Diagnosis of Bovine Abortion Jessica Rush, DVM, DACT Auburn University College of Veterinary Medicine Auburn, AL

Pregnancy loss can be divided into three categories: embryonic death, abortion, and stillbirth. Embryonic death is defined as loss of the conceptus before organogenesis which occurs around day 42 of gestation in cattle. Once organogenesis has occurred the conceptus is considered a fetus. Pregnancy loss after organogenesis is defined as abortion. Abortions in cattle are of great economic concern to producers as an abortion of mid-gestation to term fetus results in a loss of \$600-1000. An acceptable average abortion rate in beef cattle is 1-2%, and reported to be as high as 10% in dairy herds. There is cause for concern when the abortion rate rises above 3-5% in beef herds. Discovering the cause of abortion in cattle can be one of the most frustrating problems a practitioner will face, as less than 50% of cases submitted to a diagnostic laboratory are accurately diagnosed. A thorough history and proper sample submission, along with collaboration with the diagnostic laboratory, will increase the success of diagnosing the inciting cause. Infectious agents are usually responsible for about 50% of abortions in cattle. Non-infectious causes of bovine abortion include toxins, genetic conditions, nutritional deficits or extremes, hormonal imbalances, and heat stress.

Key words: Bovine abortion, pregnancy loss

It is crucial to develop a protocol with producers on how to handle abortions on their farm. Many abortifacient agents are also zoonotic and precaution should be taken during sample collection. The female should be identified and isolated. Both fetus and placenta (when available) should be obtained and refrigerated until submitted to the diagnostic laboratory. Producers should contact their referring veterinarian as soon as possible. Helping the producers to maintain accurate herd records will aid in the diagnosis. It is important to manage the producer's expectations. Make sure they understand that accurate diagnosis is not possible for every case. The process can be time consuming, expensive, and often unrewarding. This does not mean that diagnosis should not be attempted. It is difficult, if not impossible, to resolve the problem without knowing the inciting cause, but the producer should understand the limitations before the process starts.

Important questions to consider when taking a herd history

- 1. Number abortions/abortion rate
- 2. Gestation age
- 3. Age of the dam
- 4. Duration
- 5. Number of females at risk
- 6. Weak calves
- 7. Clinical signs
- 8. Females sick/retained placenta
- 9. Previous abortions in herd
- 10. Vaccination protocol
- 11. Breeding program
- 12. Nutritional program
- 13. Herd recently worked
- 14. Weather
- 15. New additions
- 16. Abortions at neighbors

Pathognomonic gross lesions are uncommon, and when present, may not be apparent in cases of tissue autolysis. Gross examination might not help in determining which diagnostic test to select. Contact the diagnostic laboratory to ensure you are submitting the correct samples in the appropriate manner. Optimal

samples for submission usually include an intact placenta, fetus, and serum samples. The placenta is a better diagnostic sample than the fetus. In cases where the placenta is not available, a caruncle can be removed from the uterus and submitted. It should be divided into two with half being placed in formalin and half sent fresh chilled. If the placenta is fresh and normal, the cotyledons will be bright red and the intercotyledonary areas translucent. As autolysis occurs, the cotyledons become a pale brown color and the intercotyledonary areas become less translucent. Changes due to autolysis can be hard to distinguish from abnormalities. Opacity to the intercotyledonary spaces can also indicates edema, fibrosis, and inflammation. Exudate on the chorioallantoic surface is also indicative of inflammation. Fibrin can be present as yellow, friable material on the cotyledons. Often maternal blood provides limited information, especially if it is a single sample taken the day of the abortion. A titer response to an organism only indicates exposure, and might not distinguish natural exposure or vaccination. In many cases, antibodies levels are high weeks prior to abortion but may be within normal range at the time of abortion. Maternal blood samples are most helpful in animals that have not been vaccinated. Additional information can be gained by submitting serial maternal blood samples taken least 3 weeks apart and submitting blood samples from unaffected females in the herd for comparison (ideally at least 10). Inspection of the fetus is important to determine gestational age and condition of tissues. If the entire fetus cannot be submitted, both formalin fixed and fresh chilled samples should be taken and include:

Formalin Fixed

- Lung
- Liver
- Kidney
- Spleen
- Heart
- Brain
- Skeletal muscle
- Thymus
- Eyelid
- Abnormal tissue

- Fresh Chilled
 - Lung
 - Liver
 - Kidney
 - Spleen
 - Heart
 - Brain
 - Abomasal contents
 - Thoracic fluid
 - Ocular fluid

When the fetal necropsy is performed on farm, care should be taken to observe for signs that the fetus was alive at the time of parturition. This includes inflation of lungs, thrombosis of umbilical vessels, hemorrhage around the vessels, and milk in the abomasum. The changes to the umbilical vessels are seen due to tearing and thrombosis which occurs during parturition. Also, take note of fluid within the body cavities. A recently deceased fetus will have clear amber fluid within the body. Corneal edema present in a recently expelled fetus indicates that death occurred 6-12 hours prior. Evaluating the liver is very important because organisms which reach the fetus by way of the umbilical veins often induce lesions in the liver. *Listeria monocytogenes*, BHV-1, *Yersinia pseudotuberculosis*, and *Samonella enterica* are known to induce liver necrosis. Evaluation of the dermis is also important. Fungal infections commonly cause thickened, raise skin lesions. An additional test that can be helpful is fetal IgG concentration. Bovine IgG can be measured in fetal blood or thoracic fluid and if elevated (>20mg/dL) suggest an active fetal immune response to an infectious organism. Serology should be performed to test for BHV-1, BVD, *Leptospira, Neospora, Brucella*, bluetongue virus, and PI3.

Abortifacient organisms invade the dam by different routes: skin (*Leptospira*), conjunctiva (*Brucella*), respiratory tract (IBR, BVD), vaginal (*Campylobacter, Tritrichomonas*, and *Ureaplasma*), and mouth (BVD, *Listeria, Leptospira*). The placenta is infected by either hematogenous or venereal routes. Placental inflammation/infection puts stress on the fetus with potential spread of the organism to the fetus. The result depends on the stage of gestation. In the first trimester, placental and/or fetal infection induces fetal death, resorption, mummification, maceration, or abortion. During the second trimester, infection causes fetal death, abortion, or mummification. Fetal and/or placental infection during the third trimester results in fetal death, abortion, maceration, mummification, emphysema, stillbirth, or birth of weak nonviable calves. The dam might develop endometritis, metritis, or retained placenta following pregnancy loss due to placental/fetal infection.

Infectious Causes of Abortion

Bacterial

There are some opportunistic organisms which can come overgrown the uterus resulting in sporadic abortion. These include *Trueperella pyogenes, E. coli*, and *Bacillus sp.* As these organisms are ubiquitous to the environment or part of the normal flora in the reproductive tract, contamination of the placenta or fetus can occur during delivery or sample collection thus caution must be taken when interpreting laboratory results. The sporadic abortions due to T. *pyogenes* usually happen in the second half of gestation. Typically, lesions are not present on the fetus, however small white foci have been reported in the lungs of feti aborted in the first half of gestation. *Bacillus sp.* causes sporadic abortion in the last trimester and the fetus has pericarditis.

Brucella abortus is a zoonotic, reportable bacterial disease in cattle. This disease is uncommon in the United States due to stringent eradication programs. Transmission most commonly occurs via contact with aborted tissues or fluids, but can also be spread through the milk or *in utero*. The organism has an affinity for the erythritol present in placental trophoblast. Abortion rates can reach as high as 80% in unvaccinated herd. The most common time for *Brucella* to induce abortion is from six months on to term. Placentitis is a consistent finding. The membranes will be thickened with yellow exudate, and greyish yellow debris is present around the cotyledons. The placenta has a leathery appearance, causing it to be referred to as 'Moroccan leather placenta'. The fetus is usually autolyzed, due to being expelled 1-4 days after death. Pneumonia is commonly found in the aborted fetus, with a characteristic cobblestone texture to the lung surface with small white foci. Metritis and retained placenta are common sequela to *Brucella* abortions. The gold standard of diagnosis for *Brucella* is isolation of the organism from placenta or fetus (lung, spleen, abomasal contents). Serology is the best method of test and is usually performed by card agglutination test.

