Ministry of Agriculture
Animal and Plant Health Regulatory Directorate

Livestock Diseases Outbreak Investigation Guideline

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Foreword

This technical document entitled “Livestock Disease Outbreak Investigation Guideline” is one of the documents in a series of guidelines and Standard Operating Procedures (SOPs) developed by the Ministry of Agriculture (MoA) in collaboration with the Ethiopian Sanitary and Phytosanitary and Livestock and Meat Marketing (SPS-LMM) Program. SPS-LMM program is financed by USAID and is implemented by the Norman Borlaug Institute for International Agriculture, Texas A & M University System. The main goal of the SPS-LMM program is to increase exports of meat and livestock to benefit Ethiopian livestock producers and exporters and to promote national economic development.

This guideline and SOP is intended to assist Federal and Regional animal health services and livestock disease investigation laboratories as well as private practitioners in carrying out early detection and rapid response to any possible livestock disease(s) outbreak.

At this point, the Animal and Plant Health Regulatory Directorate (APHRD) would like to thank the SPS-LMM program and USAID for developing and publishing this guideline and SOP.

Last but not least, I would like also to thank Drs. Nega Tewolde and Wondwosen Asfaw for preparing this guideline and SOP.

Berhe G/ Egziabher (PhD)
Head, Animal and Plant Health Regulatory Directorate (APHRD)
Ministry of Agriculture (MoA)
Addis Ababa, ETHIOPIA

1. Introduction

Because all outbreaks and all livestock operations are different, there is no “recipe for success” that covers all possible situations in the field. The investigating veterinarian must be able to adapt these techniques to each specific situation. The approach outlined here does not have to be followed in the order presented. Often, individual steps are repeated many times before the information necessary to implement a successful control process becomes apparent. The techniques described are sufficient in many investigations. However, situations requiring more complex analysis demand consultation with specialists, including epidemiologists and laboratory diagnosticians.

The role of detective or outbreak investigator can be a refreshing break from the routine of daily practice. The investigation of disease outbreaks provides an opportunity for the veterinarian to show livestock owners (clients) the advantages of a herd health program and the value of a good record-keeping system.

The decision to conduct an in-depth investigation should depend on the opportunity to control the current problem and minimize the potential for future problems, the severity of the problem and the risk to other producers, the potential risk to public health, and, in some situations, the opportunity for research and training. A functioning support system for outbreak investigations can assist veterinarians in resolving individual herd problems, while also providing benefits for the livestock industry by enhancing active surveillance for emerging disease problems.

In addition to excellent observation skills and a good understanding of production systems, veterinarians require the tools of epidemiology for the successful investigation of disease outbreaks.

The methods for investigating outbreaks of clinical disease are equally applicable to the identification of factors responsible for suboptimal productivity or changes in herd performance. An outbreak is indicated when disease rates are higher than normal or productivity is lower than normal for a particular population. The expected incidence and consequences of different diseases vary considerably, and therefore the threshold of a disease that triggers an investigation may vary depending on the disease. An extensive outbreak investigation could be triggered by just one case. For example, the diagnosis of a persistently infected calf in a herd that was thought to be in a bio-secured facility may require a comprehensive investigation.

The immediate goal of the practitioner is to take steps to minimize additional herd losses. Actions can often be taken to stop the problem before the specific cause is identified. For example, if a toxic exposure is suspected on pasture, animals can be moved to a different field while an investigation takes

place to identify the source and type of toxin. After any necessary actions have been taken to address the immediate threat, the next step is to identify the factors that contributed to the development of disease or change in productivity. The practitioner must then determine which of these factors can be controlled by management to reduce the risk of future outbreaks. These objectives can be achieved by addressing the following questions for each outbreak (what, who, when, where, and why). *Define the problem* - what and how much? *Identify groups for comparison* - who, when, and where? *Preview important risk factors and identify key determinants* - why?

This guideline outlines the primary questions a practitioner should systematically address and summarize to determine the causes of an outbreak and minimize further losses.

2. Objective

This guideline is aimed at

- serving as a quick reference for conducting a through investigation to livestock disease outbreaks.
- Carrying out early detection and rapid response to any possible livestock disease(s) outbreak.

3. Users

This field guideline is intended for use by all formally trained Federal and Regional animal health services and livestock disease investigation laboratories both at Federal and Regional levels. Other public livestock health personnel, as well as private practitioners, may also use this guideline.

5. Steps of an outbreak investigation

It useful to follow a systematic approach such as outlined below. The steps are in conceptual order. However, several of these steps may be applied at the same time, or they may be done in a different order. For example, control measures should be implemented as soon as the source and mode of transmission are known, which may be early or late in any outbreak investigation.

5.1. Preparing for a field work

Anyone about to embark on an outbreak investigation should be well prepared before leaving for the field. Preparations can be grouped into three categories: (a) investigation, (b) administration, and (c) consultation. Good preparation in all three categories will facilitate a smooth field experience.

Investigation: First, as a field investigator, you must have the appropriate scientific knowledge, supplies, and equipment to carry out the investigation. You should discuss the situation with someone knowledgeable about the disease and about field investigations, and review the applicable

literature. You should assemble useful references such as journal articles, books and sample questionnaires. Before leaving for a field investigation, consult laboratory staff to ensure that you take the proper laboratory material and know the proper collection, storage, and transportation techniques for samples. If possible, arrange for a portable computer, tape recorder, camera, and other supplies. *Administration*: You must pay attention to administrative procedures and get them approved. You also need to take care of personal matters before you leave, especially if the investigation is likely to be lengthy. *Consultation:* As an investigator, you must know your expected role in the field. Before departure, all parties should agree on your role. For example, are you expected to lead the investigation, provide consultation, or simply lend a hand? In addition, you should know who your local contacts will be. Before leaving, you should know when and where you are to meet with local officials and contacts when you arrive in the field.

5.2. Define and describe the problem - is it worth investigating?

The first and most critical task early in the investigation is to clearly define the problem. An early working definition of the problem provides direction for the investigation. If the veterinarian has an established close working relationship with the producer(s), he/she should be better able to clearly define the problem. The initial complaint often encompasses a number of different types of health and production issues. The initial complaint can include unexplained mortality, clinical disease, sub-clinical disease, impaired performance, and falling trends in productivity, etc. Other issues related to a complaint might involve potential public health concerns about food safety and environmental issues. The use of herd record systems or disease outbreak reports may allow monitoring trends in herd productivity measures over time or benchmark comparisons. Such systems can identify a problem before an initial complaint is made. The field veterinarian is also more likely to be aware of any recent changes in management that may have a connection with the initial complaint.

