Strategic Laboratory Use:

How to Use Hypothesis-based Laboratory Testing

Dale Hancock, *DVM, PhD*
Field Disease Investigation Unit
Washington State University
Pullman, WA 99164-6610

© Copyright American Association of Bovine Practitioners; open access distribution.

Semantically speaking, "diagnosis" used in reference to microbiological and pathological findings is not what we are after in herd disease investigations. Problem solving in livestock operations requires epidemiologic diagnoses in which key determinants (critical control points) are identified. Laboratory diagnosis can be an important adjunct to problem-solving efforts, but only if used strategically to test hypotheses about key determinants.

How do we react to a problem of excess disease in a herd? The answer seems elementary. We sample the sick and dead animals, and by defining the infectious agents and/or pathological processes, we determine what is causing the problem. Only by finding the cause can we hope to reach a solution. At least, this is the time honored dogma. An assumption of this approach is that the causes of excess disease in populations are mainly external and visited upon livestock operations through no fault of their own. Under this assumption, the veterinarian functions mainly as a courier, sending the samples off and waiting for the answer and the right elixir or vaccine to arrive via return parcel (Figure 1).

Consider four scenarios from the Field Disease Investigation Unit at Washington State University which are intended to create doubt about the delivery-person-vet mode of investigation and will set the stage for descriptions of an alternative strategy.

Scenario 1:

Five steers were brought in for necropsy from an 18,000 head feedlot. They were representative of approximately 200 cases which had occurred over the past couple of months. The steers first showed respiratory signs and after several days became diarrheic. In about half the cases the feces contained blood and fibrin. *Salmonella typhimurium* was found in various tissues of all 5 steers and lesions characteristic of enteropathic salmonellosis were observed. Lesions of resolving fibrinous bronchopneumonia were observed in 3 of the steers. A laboratory diagnosis was achieved. Was a solution at hand? No.

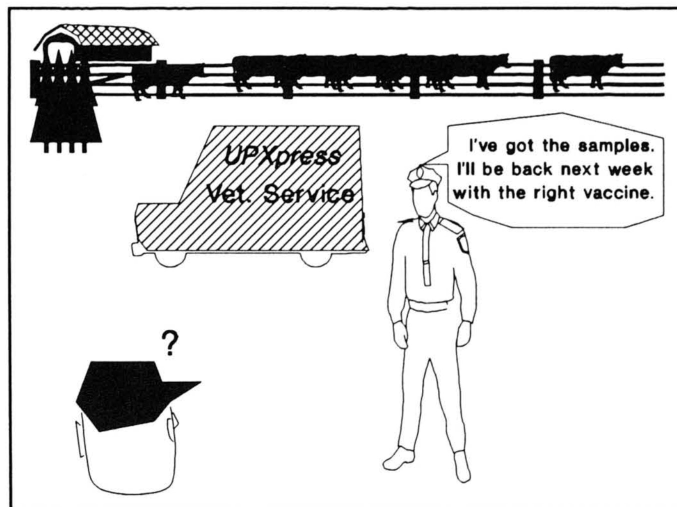

Figure 1. The opposite of strategic sampling is delivery-person-vet sampling in which all causes are assumed to be external and in which the veterinarian functions as a mere courier.

Scenario 2:

A dairy herd had a severe diarrhea problem in calves. Morbidity has approached 100% and mortality has been about 25%. Numerous fecal samples have been sent to the diagnostic laboratory and have revealed the presence of rotavirus, coronavirus, and cryptosporidia. At necropsy, there have been no particularly outstanding gross lesions — just emaciation and dehydration. Villous atrophy of small intestine has been a consistent finding. A laboratory diagnosis was achieved. Was a solution at hand? No.

Scenario 3:

A cow-calf operation had a high rate of weak calves, many of which died shortly after birth. The weather

had been quite cold. Two of the calves were necropsied. Lesions consistent with cold stress were observed. No infectious agents or lesions consistent with an infectious process were observed. A diagnosis was achieved. Was a solution at hand? No.