Campylobacter fetus subspecies *venerealis*, also known as Vibrio, is a bovine venereal disease causing early embryonic death, infertility, and occasionally abortion. It is an obligate pathogen of the bovine reproductive tract. The organism is deposited in the vagina after coitus with an infected bull and ascends to the uterus within two weeks. Embryonic death occurs within the first 2 months of gestation. The conceptus is usually lost after maternal recognition of pregnancy resulting in a prolonged interestrus period. Therefore, the most common indications seen in the herd are repeat breeders and infertility. Conception rates have been reported as low as 10% during a herd outbreak. Although uncommon, abortions can also occur between 4-6 months of gestation. Fetal lesions include fibrinous pleuritic, pericarditis, and peritonitis. Placental lesion are similar to those seen with *Brucella* (leathery intercotylendary areas) however the placenta is typically expelled. Diagnosis can be achieve by multiple methods. Organisms can be seen in abomasal fluid by dark field microscopy or cultured from aborted tissues. If submitting for culture, it is best to ship samples in Amies transport media, with or without charcoal.

Chlamydia is an intracellular gram negative bacteria. Clinical signs include abortion, polyarthritis, encephalomyelitis, keratoconjunctivitis, pneumonia, enteritis, hepatitis, vaginitis, and infertility. *Chlamydophila abortus* is the most common *Chlamydia* species to induce abortion in cattle, however it is still an unlikely cause of bovine abortion. *Chlamydia* abortions are more sporadic in cattle than small ruminants. Abortions typically occur during 6-8th month of gestation and are accompanied with a necrotizing placentitis. The fetus may have edema, ascites, pleuritic, or peritonitis. Histologic examination of the cotyledons can be diagnostic as the organisms multiple in the cotyledons. Isolation of the organism is gold standard for diagnosis, but this requires specialized media. Members of the *Chlamydia* family are found in the gastrointestinal tract of cattle, so false positives can occur in cases of fecal contamination on aborted tissues. Serial titers can be helpful, as the level is usually normal at the time of abortion but rises 2-3 weeks later.

Leptospira interrogans serovar *hardjo* and *Pomona* are the most likely to cause leptospirosis abortion in cattle. The *hardjo* strains are host adapted to cattle whereas cattle are an incidental host for *Pomona*. The organism is transmitted by urine, transplacental, venereally, orally, and across the conjunctiva. *Leptospira* localizes to the reproductive and urinary tracts. Bacteremia occurs within two weeks after inoculation. Abortion occur during the last trimester, and infection can also induce delivery of weak infected calves. Abortion rates due to *Pomona* are much higher than *hardjo*, 50% versus 10-30% respectfully. The aborted fetus is usually expelled autolyzed and may be icteric. Intercotyledonary edema is present in the placenta and occasionally yellowish brown fluid and necrotic cotyledons are present. This is similar to what is seen with BHV-1 abortions. Good samples to submit for isolation of the organism include kidney, pericardial fluid, and abomasal fluid. A diagnosis can be made by serology using microscopic agglutination test. Immunohistochemistry (IHC) for the organism can be performed on formalin-fixed tissues.

Listeria monocytogenes causes meningoencephalitis, neonatal septicemia, and abortion in cattle. It most common route of transmission is by ingestion of contaminated silage. The organism can spread to the placenta via the blood in 5-12 days inducing fetal septicemia and death. Abortions most commonly occur in the winter months, when cows are in the last trimester of pregnancy. The dam typically is pyrexic and anorexic prior to abortion. The fetus is expelled a few days after death resulting in autolysis of tissues. This masks any lesions that may be present on the fetus. The placenta will have cotyledonary necrosis and intercotyledonary placentitis characterized by greyish white to reddish brown exudate. Often the placenta is retained. Other clinical signs seen in the cow include circling and blindness due to encephalitis. The dam can be clinical ill pre-, para-, or post-abortion. The dam can also become re-infected and abort again if continues to be feed contaminated silage. Diagnosis is commonly made by organism isolation, although culture can be slow (up to one month). Gram staining of abomasal fluids or impression smears of tissues can also reveal the presence of the organism.