The development of a *case definition* is an important step in describing the problem. This case definition is critical when comparing "*cases*" with "*non-cases*" to determine the importance of potential risk factors for disease. This definition must be reevaluated and refined as more data become available. A simple, easily recognized and applied definition facilitates the consistent reporting of cases by the herd owner(s), other farm workers, and field animal health personnel. A case definition for pneumonia in neonatal calves might include all febrile or depressed animals less than three months of age with evidence of increased respiratory effort or coughing. The inclusion of unrelated cases (e.g. all calves with an increased respiratory rate) can result in errors in recognizing factors that might have contributed to the outbreak. In some outbreaks, such as abortion, outlining the case definition is a relatively simple procedure. However, when morbidity is the primary complaint, a precise case

definition helps to avoid differences in treatment thresholds that may exist between various producers or farm workers. For example, using a specific temperature "*cutoff*" as part of the case definition for respiratory disease creates a more specific case definition than a case definition that includes just those animals that are assessed as ''sick.

After developing a working description of the clinical disease or impairment in productivity, the veterinarian must determine the extent of the problem that is present in the herd/flock. The answer to this question helps the investigator decide if there really is a problem and then determine the appropriate amount of resources that should be allocated to the investigation.

To determine if the outbreak is real, the productivity and disease frequency in the herd are first measured and then compared with published benchmarks, historical expectations, disease outbreak records, and goals of farmer, herders, and produces, or the performance of neighboring herds/flocks. Veterinarians that use computerized records (e.g. disease outbreak reports) may be able to benchmark the performance of livestock from year to year and between populations or groups. The veterinarian must have a good understanding of the "normal'' production and disease levels for livestock raised under similar management conditions to be able to determine whether or not the observed values are within an expected range. The most appropriate benchmarks against which to compare the production of any herd/flock vary because of economic constraints, physical restrictions, time and management limitations, and individual differences. The targets for performance vary over time for a particular herd/flock as these constraints change.

Most outbreaks are identified and reported by the livestock owner. The severity of problems presented to the veterinarian varies with the individual owner's threshold of concern. Large-scale disease outbreaks may not be brought to the attention of the local animal health personnel until significant mortality or production losses have occurred. In these situations, the veterinarian may find it difficult to reconstruct the epidemic because of insufficient client records and the loss of valuable diagnostic material. In some cases, the disease progresses slowly and by the time the veterinarian is asked to investigate, many of the animals have chronic disease. In such cases, determining the original factors that contributed to disease onset may be difficult.

On the other hand, some herd owners are alarmed by losses well within expected rates. For example, expectations of acceptable risk of abortion, incidence of calf treatment, and herd average somatic cell count vary wildly among herd owners. By confirming the existence and severity of the problem, the investigator decreases time and resources spent on investigating epidemics that do not exist.

5.3. Identify potential risk factors by comparing groups within the herd/flock

Given that there is sufficient evidence to proceed with an investigation, the immediate goal of the veterinarian is to identify risk factors that can be manipulated to resolve the outbreak or improve herd/flock production and profitability. Risk factors are characteristics of the **host** (animals), **agent** (causative factor), or **environment** (actual climatic factors or other management factors) that can increase the occurrence of disease(s). For example, some of the documented risk factors for increased neonatal mortality in diary cattle include poor cow-body condition at calving, a high degree of crowding and contamination of the calving ground, and a high percentage of heifers in the herd. The risk factors that management can control and alter to affect disease rates or production levels are sometimes referred to as **key determinants**. Pre-calving nutrition and calving-ground density are examples of important key determinants of neonatal mortality in dairy cattle.

The identification of the key determinants that can be most readily manipulated to control the outbreak is called a **working diagnosis**, an **epidemiological diagnosis**, or a **diagnosis of ‘‘best fit’’**. The first steps in identifying these risk factors are to determine which groups of animals or poultry are affected (i.e. the host characteristics, such as age, sex and breed), when they became affected (i.e. time, particularly the date of onset), and where the problem was reported (i.e. place or location of affected animals/birds or groups of animals/flocks).

5.4. Describe which animals/birds are affected

The amount of information available varies greatly between herds/flocks, depending on management, livestock identification, and record-keeping practices. The veterinarian should collect all relevant and available information on the diseased animals. Sources of this information can include identifying brands, tags (plastic and metal), or tattoos or owner’s name. Types of information can include

- age, sex, breed, and color of animals;
- use of animals;
- origin of animals (i.e. purchased or born on farm);
- an animal’s feed and water source;
- the nature of the animal’s housing;
- stage of reproductive cycle or gestation;
- lactation status;
- information about parity;
- relevant clinical, pathological, or laboratory reports and
- processing, vaccination, and treatment histories.

A complete and detailed individual animal /flock line listing or inventory is very useful, but often is not readily available.

Where herds/flocks are managed in distinct groups, much of this information can be collected and summarized at the group level. Often the herd/flock owner can describe the number of animals/birds in a pen or pasture or scavenging group and the number affected, while a record of which animals/birds were affected might not be available. In most situations, if individual animal /flock data are available, they should be retained throughout the entire investigation process. This allows the correlation of individual animal /flock laboratory data to individual animal /flock outcomes. If this is not possible, then the collection of data at the next smallest group level is the most appropriate way of proceeding. Simple spreadsheets or databases can be used to enter and sort herd/flock data as they are collected. Other information, including laboratory results, can be added later. Information should be examined for unaffected as well as affected animals/birds and groups within the herd/flock. In some cases, information on neighboring herds/flocks can also be useful. Information can be collected by a variety of methods, but personal interviews as part of a herd/flock visit are best.

5.5. Determine when the problem occurred

The temporal pattern of disease can provide important clues about the origin of disease in the herd/flock.

- Is this a new or a long-standing problem in the herd/flock?
- Are the numbers of new cases increasing, have they stabilized, or have they peaked and started to decrease?

The pattern of disease can be described in **an epidemic curve** by plotting the time of onset of each case (X-axis) in appropriate intervals against the number of cases recognized at each time interval (Y-axis). The distribution of cases in a dairy herd abortion storm is provided as an example (Fig. 1). The most appropriate time interval varies with intensity of the outbreak and may be hours, days, weeks, or even months. For example, following the exposure to a potent feed or water toxin, tracking the pattern of disease over a period of hours and days might provide clues to the source of the problem. Important events and management changes should be indicated on the epidemic curve to help the veterinarian visualize the sequence of events.

