Autosomal recessive (86)	

TABLE IV
Examples of Specific Defective Development with Known Etiology in Horses

Organ System	Defect	Etiology (Ref)	Other Associated Problems
Skin	Albinism	- Autosomal dominant (87)	
	Lethal dominant white	- Autosomal dominant (88)	
Musculo-skeletal	Anterior/medial deviation of carpal contracted digital flexor tendon	- Maternal environment and hereditary (89)	
	Multiple exostoses	- Autosomal dominant (90)	
	Arthrogryposis	- Autosomal recessive (91)	
Central Nervous System	Cerebellar hypoplasia	- Autosomal recessive (92)	
Cardio-vascular system	Antihemophilic globulin deficiency	- Sex-linked recessive (93,94)	- Multiple hemorrhages
Digestive tract	Parrot mouth	- Autosomal dominant? (95)	- Malnutrition
Repro-ductive system	Cryptorchidism	- Multiallele? (96)	
	Male pseudoherma-phroditism	- Nondisjunction of chromosomes (97) - Freemartinism (98) - Double fertilization (99) - Blastocyst fusion (99)	
Lymphoid system	Combined immunodeficiency	- Autosomal recessive (100)	- Adenovirus pneumonia

TABLE V
Abnormal Development Associated with Some Infectious Agents

Agent	Species Affected	Defective Development (Ref)
Akabane virus	Cattle Sheep Goats	- Abortions (28,30,101) - Premature births (102) - Arthrogryposis - Hydranencephaly (101)
Bovine virus diarrhea virus	Cattle, sheep	- Cerebellar dysplasia - Ocular defects - Internal hydrocephalus - Intrauterine growth retardation - Impaired immunological competence - Brachygnathia inferior - Hypomelinogenesis (33,36,38,103,104,105,106)
Bluetongue virus	Sheep	- Hydranencephaly (57,60,107) - Porencephaly - Arthrogryposis
	Cattle	- Abortion, stillbirths - Arthrogryposis (108,109,110,111) - Prognathia - Domed cranium - Hydranencephaly - Kyphosis - Scoliosis
Border disease virus	Sheep	- Hypomyelinogenesis congenita (61) - Abortion, stillbirth - Weak lambs
Hog cholera virus	Pigs	- Multiple CNS defects (80,112,113) - Cirrhosis - Kidney defects - Pulmonary hypoplasia - Congenital tremors (114)
Swine influenza virus	Pigs	- Pulmonary hypoplasia (115)

Limited examination of the sire can be undertaken. This should include sperm morphology, blood karyotyping (117), and, if the epidemiological picture warrants, analysis of the semen for heavy metals, as semen contaminated with some heavy metals has been shown to be teratogenic (130).

Genetic Analysis

It is important to define the genotype, in order to eliminate or incriminate possible genetic causes of defective development. Breed, sex of the offspring affected, and introductions into the herd/flock should be noted. If possible, the present placement of animals sold from the herd/flock, and relevant history of anomalous development should also be ascertained. If herd records are good, the extended pedigree should be obtained and examined. This may entail coding animals in the pedigree so that anonymity and confidence of the farmer are retained.

Genetic analysis of a herd requires enumeration of the normal and defective animals and each of their relationships. Hence attempts to develop a pedigree should be undertaken even if an extended pedigree is not available. Assessing the relationships of normal and defective animals can be difficult, and, unless patterns are obvious, a geneticist should be employed.

Genetic analyses proceed in two ways. First, data on full sib families containing one or more defective offspring are subjected to segregation analysis to determine whether or not the defective gene(s) follow(s) a simple Mendelian segregation pattern (131). Secondly, the patterns may be analysed by a variety of comparisons, namely: comparison of close relatives with distant relatives, testing the occurrence of the defect in both members of a twin pair, and a search for inbreeding. Finally, the comparison with an animal model with homologous hereditary defects may give important clues about genetic causality (27).

Analyses proceed from the simplest to the more complex modes of inheritance. Models based on two alleles at a single autosomal or sex-linked locus are first tested. Next, modifications that are caused by variable expressivity, and incomplete penetrance, phenocopies, or spontaneous mutations, are examined (27).

