Bovine Abortion: Causes and Diagnosis

BY JEROME C. NIETFELD, DMV, VETERINARY DIAGNOSTIC LABORATORY, DEPARTMENT OF DIAGNOSTIC MEDICINE/PATHOBIOLGY

During the winter and spring, the number of bovine abortions seen at veterinary diagnostic laboratories increases. As most veterinarians know, a cause is not found in over half of the abortions examined at veterinary diagnostic laboratories. There is no doubt that certain infectious agents are capable of causing abortion and, in many cases, are capable of affecting large numbers of cattle within a herd, which can be economically devastating. However, I am convinced that many abortions are not caused by infectious or toxic agents, which means that many cases will remain unsolved. Also, there are infectious agents, such as Actinomyces pyogenes and fungi, which are important causes of abortions but are sporadic in occurrence and do not constitute herd problems.

It is important to accurately identify cases caused by agents that cause sporadic abortions; cases with no identifiable cause; as well as cases caused by agents capable of causing large epizootics in order to give accurate advice to the herd owner. This makes it especially important to collect and submit the samples that will maximize the chances of properly determining the etiology in cases with an identifiable cause, and of correctly ruling out infectious causes in the remaining cases.

Submission of Samples
The fetus and its placenta may be submitted in their entirety, and they constitute the best samples. However, because of the size, it is often not practical to send an entire bovine fetus through the mail. Instead, the fetuses may be necropsied and the following samples submitted:

Refrigerated or frozen:
I. Fetal heart blood or thoracic fluid—1 to 3 ml collected with a sterile syringe and placed in a sterile tube. Can be used to test for antibodies to agents such as BVD virus, Neospora caninum, and Leptospira.

II. Stomach contents—1 to 3 ml. Collect with same syringe used to collect heart blood. If collected properly, stomach contents should not be contaminated and is easily the single best sample for bacterial culture. Can also be examined for Tritrichomonas foetus.

III. Lung, liver, kidney, spleen, and heart—these are primarily used for virus isolation and fluorescent antibody testing for viruses and leptospires.

IV. It is important to include placenta when it is available. Send several cotyledons and the intercotyledonary tissue. Send anything that appears necrotic or thickened.

V. Serum from the dam. Do not freeze whole blood. Serum may be refrigerated or frozen.

Fixed in formalin:
VI. Lung, liver, kidney, brain, skeletal muscle, heart, placenta (include 2 or 3 cotyledons) and any tissues that contain lesions.

Continued on page 2.
Comments on Abortion Causing Agents and Their Diagnosis

Following are a few comments on diagnosis of bovine abortion. This is by no means a complete list of causes of bovine abortion, as veterinarians and cattle owners already are aware of the economically important causes. One should remember that several very important abortion causing agents, such as Brucella abortus, Campylobacter, and Leptospira, are rare or uncommon because of control measures. However, it is important to continue to test for these agents because they have not been eliminated and it would be disastrous if they made a comeback because we quit monitoring for them.

Placenta

The placenta is especially important for diagnosing fungal and bacterial placentitis. Fungal placentitis is relatively common and the majority of cases cannot be diagnosed unless the placenta is examined. Common bacteria, such as Actinomyces pyogenes, Bacillus spp, Escherichia coli, Pasteurella spp, etc. that we do often not think of as causes of abortion do in fact cause sporadic abortions. Also, these bacteria commonly contaminate samples and unless they are isolated in pure culture from the stomach contents and there is placentitis, one cannot be certain these bacteria are the cause of the abortion or are just contaminants. In many cases the placenta simply is not available. However, it has been my experience that if one explains to the owner that having the placenta will increase the chances of correctly identifying the cause of the abortion(s), some owners will retrieve the placenta. Sometimes they do not bring the placenta with the fetus because they do not know that it is important.

Infectious Bovine Rhinotracheitis Virus

In a recent 10-year survey of 8,900 bovine abortions examined in a Midwestern diagnostic laboratory, initially IBR virus was the most commonly diagnosed cause of abortion. During the survey, the incidence of IBR abortion decreased steadily so that in the last few years of the study IBR virus was diagnosed only infrequently. However, IBR abortions still do occur and IBR virus can cause serious epizootics with over half of the cows, especially heifers, aborting. If pregnant cows are vaccinated intramuscularly with an attenuated IBR-BVD virus vaccine, the IBR virus is the more likely of the two to cause problems.