Scenario 4:

A severe outbreak of pneumonia occurred in a feedlot. Necropsy and histopath of 4 dead animals revealed lesions consistent with fibrinous bronchopneumonia. *Pasteurella hemolytica* was isolated from the lungs of all 4 dead calves and the nares of 7/12 clinical cases. Paired serology collected acutely and 2 weeks later revealed widespread seroconversion to IBR virus, PI3 virus, and BRS virus. A diagnosis has been achieved. Was a solution at hand? No.

In each of the above scenarios a laboratory diagnosis was achieved that was about as solid and defensible as one is likely to obtain. What is missing, if a solution is to be found, is knowledge of how the livestock operations involved differed from other, similar, livestock operations which did not have excess disease problems. Consider some unanswered questions:

Scenario 1:

Multitudes of animals enter a feedlot each month; a few are inevitably *Salmonella* infected. Also, among the thousands of truckloads of feeds that are received yearly, some are bound to be contaminated with *Salmonella*. These sources exist for all feedlots, but most do not have salmonellosis outbreaks. What is different here? Where and how is the infection spreading? What is the reservoir? Note that the sampling of the dead steers did not answer any of these fundamental questions.

Scenario 2:

The agents of calf diarrhea are present on every dairy farm (and cow/calf too). Infection with some diarrheal agents, notably the 3 mentioned above, is universal in dairy calves and generally inapparent or subclinical. What is it about this herd that is different? Note that the sampling of the dead calves did not answer this fundamental question.

Scenario 3:

Cold weather was present throughout the region yet most cow-calf operations did not experience the high rate of calf losses. What is peculiar about this operation? Note that the sampling of the dead calves did not answer this fundamental question.

Scenario 4:

Virulent strains of *Pasteurella hemolytica* are present in all groups of cattle. Infection with respiratory viruses is extremely common in recently received feedlot animals. This is true in groups of cattle that do not experience outbreaks of respiratory disease as well as those that do. What is different about the cattle experiencing the outbreak? What factors in this particular feedlot favor an excessive rate of respiratory disease? Note that the bacterial cultures, histopathology, and virology did not answer these fundamental questions.

The term "diagnosis" can be something of a stumbling block in solving herd disease problems. It is used in several different contexts (Table 1), only one of which is targeted directly at solving herd problems. The ultimate goal in epidemiologic diagnosis is the identification of risk factors over which we have control and which can be manipulated in an effort to control and prevent the disease. Such risk factors are called ***key determinants*** or, synonymously, ***critical control points***. For example, known key determinants for excess diarrheal morbidity/mortality in a dairy operation reside in the broad areas of housing, nutrition, and passive immunity. Identification of temporal and risk group patterns and detailed management evaluation are critical to the identification of key determinants.

Table 1. Different meanings of "diagnosis".

Diagnosis	Goal	Central question
Clinical	Define problem in individuals.	Descriptively, how does this individual differ from normal?
Laboratory	Define associated agents and pathology in individuals.	Homeostatically, how does this individual differ from normal?
Epidemiologic:		
Descriptive	Define problem in population.	Descriptively, how does this population differ from normal?
Ecologic	Identify key determinants (=critical control points).	Homeostatically, how does this population differ from normal?

Now test your skills with a *5th scenario:* Ten of your good beef cow/calf clients have reported excessive calf losses this year; according to the livestock agent, other herds are having problems as well. Outline a problem-solving strategy then read the proposed strategy in Table 2.

Two aspects of Scenario 5 and the proposed strategy in Table 2 merit consideration. First, naming the problem won't solve it. The only purpose of the clinical exams and—in the problem definition stage—the lab submissions, was to help provide a case definition for individuals. In conjunction with a quantitative boundary (e.g., percent of calves within a herd or group which have been affected), we use our case definition to identify which herds or other groups are affected and which are not.

Secondly, the two most important conceptual ques-

© Copyright American Association of Bovine Practitioners; open access distribution.

Table 2. Proposed outline for investigating problem in Scenario 5.

I. Define. . .
  A. . .the syndrome by age, clinical, path., lab findings. (what is a case?)
  B. . .the boundary for "excessive calf losses" (what is a case herd?)