Ureaplasma diversum is part of the normal flora of the bovine reproductive tract. In cases of overgrowth, this organism can cause early embryonic death, late term abortion, stillborn/weak calves, and neonatal pneumonia. Typically, the dam will not have clinical signs of illness but will retain the placenta. It induces a characteristic hemorrhagic amnionitis. The membranes will be thickened and opaque with ecchymotic fibrin, necrosis, and fibrosis. Diagnosis is usually by culture of the organism from abomasal fluid, placenta, or lung.

Viral

Bovine herpes virus 1 (BHV-1) is an alphaherpes virus, causing abortion, genital disease, respiratory disease, and encephalomyelitis in cattle. BHV-1.1 and 1.2a are more associated with abortion and 1.2b with genital disease. Adult cattle have mild clinical signs including conjunctivitis and respiratory signs. Abortion most commonly occurs in the second half of gestation, weeks to months after clinical disease. The virus spreads to the placenta then the fetus. The fetus is expelled autolyzed with pin-point white foci on the liver. Placentitis is similar to that seen with Leptospirosis. Histopathology of fetal lung, liver, spleen, kidney, and placenta can be diagnostic, but should be confirmed by IHC, PCR, fluorescent antibody detection, or virus isolation.

Bovine viral diarrhea virus (BVD) is a pestivirus that causes significant reproductive loss in cattle. Effects on reproduction include genital infection, embryonic death, birth defects, abortion, delivery of small calves, and persistently infected calves. Transplacental infection occurs during the viremic state in the dam. Infection will have a different outcome depending on the stage of gestation at time of infection. Fetal infection in the first trimester causes early embryonic death and resorption. In the second trimester, infection results in abortion and mummification. Abortions most commonly occur 4 months to term. Persistently infected calves develop when infection with the noncytopathic strain occurs before day 125 in gestation. Infections occurring between days 100-150 of gestation can develop birth defects such as hydrocephalus, cerebellar hypoplasia, microphthalmia, retinal dysplasia, cataracts, and brachygnathism. Diagnosis can be made by PCR, IHC, antigen capture ELISA, fluorescent antibody test, and virus isolation. Sample types needed depend on the test being submitted. An ear notch is the most common sample taken to test for persistently infected animals and can be submitted for immunohistochemistry, antigen capture ELISA, or PCR.

Bluetongue is an arthropod spread orbivirus with a worldwide distribution. The disease may appear seasonal, depending on the density of the *Culicoides* midge. Clinical signs in adult cattle typically manifest as ulcers in the mouth and tongue. Hyperemia and ulceration may also occur at the coronary band, progressing to sloughing of hooves in severe cases. Bluetongue virus replicates in endothelial cells, macrophages, and lymphocytes, resulting in cell death. Reproductive signs associated with infection are congenital abnormalities, abortion, mummification, and stillborn calves. Infection between 70-130 days of gestation can cause abortion and hydranencephaly. After day 150 of gestation, encephalitis or premature delivery occurs

but not malformations. Diagnosis can be difficult as the virus may no longer be present at time of abortion, but virus isolation from blood, spleen, or lymph nodes is the best test.

<u>Protozoal</u>

Neospora caninum is a protozoal parasite of canines. Cattle serve as an intermediate host by ingesting canine feces. Vertical transmission occurs in cattle and can pass through multiple generations of females via infection *in utero.* Abortion may be the only clinical sign present in the herd, typically occurring between 5-6 months of gestation. Calves can be stillborn, mummified, malformed, or born alive. Aborted fetus are usually autolyzed. The placenta may have cotyledonary necrosis with soft areas of dark discoloration. The parasite causes lesions in the fetal brain. IHC can be used to identify the parasite in tissues. Interpretation of maternal titer can be complicated as levels fluctuate or may decline over time and some cows become persistently infected. Pre-colostral serum sample of calves can help to interpret infection in suspected cows, as calves infected in utero will have high titers.