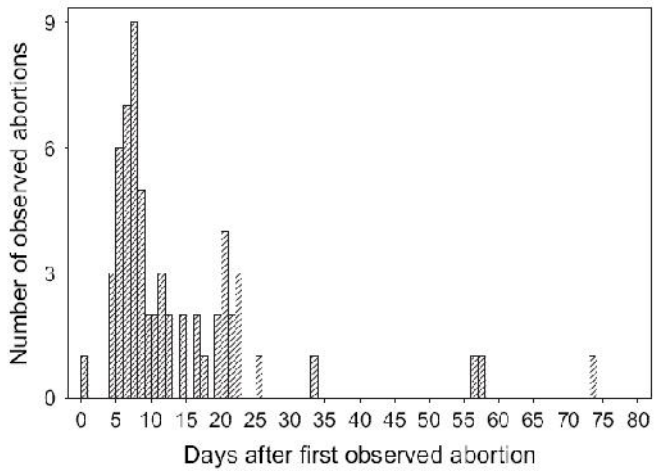

Fig. 1. Epidemic curve demonstrating abortion outbreaks in dairy herds

The epidemic curve can be used to infer whether the problem is endemic, epidemic or sporadic in the herd/flock.

- An endemic disease occurs at a consistent level in the herd/flock with only minor fluctuations over time. For example, sub-clinical mastitis is endemic in most dairy herds. The percentage of animals affected may be very low or include most of the herd.
- Epidemic is a relative term used to describe disease within a given time interval that is clearly above its expected rate of occurrence. For example, respiratory disease in dairy cattle is often epidemic shortly after weaning.
- A disease is sporadic if it occurs rarely and follows no regular pattern. An example of sporadic disease might be the death of apparently healthy animals due to anthrax.

The distribution of cases can suggest whether the problem resulted from exposure to a single-point source or is caused by an infectious agent moving from one animal to the next. For example,

- an epidemic curve showing a dramatic upward slope indicates many new cases in a short span of time. Such a curve would be expected when the causative agent is a point source. A dramatic increase of cases followed by a rapid decline might be expected following exposure to a toxicant on pasture followed by removal of the cattle at the start of clinical signs.
- A gradual upward slope followed by a steep down slope with one or more additional peaks can suggest a propagated agent. For example, in an outbreak of calf scours in cow-calf herd, data might show a gradual accumulation of cases to a peak, followed by a downward slope, with additional peaks occurring as the epidemic moves through different management groups. The shape of the curve reflects three factors.

- One is the incubation pattern, which starts from infection and continues to the time the animal becomes contagious.
- The second is the timing of transmission.
- The third is the opportunity of transmission.

The location and identification of the first case (i.e. index case) can be a clue to the source of infection in the group. As an example, several cases of salmonellosis due to *Salmonella typhimurium* may be identified in dairy cows after visiting a local veterinary clinic. Using clinic records, the index case was identified as a calf that had died in the clinic several weeks earlier. Identification of index cases can be difficult, particularly when the index case is sub-clinical, such as in the example of a non-symptomatic animal shedding Salmonella, bovine viral diarrhea virus, etc. However, when a notifiable disease is diagnosed, an intensive "trace back" is initiated by the investigating team to determine the origin of the first sick animal and potential contacts with other herds/flocks. Determining how much time passes from when the animals/birds are first observed sick until they are found dead can also be useful. Where treatment records are limited, however, only the date of death might be available.

5.6. Describe where the problem occurred

Differences in places where affected animals/birds were housed and pastured or freely scavenging in relation to unaffected animals/birds can provide additional clues about origin of the outbreak or productivity shortfall. A detailed sketch or map of the farm or locality and surrounding area can be used to record the location of all management groups. For example, dairy heifers can be separated into different facilities based on age and reproductive status. Important risk factors can be identified by investigating the timing of group movement between different housing facilities or pastures and the movement of animals between different management groups. The location of management groups in relation to neighboring herds/flocks and the potential frequency of contact with neighboring herds/flocks should also be examined. Potential contact information can usually be gathered during owner or producer interviews. The role of contact with other herds/flocks may be especially important if the owner or producer uses communal grazing/scavenging field or if there may have been unusual contact with another herd/flock. An unusual contact would be, for example, when a neighbor's bull enters a pasture.

A point map of the farm or locality should include a sketch of housing facilities, corrals, pastures (including details of barriers), and free scavenging areas, feeding facilities, and watering sources. If possible, the location of neighboring herds/flocks could also be identified. The location of each management group at the time of the outbreak and the location of individual cases can be overlaid on the map. This map can be used for detecting spatial patterns of disease and

developing hypotheses about the location of exposure. A series of maps showing animal/bird movements and patterns of disease over time can also be useful.

5.7. Generate hypotheses about key determinants by comparing groups within the herd/flock

The analysis begins with organizing and summarizing the data for the entire herd/flock. A simple spreadsheet is often needed to list livestock identifications and other information such as breed, age, laboratory results, pen/house conditions, and other risk factors. The next step is to quantitatively describe the breed and age structure of the population, the physical examination, and laboratory results. Then the task is to characterize the problem using descriptive statistics. Groups are identified within the herd/flock based on whether or not the animal/bird represents a case. Animals/birds are further sorted by characteristics, locations, and times when they were found sick.

These comparisons form the foundation of information required to determine why the outbreak occurred. Sometimes these comparisons identify one or more key determinants for the disease outbreak. This process may also raise specific questions that can be addressed by obtaining additional information from the herd/flock owner or laboratory testing.

5.8. Develop recommendations, provide a written report, and follow up to determine the effectiveness of the intervention

As more information becomes available over the course of an investigation, recommendations for control can be supplemented and modified to reduce losses. The action list evolves as the working diagnosis changes in response to new information. The recommendations for control of the herd/flock problem are developed by addressing the key determinants identified during the investigation. Possible interventions vary depending on the severity of the problem, its potential consequences, and the risks to other producers and public health. Actions available for consideration include

- changes to the environment,
- client education,
- quarantine of affected and suspect animals,
- mass vaccination and treatment

For example, changes to the environment are often the most effective method of treating an outbreak of calf scours, while mass treatment or metaphylaxis is the most effective response to shipping-fever pneumonia in weaned calves.