II. Determine what changed in case herds: e.g., forage program, calving area.

III. Prepare temporal chart of cases. Compare to weather and management changes.

IV. Perform risk group analysis.
  A. Among individuals within case herds, e.g., age breed of dam, dystocia history.
  B. Among affected and unaffected herds, e.g., Forage type (variety, protein conc., etc.), calving area variables, dystocia assistance policies, mineral supplement variables, vaccination variables, many others possible.

V. Prepare a map to identify clustering or predilection toward terrain type.

VI. Hypothesize key determinants; test using strategic lab sampling, prospective data collection, and/or intervention trials.

Grade yourself: Each separate item (e.g., II, IVA, V) is worth 10 points.

tions of this or any other herd disease investigation are: (1) How do the affected groups or populations differ from themselves when there was no disease problem? (2) How do affected groups or populations differ from those which are not affected? In attempting to answer these two questions, we place ourselves in a position to intelligently create a ruleout list (or hypothesis list). The items on this list must be ***things that can be changed*** (ie., critical control points), not the names of infectious agents, toxins, or pathologies. The overall approach is directly comparable to that we would use to investigate a disease problem in an individual animal. We just need to keep in mind that we are looking for things that can explain the failure of homeostasis at the herd-level rather than in individuals. Once we have a ruleout list, we can begin to consider strategic laboratory sampling which might be useful.

Strategic laboratory sampling is that aimed at testing management level hypotheses (= ruleouts). Another characteristic is that, if you can't define a decision-rule (this result: this conclusion; that result: that conclusion), then it's not strategic sampling but blue-sky sampling ("we'll send these samples off and see if something hits us out of the blue sky when the results come back"). Over and above the tendency of blue-sky sampling to squander time and resources, it's also very difficult to plan in terms of number and selection of individuals to be sampled. In strategic sampling, by contrast, intelligent planning is easier because management-level hypotheses can be framed in terms of one of the three questions: Is some agent or attribute present in a group or population? What proportion of a group or population have some agent or attribute? What is the average level of some quantitative attribute in a population or group?

The following is a discussion on sample size determination for the three major types of questions. The Appendix contains sample size tables.

Sampling to detect an attribute.

One of your cow-calf clients who feedlots out his own yearlings has been getting dinged at the slaughter plant for a high rate of liver condemnations. Liver flukes have been reported in some cases. The calves are born on the client's home ground and grazed on leased ground. Both areas have traditionally been thought of as fluke-free, but you decide to test the cow herd for flukes to test the hypothesis that the home place is fluke free. How many should you test? If you want to be certain (within the limits of the test) that there are no flukey cattle, you would test all 600 of them. If, instead, you are willing to ask: "How many do I sample in this 600-cow herd to be 95% certain of finding at least 1 positive animal if the prevalence is 5% or more?" then a sample size can be computed which will not include the entire herd (unless the herd is very small). Cannon and Roe[1] proposed the following equation:

$$n \approx (1 - \beta^{1/d}) \quad (N - \frac{d-1}{2})$$

where n is the computed sample size requirement, N is the size of the population or defined subgroup of interest, d is the number of animals within the population which possess the attribute, and β is the probability of drawing no positives in a sample of size n. Fractions are always rounded to the next highest integer.

Tabulated values are shown in Appendix 1 for 90%, 95%, and 99% certainty. In the fluke example, you would need to sample 56 cows to be 95% certain of detecting at least one positive if the true prevalence were 5%. If you would be happy with a 90% confidence you would need to sample only 44 cows. If you want to be 95% certain of detecting a 1% prevalence, you'll need to sample 191 cows. Of course, you may not need to actually test all of the sampled cattle—if you find a positive sample on the 30-th sample you run, you could stop there.

Targeted sampling can often make such hypothesis tests much stronger. For example, to test the hypothesis that a herd is free of flukes, testing the aged cows would conceivably provide a stronger test since they've been grazing on the putatively flukey ground the longest. In such cases, one posits a detectable preva-

© Copyright American Association of Bovine Practitioners; open access distribution.

lence within the subgroup of interest and then, based on the size of the group and the level of certainty desired, a sample size within the target group can be determined from Appendix 1 or Equation 1.