Tritrichomonas foetus is a flagellated protozoal parasite that causes bovine venereal disease. The organism is deposited into the vaginal during coitus with an infected bull. The organism ascends to the uterus, inducing early embryonic death after maternal recognition of pregnancy. Cows show a prolonged inter-estrus period before returning to estrus. Rarely pyometra occurs in infected cows. Late term abortion occasionally occurs, with fetal lesions of bronchopneumonia and enteritis. Diagnosis is made by culture and/or PCR of vaginal fluids or fetal abomasal fluid. Commercially available media is available for shipment and culture.

<u>Fungal</u>

The most common mycotic abortions are due to *Aspergillus fumigatus*, other fungal agents that are routinely involved include *Mucor* and *Rhizopus*. Clinical sign are absent in the dam. Placentitis develops slowly, disrupting fetal nutrition leading to death. Abortions occur in the last trimester. The placenta is usually retained and has a leathery appearance, similar to *Brucella*. Fungal organism form lesions along the periphery of the placenta and tend to run with blood vessels. Irregular tan plaques develop on fetal skin. Diagnosis is made by culturing the fungi from aborted tissues or abomasal fluid, or by fungal identification on KOH wet mounts

<u>Rickettsial</u>

Anaplasma marginale induces a rickettsial disease in cattle and other hoofstock. The organism is transmitted by arthropods or fomites (such as reusing needles). It is able to cross the placenta to the fetus. The organism invades erythrocytes inducing extravascular hemolysis. Clinical signs seen in adult cattle include icterus, anemia, fever, weakness, and inappetence. Fetal loss occurs due to the maternal systemica illness, including severe anemia, stress, hypoxia, and pyrexia. Abortion can occur at any point in gestation. The aborted fetuses will have splenic enlargement, as well as lung and liver petechiation. Giemsa stained blood smears will reveal the presence of the organism on erythrocytes in acute cases, however this is not the case in carrier animals. Serology and PCR are available to aid in diagnosis.

Noninfectious Causes of Abortion

Nutritional

A wise farmer once told me "you can't starve a profit out of a cow". This is especially true for the pregnant cow. In most cases, starvation will result in termination of pregnancy, as the body prioritizes. Nutritional deficiencies are associated with infertility and neonatal mortality. Nutritional excesses have also been reported to cause reproductive issues.

Deficiencies

- Protein premature calves, dystocia, and high neonatal mortality of poorly muscled calves (known as 'weak calf syndrome')
- Vitamin A later term abortion, abnormal musculoskeletal and neural development, and weak, blind calves

- Iodine hyperplastic goiter and hairless, weak calves
- Selenium/Vitamin E premature calves, stillbirths/abortions, and weak calves

Excesses

- Iodine abortion
- Selenium/Vitamin E abortions

Heat Stress

- Embryos are extremely sensitive to heat stress induced by high ambient temperatures.
- Can induce fetal hypotension, hypoxia, and acidosis

Hormonal abnormalities

- Endotoxemia and metritis induce inflammation which stimulates the release of endogenous $PGF2\alpha$ causing lysis of the corpus luteum.
- Estrogen
 - \circ Silage, Legumes, Poultry litter \rightarrow Abortion

Genetics

- Pregnancy loss before day 90 of gestation
- Chromosomal abnormalities
- Lethal genes

<u>Toxins</u>

- Nitrates
 - \circ Methemoglobin \rightarrow Hypoxia \rightarrow EED & Abortion
- Mycotoxins zearalenone
- Abortions
- Ergot alkaloids
 - \circ Abortions

Investigation of pregnancy loss in a herd can be a labor intensive, frustrating process. Since there are numerous noninfectious and infectious causes, diagnosis can be complicated. A detailed history and appropriate sample collection are key to success. It is important to submit the fetus, placenta, and maternal serum for laboratory testing and work with the diagnostic laboratory for optimal sample handling and appropriate test selection. The clinical findings should be combined with laboratory test results and herd investigation findings to determine if the presumptive diagnosis fits the clinical picture. An infectious cause is identified less than 50% of the time, in such cases noninfectious causes should be explored. As a bovine practitioner, investigating pregnancy loss in a herd can be challenging, but important undertaking with the goal of improving herd health and ultimately profitability.