Preliminary reports summarizing initial findings should be issued shortly after the first herd visit. A final report can be prepared when all laboratory analyses are completed. The report should be well organized, concise, and explain the findings and recommendations of the study without excessive scientific or industry-specific terminology. The

recommendations should be presented with sufficient detail to avoid confusion. Both short- and long-term recommendations may be necessary. The potential costs and benefits should be explained to the herd/flock owner.

Many investigations are done retrospectively with insufficient information to reach definitive conclusions. To address outstanding questions, these cases require protracted herd/flock health and productivity monitoring and, occasionally, planned follow-up studies. In outbreaks that were quickly contained through control measures, follow-up field studies may be required to determine if the control measures were effective. In some cases, pharmaceutical companies may be of assistance if a specific vaccine or antibiotic is involved in the outbreak. Field veterinarians and, occasionally, veterinary school investigation teams are also a valuable resource in situations where the outbreak is extremely unusual, or when follow-up field studies may be required.

6. Overview of the process

The basic process of defining and orienting the problem and analyzing data by animal/flock, time, and space is common to all outbreak investigations. The steps in this process often overlap and parts of the process may have to be repeated to resolve the problem. The veterinarian must be able to adapt these strategies to a wide variety of outbreak situations. The practice of outbreak investigation requires preparation, organization, and communication skills from the initial contact with the herd/flock owner through implementation of the recommendations in the final report. Some suggestions for a structured and consistent approach to field investigations are presented below.

6.1. Initial contact and preparation for the herd/flock visit

Herd/flock problems can become apparent from a question raised or from an observation while on a visit for a routine procedure. Many herd/flock problems may be initially presented as a complaint about a specific vaccine or treatment recommendation. Problems affecting herd/flock productivity should not be casually diagnosed by phone. Although many herd/flock owners can describe the problem very well, incomplete or misleading second-hand information could result in inappropriate recommendations. The veterinarian should encourage the herd/flock owner to describe the problem as completely as possible. Where the problem appears to warrant immediate further investigation, a herd/flock visit should be scheduled. More subtle changes in productivity may be pursued as a part of the next routine herd/flock health visit.

Communication and preparation before the herd/flock visit can save time on the farm or locality. Background information for all cases must include complete name, address of the herd/flock owner, and contact information. Have the herd/flock owner collect copies of any previous examination or laboratory-testing reports related to the herd/flock.

Determine whether or not any other veterinarians are involved in the case. If so, they should be contacted.

Encourage the client to gather all potentially relevant herd/flock records before the planned visit. These may include herd/flock record books, sales receipts, calendars, invoices for purchased feed, and other data, depending on the type of operation. Time can be saved by having the client fill out a short preliminary questionnaire covering basic herd/flock background information. Useful information would include

- a summary of the herd/flock inventory and any recent changes to inventory,
- breed and age distribution of the herd/flock;
- an outline of management groupings; and
- an overview of feeding and vaccination practices.

The number of animals/birds to be examined and the number and type of laboratory samples to be collected can be estimated before the herd/flock visit if herd/flock inventory numbers are available.

Whenever possible, arrange to examine the herd/flock in its original location before the herd/flock is disturbed and confined for detailed examination. If the visit is scheduled during feeding, the veterinarian can make first-hand observations of both animal/bird behavior and herd/flock management with minimal disturbance to the herd/flock. Close examination of the herd/flock, when possible, could be scheduled during some routine management procedure (e.g. pregnancy testing, vaccination, or branding). More than one herd/flock visit might be necessary to make both the required herd/flock and individual-level observations. In addition to visiting the herd/flock owner, the veterinarian should arrange to visit other family members and farm workers who can provide critical information on the history of the problem and herd/flock management practices. In some cases, these individuals may be more knowledgeable about details of animal/bird management than the actual owner.

A crude working-case definition can be developed from the information provided during the initial contact. The next step, particularly for unusual or complex problems, is to briefly review the current literature on known risk factors. Online access to electronic journals is making access to current information in remote areas easier. Several authors have suggested using a diagram to summarize relationships between known risk factors. This diagram is sometimes called **a path model**. The purpose of this diagram is to assist in planning for the herd/flock examination and to facilitate discussions with the client.

Taking the time to review the potential risk factors before the herd/flock visit decreases the chance that some area of herd/flock management or the collection of important samples is overlooked. In addition to listing risk factors for the disease, published path models depict the interrelationship

among risk factors and their effects. A diagram showing the potential relationships among risk factors can help communicate to the client that herd/flock problems usually have more than one cause, that infectious agents are usually only part of the problem, and that many areas of management should be examined (multi-factorial).

Access to extensively managed animals for repeated sampling is often an important limiting factor in outbreak investigations. An animal health technician can help organize sample collection and ensure proper labeling and packaging for transport. Contact the laboratory before the visit to verify the manner of sample collection, quantity of sample, type of container to be used, and packaging and transportation recommendations.

6.2. The herd/flock visit

During the herd/flock visit, the investigator

- collects additional history,
- examines individual animals/birds from a herd/flock,
- conducts necropsies,
- observes herd/flock management,
- examines the environment,
- obtains samples for laboratory examination, and
- examines the herd/flock records.

If no clinical cases or mortalities are available, the veterinarian may want to make arrangements to have those cases submitted or to be notified when the cases occur. The necessary steps and order of completion vary between problems and often overlap. The objective of data collection during the herd visit is first to determine how the affected or diseased animals/birds differ from those unaffected and then find which of the factors that differ among these groups can be altered by intervention to decrease the risk of disease. The unique characteristics of the nutrition, management, and the environment related to the affected animals/birds become a focus of the examination.

Detailed written notes of all observations are important for constructing a meaningful and accurate report for the herd/flock owner. Few things destroy credibility faster than making a mistake in describing the operation. Digital pictures are inexpensive and useful memory aids as well as great tools for client education and presentations to producer groups. Photos can be particularly useful for recording subjective observations, including body conditions and farm hygiene. Changes over time can be monitored by comparing representative pictures from different dates. When trying to show the size of an object, include a standard reference in the picture, such as a meter stick or a person of a known height. If specific animal/bird identification is important, include the ear tag and brand as applicable in the photo. Alternatively, include a small sign or card in the picture with the necessary

information. Video cameras can be used to record behavioral or postural abnormalities and unusual sounds.

All records should be in ink, dated, and signed. Photos and video records can also provide critical information for the visit report. Photos and video should be date- and time stamped. Record where and when the pictures were taken and include detailed voice narration on the video describing what was seen.