Using Equation 1 or Appendix 1 for planning the sampling for transient infections can be tricky. Say, we want to sample within pens of feedlot cattle to determine if *Salmonella* infection exists. In the fluke prevalence example we are assuming that, whatever the prevalence is in the herd, it is fairly stable and will be identical or very similar next week. In sampling for transient infections (say, *Salmonella*), the observed infection prevalence is a chaotic variable, meaning that it changes daily. The average prevalence over, say, a week's period might be 10% but it will not be 10% at every instant within the week. We are effectively sampling animal-instants rather than individuals, and thus we must use the sample size for infinite populations. For example, to give a 95% chance of detecting at least one *Salmonella*-positive animal if the underlying prevalence were 10%, we would need to sample 29 animals (Appendix 1).

Sampling to estimate a proportion.

You are investigating reasons for historically high neonatal calf losses in a large cow-calf operation. You hypothesize that failure of passive transfer is excessive among calves born into this 400-cow herd and would like to estimate the proportion of 2-7 day old calves with inadequate passive transfer levels (say, < 500 mg/dl IgG). How many calves will you need to sample to obtain a good estimate. The formula for computing the needed sample size is as follows:

$$n_i \approx \frac{Z^2 p\,(1-p)}{d^2}$$

$$n \approx \frac{1}{\frac{1}{n_i} + \frac{1}{N}}$$

where n is the computed sample size requirement, n_i is the sample size for an infinite population, Z is the reliability coefficient (e.g., 1.96 for 95%), p is the rough estimate of prevalence, d is the desired confidence bound, and N is the size of the population or defined group from which samples are being collected. Fractions are rounded up.

Since the variance of the estimate of a proportion is dependent somewhat on how close the proportion is to 0.5 (the variance increases closer to 0.50), one must make a crude estimate of the general prevalence, erring toward conservatism. In the present case, you'd be surprised if the proportion of inadequate passive transfer was more than 25% in a beef herd, thus you choose 25% as p. Next, you must choose how wide you want the confidence interval to be and at what level of confidence (e.g., 90%, 95%). Let's say you are willing to settle for an estimate that has a 95% confidence bound of 5 percentage points either side of the estimated proportion. From Appendix 2b, you find the number 168. This seems higher than the number you had in mind, so you decide to check the sample size needed for a 10 percentage point bound. In Appendix 2b you find the number 62. It's a good thing you looked up a sample size to guide your efforts since, otherwise, you would have been inclined to only sample 10 or 15 calves—which would not have been enough to make any strong conclusions.

Sampling to estimate an average.

You have hypothesized that selenium deficiency is part of the problem in a feedlot with a long-term excess pneumonia problem. You want to estimate the average whole blood selenium level in recently arrived pens of 200 feedlot cattle. From previous sampling, you estimate the standard deviation to be about 6 ug/g and you want to estimate the average to within 3 ug/g. You could use the following equation:

$$n_i \approx \frac{z^2}{d_2}$$

$$n \approx \frac{1}{\frac{1}{n_i} + \frac{1}{N}}$$

where n is the computed sample size requirement, n_i is the sample size for an infinite population, Z is the reliability coefficient (e.g., 1.96 for 95%), d is the desired confidence bound expressed in fractions of the standard deviation, and N is the size of the population or defined group from which samples are being collected. Fractions are rounded up. An alternative to using the equation is Appendix 3. For a 95% confidence interval find the sample size of 15 under the column headed 0.50 in Appendix 3b.

Practical comments on the use of sample size formulas.

Sample size tables are not, strictly speaking, essential to strategic sampling; the only critical feature being that testable hypotheses should form the basis for sampling. Without some guidelines on appropriate sample size, however, many of the hypotheses posited would not be adequately tested. Occasional reference to sample size tables will create a sense of the magnitude of sampling that is appropriate. Another benefit to considering sample size is that it forces us to state our hypothesis explicitly, a point at which many of our notions will evaporate into the blue sky.