Bovine Virus Diarrhea Virus

In the same 10-year survey, BVD virus was the second most commonly diagnosed cause, barely behind IBR virus. In the last few years of the study, BVD virus was diagnosed more often than BVD virus, and the incidence of BVD virus remained essentially unchanged over the 10 years. BVD virus can be one of the harder agents to identify in aborted fetuses because some fetuses eliminate the virus before being aborted. If a herd has had several abortions but nothing can be identified, I would not rule out BVD virus until several calves have been examined.

Miscellaneous bacteria

Bacteria such as Actinomyces pyogenes and Bacillus spp actually cause more abortions than agents such as Brucella abortus, Campylobacter spp, and Listeria. In fact, if Actinomyces pyogenes is isolated from a fetus it probably should be considered as the cause of abortion whether or not one is able to examine the placenta. The good thing about these miscellaneous bacteria, and there is a long list of them that have been reported to cause abortion, is that they are not contagious and the owner does not have to worry about additional problems.

Neospora caninum—Neospora is common in dairy cattle throughout the United States. It can cause abortion storms or it may be a chronic problem and cause a yearly abortion rate of 10 to 20%. Recently, it was reported that N. caninum is carried by dogs and shed in their feces. The abortion storms are the result of contamination of feed by dog feces. It is also known that some cattle are born infected and are persistent carriers. The fetuses of some persistently infected cows become infected during gestation and this can result in fetal death or birth of an infected calf that is clinically normal or is born with symptoms of central nervous system disease. Cows have been reported to abort more than once from Neospora infection.

Serologic tests

A single antibody titer to an infectious agent at best is an indication of past exposure to that agent. When attempting to correlate an abortion with a titer you must be cautious. Keep in mind the frequency with which cattle are exposed to the agent in question, the frequency with which owners vaccinate for the agent and the length of time antibodies persist. For example, in most areas, vaccination is common and over half of the cow population will have titers to BVD. The combination of vaccination with live virus vaccines and exposure to field virus can result in very high titers, well above 1:1000, which persist for quite a long time. Thus, a high titer, especially in an animal that is several years old, is just as likely to indicate that she was immune to BVD virus rather than she aborted because of BVD virus. Examination of paired serum samples from 10 animals or 10% of the herd can give much more valid results. Often, the animal that aborted has reached the peak of her antibody response by the time she aborts, but other animals in the herd have not. If a 4-fold increase in antibody titer is found in herdmates, it proves that the agent was circulating in the herd at or shortly before the abortion.

Wanted

Assistance from veterinarians to diagnose cases of abomasal bloat/ulcers in beef or dairy calves. We are seeking diagnostic specimens or live animals to collect samples. Please contact Dr. Brad DeBey at (785) 532-5650, Dr. T.G. Nagaraja at (785) 532-4403 or Dr. Gerald L. Stokka at (785) 532-5694.

LCI Offers Spanish Version of Cattle Handling Video

(LCI News: Nov/Dec 98) The Livestock Conservation Institute’s award winning Cattle Handling and Transportation video was recently translated into Spanish thanks to funding from Pharmacia Upjohn. Copies are available through LCI for $34.95. Plans are to translate additional educational videos in the near future. To order a copy, call LCI at 502-782-9798. We have a copy that we can make available to Kansas veterinarians for use in quality assurance training for their clients and client employees.
New PCR for Escherichia coli and toxins

BY JEROME C. NIETFELD, DMV, VETERINARY DIAGNOSTIC LABORATORY, DEPARTMENT OF DIAGNOSTIC MEDICINE/PATHOBIOLGY

The KSU Veterinary Diagnostic Laboratory is pleased to announce a new multiplex polymerase chain reaction (PCR) test for identification of pilus adhesions and toxins produced by Escherichia coli. The PCR was developed by researchers at the National Animal Disease Center, Ames, Iowa. The multiplex PCR was designed to test E. coli isolated in the laboratory rather than to directly test clinical samples. The test will identify genes for the following:

K99 (F4) pilus
K99 (F5) pilus
987P (F6) pilus
F41 pilus
F18 (F107) pilus
Labile toxin (LT)
Stable toxin a (STa)
Stable toxin b (STb)
Shiga-like toxin IIe (SLT-IIe)

K99 and F41 pili are associated with enterotoxigenic E. coli (ETEC) that affect calves and pigs, and on occasion foals, lambs, and puppies. Normally E. coli isolates that express these pili can only colonize neonates up to 3 to 4 days of age. There is some evidence that viral infection can extend for a few days—the period for which these E. coli can colonize. F41 is rarely expressed by itself. Therefore, many labs do not test for it, because if one detects E. coli expressing other pili they will nearly always also detect the F41+.