6.3. History

Have the herd/flock owner restate the complaint and encourage him or her to clarify the time sequence of events. Questionnaires prepared for the problem under investigation can help ensure that nothing is overlooked during the interview. Such questionnaires are especially valuable for those inexperienced in this field. Several examples of lists of questions are available for addressing specific herd/flock problems. However, while strict adherence to standardized questionnaires is essential, this same strict adherence to a prepared questionnaire can hinder the outbreak investigation process. Insistence on following a preformatted series of questions often interferes with the chronological order of the story and no standard questionnaire can fit every situation. Rather than a formal questionnaire, a simple list of required information can be prepared and tailored to the particular problem. This list is used as a *"check list"* to fill in missing areas not covered in the initial disclosure of information after the herd/flock owner has told his or her story.

Good listening skills and careful questioning can increase the accuracy and reliability of the information collected. Carefully worded open-ended questions allow the possibility of answers not previously considered by the veterinarian. Unnecessary professional jargon and scientific terminology should be avoided. Each question should ask for one piece of information at a time, and time references in all questions should be unambiguous. Unnecessary interruptions or *jumping to conclusions* can disrupt the chronological presentation or lead the herd/flock owner to tell the story that supports the veterinarian's assumptions about the cause of the problem.

Emotionally loaded or leading questions can also suggest one answer is better than another and bias the results of the interview. The herd/flock owner can be tempted to give the perceived *"correct"* or more *"socially acceptable"* answer and not describe what actually happened. When doubts arise about the completeness of the information provided, alternatives for getting the information should be considered. For example, clues to actual treatment patterns may be discovered through inquiries into the total volume of antibiotic purchased in the previous few months and by investigating remaining inventory. By asking the herd/flock owner to refer to relevant records and verify dates and numbers, the veterinarian improves the accuracy of information collected during the visit. Where records are not available, a binder filled with examples of

labels or parts of the box from common drugs or vaccines can be used when herd/flock owners have difficulty remembering brand names of relevant drugs, vaccines, electrolytes, milk replacers, or mineral mixes.

In addition to basic information about the herd/flock owner, history, and current status of the operation, the veterinarian should tactfully ask about other employment and off-farm sources of income. A part-time job as a stock /flock person in another herd/flock or at a market, for example, could present important bio-security problems. When there is the potential for zoonotic disease or simultaneous exposures to toxins, the veterinarian must also tactfully question the herd/flock owner, employees, and family members about concurrent illnesses. While most of the interview should be focused on collecting the owner's observations, the veterinarian should also ask the herd/flock owner for his or her opinion about what is causing the problem.

6.4. Clinical examination of individual animals/birds from the herd/flock

General distant observations should be made of the group and individual animals/birds before they are confined and restrained for detailed exams. The distant visual examination of the group could include an assessment of a variety of conditions and characteristics (Table 1).

In outbreak investigations, rarely is it possible to confine and individually examine every animal/bird in the herd/flock. The visual check of different groups in the herd/flock when they are minimally confined or during feeding or grazing is a valuable tool that should not be overlooked. When animals cannot be confined, the body condition can be scored visually from a short distance. Visual appraisal is comparable to palpation, unless the animals have long hair. Information on diet composition and recent changes or accidental ingestions can also be verified during an examination in the home pen using observations of the amount, consistency, and appearance of fecal material. For example, fecal consistency provides information on the amount of dry hay relative to silage, green grass, or concentrate. Fecal content can reflect recent grain overload or exposure to environmental toxins, such as spilled oil.

Each animal can then be confined and examined individually during the initial visit, if warranted by the severity of the problem, or later, when the examination can be combined with other routine processing activities.

Box 1. Possible factors to assess during distant visual examination of group

- Behavior, mental status, temperament, and exercise tolerance
- Degree of crowding, dominance and aggression problems within the group, and the degree of access and availability of bedding, food, and water
- Respiration rate and character, coughing, and unusual noises or discharges
- Appetite, mastication, and swallowing
- Abdominal shape or contour (e.g. bloated, gaunt)
- Defecation characteristics (e.g. frequency, consistency, straining) and urination patterns
- Reproductive status, signs of estrus or mounting activity, discharges from the reproductive tract, maternal behavior, udder conformation, and lactation status
- Evidence of libido or successful mounting activity for breeding males
- Posture, gait, lameness and progression of movement
- Body weight and condition score
- Relative frame size and conformation
- Health and cleanliness of the skin and hair coat

The procedure for detailed examination of individual animals/birds has been well documented and will not be reviewed here.

Another way to approach an examination is to confine and examine subsets of severely affected, moderate or mildly affected, and not apparently affected animals/birds from the herd/flock. While no specific guidelines are available for how many animals/birds should be examined, some investigators have suggested a minimum of four severely affected, four mild cases, and four normal animals. Others recommend examining the first few animals/birds that became sick as well as any recent additions to the herd/flock. Identifying and examining early cases that have not yet been treated might also be a valuable diagnostic tool. To minimize the potential for transmitting disease within the herd/flock during the examination, the veterinarian should begin where possible by examining the *"normal"* animals/birds.

The reproductive status and body condition score can also be individually recorded during the detailed examination. For any investigation potentially examining reproductive performance, all animals should be palpated for pregnancy status or evidence of estrus activity and herd bulls should be tested for breeding soundness. This individual animal listing and record of reproductive status are particularly important if the problem requires monitoring over time or there is a potential for ongoing losses.

6.5. Necropsy examination of dead or sacrificed animals/birds

Detailed instructions for postmortem examination are not reviewed here as these are available from most diagnostic laboratories and other sources. However, some additional factors should be considered when examining losses associated with outbreak investigations. The results from both gross and histological examination can be critical to refining the case definition where clinical signs are vague and nonspecific. All available cadavers should be examined whenever possible. One or two cases from a major disease outbreak might not be representative of the underlying problem. For example, an outbreak of a severe scours (>70% herd mortality) in calves greater than 4 weeks of age, the first two calves examined may be found to have died from causes unrelated to the majority of herd losses. One death might be caused by pneumonia. The other may be the result of an abscessed umbilical vein. In this example, histopathology on a series of additional calves at different stages of the disease might be necessary to define the type of intestinal lesion.