It's also noteworthy that other sample size formulas exist, some of which include additional variables for test sensitivity and specificity. Undoubtedly, imperfect tests will influence the sample sizes needed, but including assumptions for test sensitivity and specificity creates some very complex equations (or many pages of tables) and require assumptions for which reliable values do not exist or are very difficult to obtain. In a prag-

© Copyright American Association of Bovine Practitioners; open access distribution.

matic world, our hypotheses must be framed in terms of group prevalences or averages as we see them through the "eyes" of imperfect tests. We also understand that there is unavoidable fuzz to sample size selection—regardless of the degree of test perfection—in that the choices for level of confidence or width of bound are somewhat arbitrary.

Scenario 1 revisited

As an example of applied strategic sampling, consider that used in the salmonellosis problem described in Scenario 1. Even though this investigation was not perfect in all aspects, it serves to illustrate how strategic laboratory sampling can be used, even under the constraints of practical circumstances, to converge on a problem.

The delivery-person-vet approach would have been to prescribe a vaccine for the feedlot with the salmonellosis cases. This was not done due to the questionable efficacy of available biologicals and the high cost which would be involved in vaccinating >50,000 head per year (18,000 capacity, 3x/year turnover). It was decided to use strategic sampling in order to determine the reservoir and mode of spread with the goal of defining critical control points. The hypotheses tested on the preliminary visit was that the *Salmonella* was spreading primarily in problem pens (those with above median morbidity) rather than in the entire feedlot or only in the hospital pens. Ten fecal pat samples were collected from each of 4 problem pens (above median morbidities) and 3 adjoining pens. (100 pens total in the feedlot). The treatment crew had almost finished treatments at the time of our arrival, and only the last 14 animals through the hospital pen could be sampled. The sample sizes here (40 from problem pens, 10 from adjoining pens, and 14 from hospital pens) were fixed by the sampling supplies available and the capacity of the laboratory, but the aggregate numbers sampled in home pens (other than hospital pens) provided a greater than 95% probability of detecting a 5% prevalence (Appendix 1, n=59). The 14 samples from hospital pens was not considered optimal, but it was felt that even this limited sampling would provide good staging data to help plan any followup sampling. Five of the 14 hospital pen samples were positive for *S. typhimurium*—all of which were of an indistinguishable antibiogram as the isolates obtained from the necropsied animals. No positives were found among the 70 samples from home pens.

This led to the refined hypothesis that the *Salmonella* infections were strictly nosocomial in this feedlot (spreading in the hospital pen). The main alternative considered was that the agent was spreading in home pens and that the concentration in hospital pens was produced by pen riders who were identifying and pulling cases very quickly. Under the nosocomial hypothesis, we expected that followup sampling would continue to show the *Salmonella* infected animals to be strongly concentrated in the hospital pen. Furthermore, if the nosocomial hypothesis were true, we expected that animals sampled on the day they were pulled would be negative and that there would be an increase in infection prevalence during the first few days in the hospital pen. Followup sampling consisted of 28 fecal pats per pen in 4 above-average morbidity home pens (n=28 gave a roughly 95% chance of detecting 10% prevalence) plus all the new pulls and all the animals in the hospital pen on two sampling visits about 2 weeks apart. Culture of the 112 home pen samples (28 x 4) revealed 2 (1.8%) to be positive for *S. typhimurium*. It was considered noteworthy that recovered animals were returned to their home pens, although the use of fecal pats prevented conclusions as to whether the positive fecals came from former hospital residents. Culture of 16 new pulls revealed 4 (25%) were positive, but all 4 positives were relapsed cases as denoted by the existence of white hospital tags in their ears. Cattle which had been in the hospital pen one day had a *S. typhimurium* prevalence of 33% (7/21), those in hospital pen for two days had a prevalence of 67% (8/12) as did those in hospital for three days (10/15). Cattle present in the hospital pen for 4 days or longer had a *S. typhimurium* prevalence of 56% All of the isolates were of the same antibiogram as those from the necropsied steers and from the first sampling visit (plasmid profiling later confirmed that all isolates were of indistinguishable fingerprint). These data appeared to be most consistent with the nosocomial hypothesis.