Genetic causes of defective development may become obvious through genetic analyses. However, this is not always the situation, in which case closer examination of the environment is required.

Environmental Analysis: An Epidemiological Approach

If there are no obvious genetic or environmental causes of abnormal development, and the crude prevalence indicates that a herd problem exists, then a more detailed examination of the herd environment and

TABLE VI
Examples of Defective Development caused by Ingestion of Various Plants during Gestation

Plant	Common Name	Species Affected	Defective Development (Ref)
Lupinus caudatus	Lupine	Cattle	- Arthrogryposis (6,11,16,134) - Torticollis, scoliosis - Kyphosis, cleft palate
L. serriceus	"	"	
L. nootkatensis	"	"	
Conium maculatum	Hemlock	Cattle	- Arthrogryposis (74,134)
Veratrum californicum	Western hellebore or skunk cabbage	Sheep Cattle Goats	- Cyclops (11,134,135) - Cleft palate - Hypoplasia of metacarpal and metatarsal bones (135,136)
Veratrum eschscholtzii		Horses	- Cyclops (136)
Astragalus spp. *Oxytropis* spp.	Locoweeds	Cattle Sheep Horses	- Abortion - Ill thrift - Arthrogryposis (134,137)
Nicotiana tobacum	Tobacco	Pigs	- Arthrogryposis (70,71,72)
Sorghum vulgare	Sudan grass	Horses	- Arthrogryposis (138)
S. sudanese	Sudan grass	Horses	- Arthrogryposis (139)

herd dynamics is required. Descriptive epidemiology, natural experiments, and the experimental method, are the three basic types of epidemiological investigation that can be used.

Descriptive Epidemiology — The purpose of descriptive epidemiology is to portray the herd environment and to look for patterns of occurrence (124) within the herd. It requires further definition of the prevalence of the defects using herd records to identify risk factors. Stratified analysis is used to determine if associations exist between these risk factors and the occurrence of developmental anomalies.

The herd environment should be carefully described so that risk factors can be identified. Information gathered should include breed affected, age of parents, geographic region, type of pasture, soil type, water source, feeding and management practices, maternal medication and vaccination records, disease status of the herd, periods of stress, handling procedures, and congenital defects observed in previous years (132,133). Time of pregnancy diagnosis should be noted, as atresia ani has been associated with this practice (45). Possible exposure to teratogenic plants (Table VI), xenobiotics in the form of garbage (e.g. discarded batteries), chemical dump sites, air or water pollutants should be established. Any history of similar congenital defects occurring in neighboring herds is noteworthy. Examination of these factors may lead the investigator to identify a number of risk factors that are associated with the anomalies.

A stratified analysis of the data involves organizing individual members of the herd into combinations of categories (called strata) for each risk factor or variable. This grouping allows one to assess the relevance of each risk factor (123). Stratification means the factor itself can be divided into subgroups and differences between those subgroups examined. Age of dam is an example of a factor that can easily be stratified. The herd is divided into three age strata, for example: dams less than four years old (young); dams four to eight years old (middle); and dams greater than eight years old (old). The prevalence of anomalies is then calculated for each stratum. Examination of the difference in prevalence of anomalies among strata can help one generate hypotheses about the etiology. For example, if the prevalence of the anomaly in offspring of old dams was three times that in the other two groups, then older dams would have had a threefold higher risk of giving birth to an affected offspring, e.g. Down's syndrome in man.

All risk factors are potential candidates for stratification. Construction of simple tables and graphs comparing strata may be all that is necessary to demonstrate obvious differences between strata. If there are sufficient numbers in all strata, simple statistical analyses can be carried out within each stratum, using a Mantel-Haenszel chi-square test for association and estimation of effect (123). If many variables or risk factors are implicated by the stratified analysis, confounding and interaction may be a problem and the use of multivariate analyses may be required. A delailed presentation of the techniques of stratified and multivariate analysis has been published (123). These tests are beyond the scope of most practitioners, and a biometrician should be employed. A simpler option would be to look for the presence of a natural experiment within the herd or to apply the experimental method to the herd.