Where the value of individual animals is low, the herd owner might also be willing to sacrifice a small number of affected animals for a complete necropsy examination. If the value of individual animals is high, the herd owner might consent to the euthanasia of one or two chronically diseased animals where the prognosis for recovery is poor. The animals/birds should be submitted for processing at a diagnostic laboratory where possible. The postmortem examination and sample collection can be completed on the carcass within minutes of death. The submission of all samples from one herd/flock to the same laboratory and a single pathologist improves communication, maintains consistency of interpretation, facilitates comparisons between individual cases and identification of trends during the outbreak, and, finally, provides the information to make a herd/flock diagnosis based on quantitative pathology. The veterinarian can then determine to what extent the various pathological lesions observed affect overall mortality and mortality within each management group. By maintaining a consistent protocol for sample collection and a log of all samples collected, the veterinarian minimizes the chance of missing important information. Tissues from all important systems should be submitted for histopathology. Reliance on the gross evidence of abnormalities as a basis for sampling can result in a much lower rate of diagnostic success. Call the laboratory in advance for instructions about any non-routine samples to verify

- the amount of appropriate sample containers (e.g. glass or plastic),
- storage conditions (e.g. frozen, on ice, or at room temperature),
- preservation, and
- shipping.

Special care improves chances for the survival of the suspected pathogen and reduces the likelihood of overgrowth of contaminants. Samples for toxicological analysis can be contaminated during collection or degrade very rapidly if the wrong container or preservative is used. Small submission errors can result in misleading and inaccurate laboratory results. Laboratory analysis is expensive and so-called "fishing expeditions" where samples are submitted for a number of tests in the hope something might come up positive, seldom provide useful information. However, because the veterinarian has only one opportunity to collect samples from a carcass, it is much better to collect tissue that might not be used than to miss important information. The initial laboratory costs and the potential for important information loss can be minimized by properly storing tissue samples. If the factor to be analyzed is stable for a known period and if the cost of collection and storage are not prohibitive, then the veterinarian may find it appropriate to bank tissue samples for future analysis. Small pieces of formalized tissue for histology, for example, do not add much to the cost of the investigation and require little, if any, additional storage space. Frozen tissue samples can be analyzed later for some types of toxins or trace mineral content. Where herd/flock losses are substantial, the veterinarian should consider asking the owner to transport at least some carcasses to a diagnostic laboratory. Examinations can be conducted by a pathologist under the more controlled conditions of the laboratory. Where this method is not practical or possible, the examinations must be completed in the field using the best possible practices. Use a camera to record gross pathological abnormalities. The identity of the subject animal/birds, the date, a size reference scale marker, and the identity of the pathologist should be included in each photograph. Table 2 contains common errors made in postmortem. Occasionally, tissues from *"normal"* animals within the herd/flock may be required as controls or for determinations of trace mineral, heavy metal status, or parasite load. Tissue from animals/birds sent to slaughter for the commercial market or for in-home use can be a potential source of reference material.

Box 2. Common errors in postmortem examinations

- Incomplete examination (e.g. failure to examine the brain)
- Inadequate documentation (e.g. no written report or photographs)
- Too much delay between the examination and preparation of the report
- Failure to collect samples for supplementary analysis (e.g. microbiology, histology, or toxicology)
- Improper collection of samples (e.g. inappropriate samples, unsuitable containers, insufficient preservation, inadequate labeling)
- Accidental damage to specimens, including contamination with spilled gastrointestinal content, or improper or rough handling of fragile tissues
- Confusion of artifacts, including autolysis, or histological damage from freezing with important pathological lesions
- Failure to consult with other experts
- Bias resulting from too much emphasis placed on the case history

6.6. Observation of herd/flock management

Most disease outbreaks in herds/flocks are the result of some limitation or deficiency in management. Very few of the disease problems important in food animal/bird practice require only the infectious agent to cause substantial herd/flock losses. Almost all outbreaks or suboptimal productivity problems result from a number of factors, including factors related to management. In some cases, the disease outbreak may provide a unique teachable moment for the veterinarian to emphasize the importance of making management changes that may have been suggested in the past but ignored. The objective of the investigation is to identify all factors that can be changed to resolve the current herd/flock problem or minimize the potential for future losses.

A management history obtained with simple questions having yes or no answers provides limited and superficial information on herd/flock management practices. For example, many management questionnaires ask whether or not animals/birds are vaccinated for disease causing organisms. This question seems straightforward. However, if the producer answered yes, the veterinarian still has no information on what type of vaccine was used, when it was administered, and if label directions were properly followed.

If management has changed its practices, information about those changes can be important in determining the cause of the outbreak. However, changes that preceded the outbreak need to be carefully differentiated from changes that resulted from the outbreak. The most useful information on herd/flock management comes from seeing how things are done, which can

require several visits and extra time. The best management assessments are often collected as part of an ongoing process where the investigating veterinarian works with the livestock owner on a regular herd/flock-health program.

6.7. Examination of the environment

Climate, housing, population density, and air quality are all important determinants of disease risk and herd/flock productivity. Historical weather data can usually be obtained from local meteorological stations to confirm observations of herd/flock owners. Weather events should not be taken lightly. For example, rain in combination with poor drainage can dramatically reduce functional pen size. A field that should be of adequate size for a given number of animals can be overcrowded and highly contaminated during certain times of the year because of water accumulation. Animal density should be calculated based on the usable space at the critical point in time and not total space in the pen. Herd visits should be made as soon as possible, as problematic environmental conditions are often transitory. Ground conditions (e.g. degree of wetness, drainage, type of cover) are also important to livestock health and production. The type and amount of bedding are important to the ground conditions, but bedding management can also affect dust levels in the pen. Air quality is particularly important in dry lots and feedlots where dust levels can potentially affect the occurrence of respiratory disease. The location and type of shelter within the outdoor pen are important. Other potential environmental risks include insect and predator problems on the pasture and the location of carcass disposal sites, garbage dumps, fuel and chemical storage areas, and nearby industrial facilities. Good ventilation, temperature and humidity control is critical to maintaining health and productivity in housed livestock of all species. The objective evaluation of indoor ventilation may require the assistance of an expert in this area. A number of parameters are typically measured, depending in part on the type of animal being housed. Such parameters include

- minimum and maximum temperature,
- the number of air changes per hour,
- humidity and condensation,
- the location and size of air inlets and fans, and
- the presence of drafts.

Specialized monitoring equipment can be used to measure levels of a number of suspected problem gases, including ammonia, hydrogen sulfide, and carbon monoxide. Other factors to be evaluated for indoor environments include

- the sanitation and hygiene,
- the type and condition of the flooring,
- the barn-cleaning and waste-disposal systems,
- stall or crate design and dimensions,
- bedding type and adequacy,

- the ease of movement for both animals and attendants within the unit, and
- the adequacy of the lighting.