A secondary hypothesis was posited that the main route of spread in the hospital pen was via direct contact and that spread was encouraged by the commingling of new pulls with chronics (i.e., the lack of any attempt at segregation based on days post entry). A segregation scheme was put in place and, after another two weeks, the prevalence of *Salmonella* was examined in the hospital cattle. A pattern very similar to that described above was observed—new pulls were negative (except a few relapsed animals) and prevalence increased with time in hospital pen such that most animals were infected by day 3 post entry. It was concluded that segregation by days post entry into hospital had not been successful and that some means of transmission other than direct animal-animal must be operational.

The major alternative hypothesis (to animal-animal transmission) was that transmission was primarily occurring via treatment equipment. Sampling of treatment equipment from the time of the first visit had revealed the presence of *S. typhimurium* (same antibiogram) on balling guns, stomach tube, and *Lactobacillus* paste dispenser. It was initially felt that inoculum doses from this route were most likely of small consequence. Also, feedlot management had been reluctant to implement an equipment sanitation program on grounds that no other area feedlot went to this trouble. But, after the failure of segregation, a sanitation program was implemented and monitored through the con-

© Copyright American Association of Bovine Practitioners; open access distribution.

tinued use of equipment cultures. The paste dispenser proved impossible to adequately sanitize and was eliminated from all treatment programs. The sanitation program was successful for other equipment such that a *Salmonella* positive sample was never again found on any piece of equipment. A month after successful implementation of equipment sanitation, only one *Salmonella* positive animal was detected in the hospital pen (at day 2 post entry); two months later no positives were found. Based on subjective reports from feedlot management, the syndrome consistent with clinical salmonellosis declined to near zero soon after implementation of the sanitation program.

The hypothesis that spread was primarily via equipment was considered to be provisionally established, although the evidence was not considered conclusive given the impossibility of providing a control group in which equipment sanitation was not practiced. It was also considered possible that nosocomial infections of *Salmonella* were normal for all feedlots and that the clinical syndrome was not related to salmonellosis. To test this hypothesis, hospital and home pen cattle in five control feedlots were cultured showing only a few sporadic *Salmonella* positives (same serotype as in feed) in a home pen of one feedlot and no positive hospital pen cattle. This lent credibility to the diagnosis of nosocomial salmonellosis as a specific feedlot syndrome with sanitation of treatment equipment as one critical control point. Since the other feedlots did not practice routine equipment sanitation, it was concluded that other (unknown) critical control points must have been involved in the initial establishment of a single strain of endemic *Salmonella* in hospital pens.

Reference

1. Cannon RM and Roe RT, Livestock Disease Surveys: ***A Field Manual for Veterinarians.*** Australian Bureau of Animal Health, Department of Primary Industry. Australian Government Publishing Service, Canberra, 1982.

Appendix 1. Sample size required to detect attribute in a population or defined group with defined level of certainty.