K98, 987P, and F18 expressing E. coli only affect pigs. Like K99 and F41 positive E. coli, 987P+ E. coli only colonizes pigs for the first 3 to 4 days of life. K98+ isolates can colonize pigs from birth until after weaning; therefore, this pilus type is associated with pre- and postweaning diarrhea. F18 (F107)+ E. coli do not colonize pigs until after weaning and are associated with postweaning diarrhea and edema disease.

LT is secreted by K88 and F18 positive E. coli and is produced by ETEC affecting pigs and people. It is closely related to and causes diarrhea by a mechanism similar to cholera toxin. STa and STb are produced by E. coli expressing all of the above pili and are produced by ETEC affecting all species. SLT-IIe is secreted by F18 positive E. coli and is responsible for the vascular damage and clinical signs seen in pigs with edema disease.

Since K99+ isolates are the only type of ETEC that colonize calves and the F41 isolates also express K99, the lab will continue to test calf samples for K99 by the ELISA test that we have used in the past. The ELISA is faster and less expensive. The cost for the multiplex PCR will be $17.50. The cost for the K99 ELISA will remain at $5.

If you have any questions concerning the E. coli PCR, contact Dr. Jerome Nietsfeld or Dr. Teresa Yeary at 785-532-5650.

New PCR Tests for Mycoplasma

BY JEROME C. NIETFELD, DMV, VETERINARY DIAGNOSTIC LABORATORY, DEPARTMENT OF DIAGNOSTIC MEDICINE/PATHOBIOLGY

The KSU Veterinary Diagnostic Laboratory is pleased to announce three new polymerase chain reaction (PCR) procedures for identification of mycoplasmas in clinical samples. For the past several years, Dr. Lloyd Lauerer at the Alabama Veterinary Diagnostic Laboratory, Auburn University, has been evaluating, improving, and developing these tests. He has spent considerable time comparing the results with culture, serology, and other methods of diagnosis and has developed a very good PCR procedure. Currently, the Auburn lab is testing approximately 7,000 samples/year by this method. This past summer the KSU Diagnostic Lab sent Dr. Teresa Yeary to Auburn to learn the mycoplasma PCR procedure and since then we have been working with the procedure in order to offer it to our clients.

The first PCR is a general test for mycoplasmas. It utilizes primers specific for members of the mycoplasma family and will detect both Mycoplasma spp and Ureaplasma spp, but will not identify the particular species present. If it is desirable to identify the particular species present, the product from positive PCR results can be evaluated by restriction fragment length polymorphism (RFLP) using four restriction enzymes. The patterns obtained by RFLP are compared with the patterns of individual species of Mycoplasma, each species gives a different pattern, and in most cases the species of Mycoplasma present can be determined. The cost for the general Mycoplasma PCR will be $15 and the cost for RFLP will be an additional $15.

The second PCR is one for Mycoplasma hyopneumoniae, the cause of enzootic pneumonia in pigs. The procedure is the same as the general mycoplasma PCR except that primers specific for M. hyopneumoniae are used. The test identifies M. hyopneumoniae without the use of RFLP. The cost of this test is also $15.

The third PCR is for Ureaplasma diversum, a cause of vaginal infections and abortion in cattle. Again, the procedure is the same as for the general mycoplasma PCR, except that primers specific for U. diversum are used. The cost is $15.