Water quality and availability are important determinants of livestock health and feed intake. Determine the ratio of animals/birds to waterers in each pen. Observe animals/birds using the water source and record any evidence of crowding and dominance problems, stray voltage, or access and footing (e.g. mud, manure/dropping buildup, etc). Verify that all automatic waterers are working properly. Is dugout, pothole, or stream water pumped to a trough or do the animals/birds actually enter the water to drink? Is the water clean and free of offensive odors, or are there substantial amounts of mud and manure/droppings suspended in the water because of animal/bird movements? Is algae growth an obvious problem?

6.8. Collecting samples

Laboratory examination of strategically submitted samples can help rule out or confirm the diagnosis, monitor exposure to potentially important risk factors, determine the necessity for and appropriate level of intervention, and, finally, assess the success of control measures. The only laboratory analyses that should be considered in most investigations are those where the results are likely to directly affect management decisions. Appropriate samples might include

- blood,
- milk,
- feces,
- urine,
- nasal and ocular swabs,
- biopsies or tissue samples from slaughter animals or cadavers,
- other fluid samples,
- feed,
- water, and
- soil samples.

A number of factors determine the success of the laboratory sampling strategy. The appropriate samples must be selected for the question being asked and the number of animals/birds sampled must be sufficient to answer the question with a reasonable degree of certainty. The appropriate technique and collection equipment must be used and the samples must be stored properly and delivered to the laboratory before sample quality is compromised. Laboratories may change their recommendations over time on recommended transport media (e.g. those for *Campylobacter fetus* or *Tritrichomonas fetus* cultures) or preferred sample form (e.g. serum or whole blood for selenium).

Finally, the laboratory test chosen must have acceptable measurement error, accuracy, and precision standards and be

able to discriminate adequately between *"normal"* and affected individuals. The laboratory should provide the sensitivity and specificity of the test and define how the value for the normal cutoff has been established.

6.9. Sample-size determination

Cost considerations often prohibit laboratory testing of every animal/bird in the herd/flock. The most common approach to testing is to submit samples for comparison of laboratory data between cases and a sample of control (i.e. unaffected) animals within the herd/flock. All available cases are often needed, depending on herd/flock size and the severity of the problem. The veterinarian must select an appropriate comparison or control group. Samples can also be submitted to compare results between animals/birds with acute and chronic disease or among animals/birds from different age cohorts, management groups, or history of exposure to some other risk factor of interest.

Probably the most common question and often the most difficult to address is "How many samples are necessary?" Often, complete herd/flock sampling and testing is too costly. There is no single correct number of samples suitable for every situation. The required number of animals/birds for sampling is based on

- the acceptable degree of uncertainty in the final estimate;
- the expected prevalence of the factor of interest, or, for continuous measurements, the variation in the factor within the population, and
- the size of the population examined.

The number of samples required also depends on the question being asked. In most cases, the veterinarian must take what is available. If few cases are available, there are some statistical advantages to having more controls than cases up to a ratio of about 4:1. Computer programs, such as Win Episcope 2.0, can be used to calculate the required sample size.

Without a sufficient number of samples across groups, the veterinarian often misses differences that are important clues to the underlying cause of the outbreak. As with postmortem examinations, the clinician often has only one opportunity to collect laboratory samples from live animals/birds during the critical time frame. Because of costs, the clinician cannot possibly direct the laboratory to analyze the samples for every potential question that could arise during the investigation. Serum banking should be considered.

6.10. Feed and water samples

The role of feeding management is central to many investigations. Expensive laboratory analysis alone cannot prove that the nutrition program is adequate for the type of

animal and environment. While chemical analysis of the feedstuffs is important, this provides only the laboratory report on the components used to formulate the feed. The report does not address what is actually ingested by the average animal and how much variation there is among animals.

Before collecting any feed samples, have the herd/flock owner prepare an inventory of all forages, grains, and purchased supplements. Purchased feeds should be listed by

- date of purchase,
- source,
- type and composition, and amount.
- Feed tags, which include analysis and instructions for use, or the supplier contact numbers should be collected for all commercially prepared feeds or supplements.

Is there any feed left from the time just before the herd/flock problem was noticed?

Good sample-collection technique is important for a meaningful chemical analysis. The samples submitted for analysis must be representative of what is being fed. Use a good quality core sampler for hay, and an auger or probe for grain and concentrates. Silage and haylage are often the most difficult to representatively sample at a single point in time. Collect multiple grab samples from different parts of the open pit or from different times while the conveyor belt is running. There might be substantial differences in quality in different parts of the pit or silo. Sampling of the final or total mixed ration, as compared with the “paper ration”, provides a check on the adequacy of the mixing process and the actual composition.

Collect, pool, and mix thoroughly five to ten samples for grain or concentrate and 20 individual samples for hay or silage from each lot of feed before submission to the laboratory. Silage samples should be placed in airtight plastic bags, stored on ice, and shipped to the laboratory as soon as possible. The laboratory should be asked to dry and grind the entire sample before analysis. Laboratories may process a sub-sample of what was submitted, unless the instructions specify otherwise.

The visual appearance and physical characteristics of the feed observed during sample collection should be recorded. Odor, color, stage of maturity, presence of foreign material (e.g. garbage, weeds) and physical form provide information necessary for proper interpretation of the feed analysis. Note the relative stem-to-leaf ratio or the grain-to-forage ratio in the stored feedstuff. Chop length and moisture content should be measured in the field where possible for silage and total mixed rations. Silage pH can also be measured in the field.

Pasture sampling is done relatively infrequently, but may be occasionally necessary in the investigation of metabolic

disease or trace-mineral deficiencies. The samples for chemical analysis should not be pulled from the ground but be cut off near the roots with scissors to minimize soil contamination. Toxic plants can be photographed and then collected and dried for identification by an expert in that area.

Before submitting the samples, determine what chemical-analysis packages are available from the laboratory. Because feed analysis can be very expensive, all requested analysis should provide necessary data and the practitioner should understand the limitations of all the tests requested. Commercial feed laboratories also vary greatly in their internal quality-control procedures and the types of analytical, estimation, and calculation procedures used.

Obtain bottles for water sampling directly from the laboratory where possible. Improperly cleaned containers can contaminate the sample. This precaution is particularly important for trace-mineral or organic analysis. Proper sampling technique, special preservatives, and rapid transportation are required for samples being tested for potentially volatile components. Practitioners should call the laboratory in advance if they are planning on requesting tests outside the routine potability parameters.