PREVALENCE	1%	1%	1%	5%	5%	5%	10%	10%	10%	25%	25%	25%	50%	50%	50%	75%	75%	75%
CERTAINTY	90%	95%	99%	90%	95%	99%	90%	95%	99%	90%	95%	99%	90%	95%	99%	90%	95%	99%
N= 10	10	10	10	10	10	10	10	10	10	6	7	8	3	4	5	2	3	4
20	20	20	20	19	20	20	14	16	18	7	9	11	4	5	6	2	3	4
30	30	30	30	24	26	29	16	19	23	8	9	13	4	5	7	2	3	4
40	40	40	40	28	31	36	17	21	27	8	10	14	4	5	7	2	3	4
50	50	50	50	30	35	42	18	22	29	8	10	14	4	5	7	2	3	4
60	59	60	60	32	38	47	19	23	31	8	10	15	4	5	7	2	3	4
70	68	70	70	34	40	51	19	24	33	8	10	15	4	5	7	2	3	4
80	76	79	80	35	42	54	20	24	34	8	10	15	4	5	7	2	3	4
90	84	87	90	36	43	57	20	25	35	8	10	15	4	5	7	2	3	4
100	91	96	100	37	45	59	20	25	36	8	10	15	4	5	7	2	3	4
125	106	114	122	38	47	64	21	26	37	8	11	16	4	5	7	2	3	4
150	118	130	143	39	49	68	21	26	38	8	11	16	4	5	7	2	3	4
175	128	144	163	40	50	71	21	27	39	8	11	16	4	5	7	2	3	4
200	137	155	180	41	51	73	21	27	40	8	11	16	4	5	7	2	3	4
225	144	166	196	41	52	74	21	27	40	8	11	16	4	5	7	2	3	4
250	151	175	210	42	53	76	21	27	41	8	11	16	4	5	7	2	3	4
275	156	182	223	42	53	77	22	28	41	8	11	16	4	5	7	2	3	4
300	161	189	235	42	54	78	22	28	41	8	11	16	4	5	7	2	3	4
400	175	211	273	43	55	81	22	28	42	8	11	16	4	5	7	2	3	4
500	184	225	300	43	56	83	22	28	42	8	11	16	4	5	7	2	3	4
600	191	235	321	44	56	84	22	28	43	9	11	16	4	5	7	2	3	4
700	196	243	336	44	57	85	22	28	43	9	11	16	4	5	7	2	3	4
800	200	249	349	44	57	85	22	28	43	9	11	16	4	5	7	2	3	4
900	203	254	359	44	57	86	22	29	43	9	11	16	4	5	7	2	3	4
1000	205	258	368	44	57	86	22	29	43	9	11	16	4	5	7	2	3	4
Infinite	229	298	458	45	59	90	22	29	44	9	11	17	4	5	7	2	3	4

Prevalence = lowest prevalence in group or population of interest detectable at defined certainty level using sample size given. Certainty = level of certainty of detection desired (90%, 95%, 99%). N = number of individuals in group or population of interest, Computations based on: Cannon RM and Roe RT, Livestock Disease Surveys: A Field Manual for Veterinarians. Australian Bureau of Animal Health, Department of Primary Industry. Australian Government Publishing Service, Canberra, 1982.

© Copyright American Association of Bovine Practitioners; open access distribution.

Appendix 2a. Sample size required to place 90% confidence bound of desired width on an estimated proportion.

Est. prop.	5%	10%	10%	25%	25%	25%	25%	50%	50%	50%	50%	50%
Bound	3%	3%	5%	3%	5%	10%	15%	3%	5%	10%	15%	20%
N= 30	25	27	23	29	27	19	13	29	27	21	15	11
40	32	35	29	38	34	23	15	38	35	26	18	12
50	37	43	33	46	41	26	16	47	43	29	19	13
60	43	50	38	55	47	28	17	56	50	32	20	14
70	47	56	41	63	52	30	17	65	56	35	21	14
80	52	62	44	71	58	31	18	73	62	37	22	14
90	56	68	47	78	63	33	18	81	68	39	23	15
100	59	73	50	85	67	34	19	89	73	41	24	15
125	67	86	55	103	78	36	20	108	86	44	25	15
150	73	97	59	119	87	38	20	125	97	47	25	16
175	79	107	63	134	94	40	20	142	107	49	26	16
200	84	115	66	148	101	41	21	158	115	51	26	16
300	97	142	74	196	121	44	21	215	142	55	28	16
400	105	161	78	234	135	45	22	261	161	58	28	17
500	111	175	82	265	144	46	22	300	175	60	29	17
1000	125	212	89	360	168	49	22	428	212	64	30	17
Infinite	142	269	97	560	202	50	22	747	269	67	30	17

Appendix 2b. Sample size required to place 95% confidence bound of desired width on an estimated proportion.