If you have any questions concerning these tests, call Dr. Jerome Nietsfeld or Dr. Teresa Yeary at 785-532-5650.
Interpretation of Antimicrobial Susceptibility Reports from the KSU Diagnostic Laboratory

BY LINDA COX, BS, MS, BACTERIOLOGY TECHNICIAN AND GEORGE KENNEDY, DIAGNOSTIC MEDICINE/PATHOLOGY

Antimicrobial susceptibility tests are in vitro attempts to measure the inhibitory capacity of a chemotherapeutic agent toward a specific microorganism. The results are usually conveyed in an interpretative statement (sensitive, intermediate, or resistant), indicating whether this inhibition can be expected in vivo through a normal dosage regimen of the particular chemotherapeutic agent. The two most common antimicrobial susceptibility testing procedures in use today are the broth dilution procedure and the disk diffusion Kirby Bauer procedure. The Kirby-Bauer disk diffusion test utilizes a measured zone of inhibited growth around each antibiotic impregnated disk to derive the interpretative statement. This system has been useful and is still used, particularly for some organisms with fastidious growth requirements. However, the Kirby-Bauer test is a more qualitative test and is quantitatively less precise regarding the minimum amount of an antibiotic necessary to inhibit a given organism, i.e., the Minimum Inhibitory Concentration or MIC. The Minimum Inhibitory Concentration is just that, inhibitory. It is not the concentration that kills bacteria, which is the Minimum Bactericidal Concentration or MBC. The MIC and the MBC may or may not be similar for a given antibiotic. The most common quantitative technique currently being used is the broth dilution procedure. This provides the clinician with information regarding the minimum plasma concentration necessary to inhibit a given organism. Most antibacterial susceptibilities at the KSU Diagnostic Laboratory are performed using microtiter plates and the Accu-Med/Sensititre System. Unfortunately, due to economics most veterinary laboratories are forced to use "breakpoints" rather than true MIC's. With the breakpoint method, a greater number and variety of antibiotics can be tested for on the microtiter dilution plate than if a true MIC is determined which requires more dilutions, and more expense.

Breakpoint susceptibility testing typically refers to selecting two appropriate concentrations of a given antibiotic that is achievable in vivo. Bacterial growth at both concentrations indicates resistance, growth at only the lower concentration indicates an intermediate susceptibility and no growth at either concentration is interpreted as susceptible.

The concentrations of antibiotics on the plates are based on the standards set by the National Committee for Clinical Laboratory Standards (NCCLS). The results are given as susceptible (S), intermediate (I), or resistant (R). For a variety of reasons, the in vitro results may not always correlate 100% with in vivo results. Currently, the KSU Diagnostic Laboratory does not routinely print out the actual breakpoint concentrations, but if a veterinarian wants to know the breakpoints of a particular drug, we can provide that information from the system.

Sometimes results will appear as NM or MN. These represent invalid results. There are several technical reasons why these may occur. If this happens, the test can be repeated upon request but the same result often recurs. Another option is to perform the Kirby-Bauer disk diffusion technique which can also be done upon request.

Mastitis samples are set on a true Minimum Inhibitory Concentration (MIC) plate. The MIC is the highest dilution (the lowest level of the drug) that prevents visible growth. Again, the results are given as susceptible, intermediate, or resistant. The numbers beside the results are based on presumed udder (milk) levels of the antimicrobials.

MIC numbers cannot be directly compared to each other because they are based on the dosage level of the individual drug. For example, it may take a smaller dose of one drug to reach 4 μg/ml than it does another drug to reach 2 μg/ml.

Sensitivities on all ocular antimicrobials, and for some fastidious bacteria, are derived from the Kirby-Bauer disk diffusion plates. These are recorded as simply susceptible, intermediate, or resistant based on the size of the zone of inhibition. If a breakpoint of MIC plate and a Kirby-Bauer are both given on a particular antibiotic, they may have different results due to different concentrations on the plates and in the disks. In that case, the breakpoint or MIC results should be used since those results are qualitative and based on concentrations approved for animal use.

A few points to keep in mind when interpreting susceptibility results include the following.

1. The breakpoints represent estimates of the mean for a population of animals. There may be significant variation within individual animals. Another potential problem is that some breakpoints are not derived from animal studies but are extrapolated from human medicine. Tetracycline is an example of a drug for which the commonly used breakpoints are derived from human studies. Examples of drugs for which breakpoints have been specifically derived for veterinary medicine include: cefiofur, for bovine and swine respiratory disease; tilimicosin, for bovine respiratory disease; amoxicillin/clavulanic acid for dogs; enrofloxacin for dogs; sarafloxacin for poultry; pirlimycin for bovine mastitis; penicillin/novobiocin for bovine mastitis; tiamulin for swine.