Water sampling for livestock use should include an assessment of total dissolved solids, sulfates, nitrites, and nitrates. Total dissolved solids in excess of 5000 ppm can reduce water intake and result in diarrhea in all classes of livestock. High sulfate levels (>1,000 ppm) can interfere with copper status and increase the risk of polio-encephalomalacia.

6.11. Examining the herd records

The quality and completeness of records vary greatly between operations. Some herds/flocks have complete records on computer for each animal/flock and others have little or no record of animal/bird health and productivity. Understanding the most commonly used on-farm computerized record systems saves time and can increase the amount of retrievable information.

Too often herd/flock records are scant or nonexistent. Persistence and ingenuity can, however, turn up useful information in unexpected places. Ask the herd/flock owner for any livestock-associated records, including records related to veterinary services, supplies, or drugs; old calendars, pocket diaries; feed and supplement bills; financial records related to animals/birds bought and sold; and bulk tank receipts showing somatic cell count and bacterial numbers.

Individual animal treatment records are often difficult or impossible to obtain in the face of a disease outbreak. During a crisis, however, herd owners might be more likely to record counts of animals treated each day or week than individual animal information. If record compliance is poor, it may be useful to have the herd owner directly identify animals

treated with a livestock marker. A different marker color can be used each day. If this method is used, a walk through the herd can provide a quick impression of how many animals have been treated and how long they have been treated.

Definitions of production and health measurements must be clear and used consistently throughout the investigation. Be cautious when comparing findings from other investigations, as there are substantial problems with standardization of terminology and methods of calculation. For example, stillbirth has been defined both as calves dead within the first hour after birth and calves dead within 24 hours of birth.

6.12. Attack rate tables

The attack rate is the proportion of the group that is affected during a given period. The attack rate can compare those that are and are not exposed with each individual potential risk factor. To easily visualize this comparison across many different risk factors, an attack rate table is used. The table helps identify the exposures most likely to be associated with disease. Alternatively, a table can be constructed to compare the risk of exposure to each potentially important risk factor between case and control animals. Table 3 shows an attack rate table comparing various risk factors across exposure status examined during an investigation. The application of the appropriate statistical test to this comparison allows the epidemiologist to avoid misinterpreting a chance difference between groups as a link to a cause. To apply statistical methods, the veterinarian must assess the probability that the results of the statistical test, or more extreme results, could have been obtained if there really were no association between the risk factor and disease. A chi-square analysis is an example of a simple, commonly used test for significance. This test compares the frequency of observed events to that expected based on chance alone.

Table 1. Attack rates and other parameter calculations

Risk Factor	# Exposed to factor				# Not exposed to factor			
	Total	Ill/dead	Well	Attack rate, AR1 (%)	Total	Ill/dead	Well	Attack rate, AR2 (%)
Age	a+b	a	b	a/(a+b)	a+b	a	b	a/(a+b)
Sex								
Other potential exposure factors								

Interpretation of the table should consider the following: (i) Highest & lowest attack rate; (ii) Greatest difference (attributable risk, AP) AP=AR1-AR2; (iii) Relative risk RR= AR1/AR2; (iv) Expected levels disease pattern; (v) Etiologic fraction EF = (AT1-AR2)/ AR1 (proportion of disease attributed to the risk factor)

The importance of different risk factors can be objectively compared by measuring the magnitude of the association between each exposure and the outcome of interest or disease. The two common indices used to measure the magnitude of effect of a

risk factor are the *relative risk ratio* and the *odds ratio*. The relative risk is the ratio of the risk (or cumulative incidence or attack rate) of disease in the animals/birds exposed to the factor of interest to the risk (or cumulative incidence or attack rate) of disease in those that were not exposed. If the relative risk is <1, then the exposure is associated with a decreased risk of disease. If the relative risk is equal to 1, then there is no association between exposure and disease status. If the relative risk is >1, then the exposure is associated with an increased risk of disease. The odds ratio can be used to measure the association between exposure and disease for any study type. However, the odds ratio is the method of choice for expressing the magnitude of effect in case-control comparisons where the history of exposure to a specific risk factor is compared between case and control animals/birds. The interpretation of the odds ratio is similar to that of relative risk. Statistical association alone does not prove the identified risk factor is a cause of the outbreak. For a risk factor to be considered a potential cause of disease or suboptimal productivity, the risk factor must always precede the outcome. Other potential supporting evidence for a causal association includes

- a relatively strong association between the risk factor and the outcome,
- a biologically reasonable link between risk factor and outcome,
- some suggestion of increasing effect with increasing exposure,
- evidence that removing or decreasing exposure decreases the risk of disease, and
- consistency of the association when examined in different studies.

6.13. Communicating investigation results and follow-up

The minimum necessary follow-up is a complete written report to the herd/flock owner as described earlier. The report should be direct and concise. To effectively communicate the findings of the investigation, the report must be read by the intended audience. The purpose of the investigation should be stated at the beginning of the report. All investigation results should be summarized using simple tables and graphs where possible. The action list to the herd/flock owner should set clear priorities and include specific details for any recommendations. The report should provide a plan for follow-up and future monitoring. The report should also clearly discuss any risks associated with the recommendations, describe any risks from the current problem to public health, and promote realistic expectations of the results following any interventions. This written report may have to be supplemented as the results become available of later laboratory tests or longer-term field studies to identify risk factors. The case definition may need to be revised and the results from the investigation herd/flock compared with other herds/flocks in the surrounding area. If the disease is contagious, consider what risk the herd/flock poses to other

livestock producers in the area and consult with the herd/flock owner on minimizing the potential for transmission beyond the herd/flock. If the exposure could potentially result in food residues, contact the appropriate authorities for direction and advise the herd/flock owner of his or her legal and ethical responsibilities. If the disease is potentially reportable, the appropriate regulatory officials should be advised of the results of the field investigation as soon as possible. If the losses are extremely severe, the herd/flock owner should be advised to consider his or her short- and long-term goals and to get advice from a financial councilor before deciding among potential alternatives for control. For herd/flock problems with significant economic implications, particularly where control or treatment is not well understood, the investigation should not end with the identification of important risk factors and the recommendations for control. Where the effectiveness of proposed control measures cannot be predicted with certainty, one infrequently used, but potentially invaluable, strategy for evaluating the effectiveness of control measures in the herd/flock is the randomized controlled trial.

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