Est. prop.	5%	10%	10%	25%	25%	25%	25%	50%	50%	50%	50%	50%
Bound	3%	3%	5%	3%	5%	10%	15%	3%	5%	10%	15%	20%
N= 30	27	28	25	29	28	22	16	30	28	23	18	14
40	34	37	32	39	36	26	18	39	37	29	21	16
50	41	45	37	48	43	30	20	48	45	33	24	17
60	47	52	42	56	50	33	21	57	52	37	25	18
70	53	60	47	65	57	36	22	66	60	41	27	18
80	58	67	51	73	63	38	23	75	67	44	28	19
90	63	73	55	81	69	41	24	83	73	47	29	19
100	67	80	59	89	75	42	25	92	80	49	30	20
125	78	95	66	109	88	46	26	112	95	55	32	21
150	87	108	72	127	99	49	27	132	108	59	34	21
175	94	121	78	144	109	52	28	151	121	63	35	22
200	101	132	82	161	119	53	28	169	132	65	36	22
300	121	169	95	219	147	59	29	235	169	73	38	23
400	135	196	103	267	168	62	30	291	196	78	39	23
500	145	218	109	308	183	63	31	341	218	81	40	23
1000	169	278	122	445	224	68	32	517	278	88	41	24
infinite	203	384	138	800	288	72	32	1067	384	96	43	24

Est. prop. = crude estimate of proportion (use 50% if no information since this will give maximum sample size). Bound = distance either side of estimated proportion that confidence interval extends. N = number of individuals in population or group of interest.

© Copyright American Association of Bovine Practitioners; open access distribution.

Appendix 3a. Sample size required to place 90% confidence bound of desired width on an estimated mean.

Bound in Std. Dev. Units	0.10	0.20	0.30	0.40	0.50	0.60	0.70	0.80	0.90	1.00
N= 30	27	21	15	11	8	6	5	4	3	3
40	35	26	18	12	9	7	5	4	4	3
50	43	29	19	13	9	7	5	4	4	3
60	50	32	20	14	10	7	6	4	4	3
70	56	35	21	14	10	7	6	4	4	3
80	62	37	22	14	10	7	6	4	4	3
90	68	39	23	15	10	7	6	5	4	3
100	73	41	24	15	10	7	6	5	4	3
125	86	44	25	15	10	8	6	5	4	3
150	97	47	25	16	11	8	6	5	4	3
175	107	49	26	16	11	8	6	5	4	3
200	115	51	26	16	11	8	6	5	4	3
300	142	55	28	16	11	8	6	5	4	3
400	161	58	28	17	11	8	6	5	4	3
500	175	60	29	17	11	8	6	5	4	3
1000	212	64	30	17	11	8	6	5	4	3
Infinite	269	67	30	17	11	8	6	5	4	3

Appendix 3b. Sample size required to place 95% confidence bound of desired width on an estimated mean.

Bound in Std. Dev. Units	0.10	0.20	0.30	0.40	0.50	0.60	0.70	0.80	0.90	1.00
N= 30	28	23	18	14	11	8	7	6	5	4
40	37	29	21	16	12	9	7	6	5	4
50	45	33	24	17	12	9	7	6	5	4
60	52	37	25	18	13	10	7	6	5	4
70	60	41	27	18	13	10	8	6	5	4
80	67	44	28	19	13	10	8	6	5	4
90	73	47	29	19	14	10	8	6	5	4
100	80	49	30	20	14	10	8	6	5	4
125	95	55	32	21	14	10	8	6	5	4
150	108	59	34	21	14	10	8	6	5	4
175	121	63	35	22	15	11	8	6	5	4
200	132	65	36	22	15	11	8	6	5	4
300	169	73	38	23	15	11	8	6	5	4
400	196	78	39	23	15	11	8	6	5	4
500	218	81	40	23	15	11	8	6	5	4
1000	278	88	41	24	16	11	8	6	5	4
Infinite	384	96	43	24	16	11	8	6	5	4

Desired bound in Std. Dev. Units = Fraction of standard deviation that the desired bound represents. For example, if the SD is 10 and the desired bound is +/- 3 then the desired bound in standard deviation units is 3/10 = .3. N = number of individuals in population or group of interest.

© Copyright American Association of Bovine Practitioners; open access distribution.