Natural Experiments — During the examination of herd records, one should carefully look for natural experiments. Here, the investigator has not actually performed a controlled experiment where all risk factors (or variables) but one are controlled. Instead, two (or more) subgroups within the herd can be retrospectively identified which, by circumstance, were treated identically, except for one risk factor. The prevalence of congenital anomalies is determined for the different groups which were defined by the natural experiment. The differences between these calculated prevalences may suggest an association between the risk factor and the congenital defects.

John Snow's investigation of a cholera epidemic in London in 1854 is a classic example of a natural experiment (141). Snow recognized that two randomly mixed populations, alike in other important respects, could be differentiated by the source of water running from the taps in their individual houses. Lambeth Company water came from an intake on the Thames River above London; Southwark and Vauxhall Company water came from the sewage-polluted river basin. The mortality associated with cholera was almost tenfold higher in the houses supplied by Southwark and Vauxhall. Snow's work clearly demonstrated the importance of water supply, even though the precise nature of the disease agent had not yet been established.

Examples of natural experiments in studies of farm animal congenital defects might include recognition of circumstances where parts of the herd were treated similarly except for one of the following: geographic region, type of pasture, soil type, water source, feed, a specific management practice, vaccinations or drugs administered, or a handling procedure.

A thorough attempt to establish that the two groups defined by the natural experiment were treated similarly except for the risk factor of interest is very important. If a second factor is also identified, examination of the primary and interactive effects of the second factor becomes necessary but may require consultation with an epidemiologist.

If natural experiments do not exist, then conclusions regarding cause and effect cannot be made. The strength of natural experiments lies in the information gained from retrospective analysis of herd records. If the problem is not a recurring one, recognition of a natural experiment may be the only way that an association between defect and risk factor can be made. In herds where the problem is recurring, an even more powerful investigative tool exists, namely, application of the experimental method.

Experimental Method — Descriptive epidemiology or the recognition of a natural experiment may provide the practitioner with an hypothesis as to etiology. These methods are retrospective in nature and cannot be controlled by the investigator. The experimental method is the most powerful tool in defining the etiology of recurring developmental anomalies. It can be used by the practitioner, with the cooperation of the farmer.

The basic approach of the applied experimental method is to take one or two of the most likely hypotheses and test them one at a time on part of the herd using other untreated animals as controls. It is important to randomly select individuals to remove sample bias (142). If the herd is large, it may be divided into a number of groups allowing for the simultaneous testing of more than one hypothesis, but this depends on the prevalence of the anomalies. Developmental anomalies of low prevalence require larger groups.

For example, following description of the problem, overwinter feed is the hypothesized cause of the abnormal development. Method: divide the herd into halves and feed one half of the herd the feed suspected of causing defects and use normal feed for the remaining half. All other management procedures should remain constant for all animals. The herd should be divided randomly with respect to which animal gets which feed. This randomization can be achieved by using a random number table (143). With proper randomization and care in treating the two groups similarly, statistical differences in the prevalence of anomalies between the two groups is evidence that the risk factor tested is the cause of the anomalies (144,145).

Records at the termination of the experiment should include reproductive problems, abortions, stillbirths, and anomalies. If desired, the practitioner can use statistical tests to determine if the differences between test and control groups are statistically significant (143,146). It should be remembered that lack of statistical significance does not necessarily "disprove" associations among groups as this often occurs in low prevalence situations with inadequately sized test and control groups (147).

Conclusions

Identification of the etiology of developmental anomalies is often extremely difficult for many reasons. First, defective development alone often does not give clues to a specific cause. Second, specific teratogens such as viruses, plants, and toxins, cannot be demonstrated at the time of expulsion of the defective fetus, or even after intensive pathological and toxicological investigations. Third, except for certain chromosomal aberrations, hereditary factors are recognized only when they occur in characteristic intragenerational familial frequencies and patterns. Therefore, when a cause cannot be demonstrated, attempts to determine patterns of occurrence must be undertaken. To do this, the practitioner often requires the assistance of pathologists, geneticists, epidemiologists, and biometricians. The method described gives a stepwise protocol to determining etiology. At many of the steps, the cause of the defective development may become obvious, in which case completion of the protocol is not necessary.

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