2. The actual dosage of a drug needed to reach a particular plasma, or tissue level is often not known, especially for many of the older veterinary drugs.

3. The laboratory conditions under which susceptibility tests are performed are ideal conditions for most of the antimicrobials. The in vivo conditions may adversely affect efficacy. For example, pH, oxygen tension, and inflammatory exudates may all affect the efficacy of an antibiotic in vivo.

4. Susceptibility patterns from a single case extrapolated to a large population of animals may be misleading both as the pathogen(s) and the susceptibility profile of the isolate(s).

5. Be sure the sample selected is representative of either the lesion if it is an individual animal or that the animal is representative of the group.

6. "Susceptible" results indicate that a correct antimicrobial regimen at an appropriate time in the disease process has a good chance of success in the majority of cases.

7. "Intermediate" indicates that an elevated dose of antimicrobial may be necessary to achieve satisfactory results. Immunocompromised animals may not respond.

8. "Resistant" suggests that the antimicrobial in question is not a good choice in most cases.
Copper Toxicity in Small Ruminants

By James Pickrell, Frederick Oehme, and R. Dalefield, Diagnostic Medicine/Pathobiology, College of Veterinary Medicine

Sheep are especially sensitive to consumption of excess copper. In fact, sheep should not be fed a total ration containing more than 15 to 25 parts per million (ppm) copper. Sources of copper can be from natural sources—soils and plants, solids contaminated by mining or smelting and soils or plants fertilized with used poultry litter or swine manure, and feed additives-mineral blocks or copper directly added to a sheep diet. Sometimes the source may be difficult to find. Since cattle will routinely tolerate rations with 75 ppm copper or greater and require more than 50 ppm copper, it is especially important that cattle rations not be fed to sheep without dilution to appropriate levels of copper. A recent copper-poisoned sheep examined at the Kansas State University Veterinary Diagnostic Laboratory had been fed a ration containing 124 ppm copper, a level clearly toxic to that species.

Complicating the problem is the interaction of copper with molybdenum, zinc, sulfur and iron. Sheep consuming excess dietary copper, or copper/molybdenum rations exceeding 10:1, will accumulate excess copper in the mitochondria and lysosomes of hepatocytes until levels toxic to the cell are accumulated. As toxic levels of copper accumulate in hepatocytes, the cells die, liberating copper into the blood stream. Excess copper levels in the bloodstream lyse erythrocytes, freeing hemoglobin and causing a hemolytic crisis. Hemoglobin is toxic to kidney parenchymal cells, leading to kidney pathology and the production of darkened (gun metal grey) kidneys and subsequent death.

During September and October, 1998, the Kansas State University Diagnostic Laboratory had 8 cases of suspected copper toxicity in small ruminants; 4 of those animals had copper levels in the toxic range with liver levels >250 ppm copper. For example, 4 ewes died suddenly with diarrhea and darkened kidneys and had 267, 300, 322 and 348 ppm liver copper, all concentrations in the toxic range. Their 30 and 180 ppm kidney copper levels further confirmed copper intoxication.

Clinical signs of copper toxicoses include the rapid onset of salivation, reduced feed consumption, colic, hemorrhagic diarrhea (sometimes with a greenish tinge), rapid dehydration, shock and death. The hemolytic crisis will cause anorexia, weakness, icterus, pale mucous membranes and dyspnea.

Laboratory confirmation of copper toxicity depends on detecting elevated concentrations of copper in affected animals' tissues. In live patients, serum is a practical sample for analysis, while liver and kidney are the tissues of choice to confirm copper poisoning at necropsy. Exposed but still asymptomatic animals may have elevated liver enzymes but serum copper levels remain within normal limits until about 24 hours prior to the onset of clinical signs.

Pathological lesions include icterus, congestion of the liver, kidneys and spleen, an enlarged pulp spleen suggesting hemolysis, dark bluish-black kidneys, and hemoglobinuria. The kidneys are often referred to as gun metal color. A recent case in a goat had a swollen liver, abnormally dark kidneys, "port wine" colored urine, and widespread jaundice with approximately 100 ml of dark-yellow clear fluid in the thorax. Renal tubular necrosis, noted histologically, was compatible with copper toxicity. Two ewes that died suddenly were found jaundiced and had renal-tubular necrosis. Many cases of copper toxicoses have been initiated by stress. Examples of such stressors include moving, working, showing, parasites, mastitis and even weather changes. A severe gastroenteritis may also occur alone or in combination with the above lesions following accidental administration of single large doses of copper containing formulations for therapeutic or pesticidal purposes.

Differential diagnoses include infectious gastroenteritis (acute salmonellosis) and leptospirosis as well as arsenic, mercury or selenium toxicoses and phenothiazine and some other anthelmintic poisonings.

Sheep may be treated for copper intoxication in several ways although the prognosis is generally poor. Injectable chelating agents such as ammonium tetrathiomolybdate aid in rapid binding and excretion of copper, or oral D-penicillamine (Cupramine R) may be dosed. For less acute cases, molybdenized copper phosphate has been sprayed on pastures. Even after removal from the copper source, sheep may continue to die due to kidney and liver damage. One treated for several days with Cupramine R had a 300 ppm copper level in liver, suggesting the chelator aided in preventing the clinical fatality, but did not reduce the stored liver copper to the range expected in non-copper exposed sheep.
An Epidemiological and Economic Simulation Model to Evaluate the Spread and Control of Infectious Bovine Rhinotracheitis in the Netherlands

BY VONK NOORDEGRAAF, J.A.A.M. BUIJTELS, A.A. DIJKHUIZEN, P. FRANKEN, J.A. STEEGMAN, J. VERHOEFF

Bovine herpesvirus type I (BHV-1), causing infectious bovine rhinotracheitis (IBR), was introduced in the Netherlands in 1971. In 1993, about 42% of the dairy cows had antibodies against BHV-1. In the future, stricter requirements are anticipated regarding the health status of exported breeding cows and material. To support policymakers in their decisions on IBR eradication, a simulation model was developed in which the epidemiological and economic consequences of various control strategies were evaluated. This paper describes the model and provides an overview of some important outcomes. In the model, dairy herds were classified into different disease states based on (1) the reproduction ratio of the disease (R, defined as the number of secondary cases caused by one infectious animal); (2) the within-herd prevalence, within each value of R; and (3) the expected number of infectious animals in an infectious herd within each prevalence range. The dynamic transition probability of a herd going from one state to another per week depends on direct contacts between animals, and other contacts such as transmission through fomites, indirect transmission through other species, airborne transmission and minor disease-specific routes such as venereal or iatrogenic transmission. Five control strategies, including both a voluntary vaccination program and a compulsory vaccination program for all dairy herds were evaluated. A voluntary vaccination program with 50% participation is not expected to lead to eradication of IBR. It appears that compulsory vaccination would be necessary to reach an IBR-free status.


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Evaluation of Bovine Viral Diarrhea Virus Control Using a Mathematical Model of Infection Dynamics

BY B.R. CHERRY, M.J. REEVES, G.SMITH

A mathematical model for infection with bovine viral diarrhea virus (BVDV) was created comprising a series of coupled differential equations. The model architecture is a development of the traditional model framework using susceptible, infectious and removed animals (the SIR model). The model predicts 1.2% persistent infection (within the range of field estimates) and is fairly insensitive to alterations of structure or parameter values. This model allows us to draw important conclusions regarding the control of BVD, particularly with respect to the importance of persistently infected (PI) animals in maintaining BVD as an endemic entity in the herd. Herds without PI animals are likely to experience episodic reproductive losses at intervals of two to three years, unlike herds with PI animals which will not see such marked episodic manifestations of infection. Instead, these herds will experience an initial peak of disease which will settle to low-level chronic reproductive losses. The model indicates that vaccine coverage for herd immunity (to avoid episodic manifestations of disease) need be only 57% without PI animals, although 97% coverage is required when PI animals are present. Analysis of model behavior suggests a program of detection and removal of PI animals may enhance the effectiveness of a vaccine program provided these animals are in the herd for 10 days or less. The best results would be seen with PI animals in the herd for 5 or fewer days.

Texas A&M University College of Veterinary Medicine and Department of Agricultural Economics present

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August 6-8, 1999

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