Bacterial						
Infectious Agent	Abortion Rate	Abortion Time	Recurrence	Lesions	Samples	Diagnostics
Brucella abortus	Up to 80%	6-9 months	Rare	Placenta – retained, necrotic cotyledons, red-yellow color, thickened intercotyledonary areas	Fetus (lung, abomasal fluid), placenta, maternal blood	Maternal serology, FAT for antibodies in placenta, Bacterial culture
Campylobacter fetus ss venerealis	<10%	Early embryonic death, 5-8 months	Rare	Placenta – mild placentitis, hemorrhagic cotyledons, intercotyledonary edema	Fetus (lung, abomasal fluid), placenta, vaginal fluid	IHC, Darkfield microscopy, Bacterial culture
Campylobacter fetus ss fetus	Sporadic	Second and third trimester	Rare	Same as above	Same as above	Same as above
Chlamydophila abortus	Sporadic	Last trimester	Rare	Placenta – placentitis, thickened yellow-brown exudate Fetus – pneumonia, hepatitis	Placenta, fetus	Acid-fast staining, IHC, FAT, PCR
Leptospira interrogans serovars pomona, grippyotyphosa, hardjo, icterohaemorrhagiae, canicola	5-40%	Last trimester	Susceptible to other serovars	Placenta – diffuse placentitis, avascular pale tan cotyledons, yellow edematous intercotyledonary areas Fetus – autolyzed	Placenta, fetus (kidney)	FAT, PCR, IHC
Listeria monocytogenes	Sporadic to >50%	Last trimester	Possible	Placenta – retained, white necrotic foci in cotyledons Fetus – autolyzed fibrinous polyserositis, small liver with white necrotic foci Dam – pyrexic, inappetence, neurologic signs	Placenta, fetus (brain, lung, abomasal contents)	Bacterial culture, IHC, PCR
Ureaplasma diversum	Sporadic	Early embryonic death, third trimester, weak born calves	Possible	Placenta – retained, white opaque amniotic fibrosis, nonsuppurative placentitis Fetal – interstitial pneumonia	Placenta, fetus (lung, abomasal contents)	Bacterial culture, PCR

Protozoal						
Infectious Agent	Abortion Rate	Abortion Time	Recurrence	Lesions	Samples	Diagnostics
Tritrichomonas foetus	Sporadic	Early embryonic death	Immunity developed but not life long	Placenta – retained, mild placentitis with hemorrhagic cotyledons and thickened intercotyledonary areas	Placenta, fetus (lung, abomasal contents), vaginal or uterine fluid	Culture, PCR, IHC, Bodian's silver stain
Neospora caninum	Up to 30%	5-6 months	Possible but decreases with parity	No gross lesion on placenta or fetus Microscopic lesions – focal encephalitis with necrosis and nonsuppurative inflammation, hepatitis	Placenta, fetus (brain, kidney, lung, liver, skeletal muscle), maternal blood	Histopathology (brain), IHC, PCR, ELISA

Viral						
Infectious Agent	Abortion Rate	Abortion Time	Recurrence	Lesions	Samples	Diagnostics
Bovine Viral Diarrhea Virus (BVD)	Low	Up to 4 months	Rare	Placenta – retained Fetus – autolyzed, mummified, hydrocephalus, cerebellar hypoplasia, microphthalmia, retinal dysplasia, cataracts, brachygnathism	Placenta, fetus (lung, liver, skin, heart), maternal blood	FAT, IHC, Virus isolation, Antigen-capture ELISA, PCR
Bovine Herpesvirus type 1 (Infectious Bovine Rhinotracheitis virus – IBR)	5-60%	4 months to term	Rare	Usually no lesions in placenta or fetus Placenta – necrotizing placentitis Fetus – autolyzed, focal necrosis in liver	Placenta, fetus (adrenal, kidney, liver, lung) maternal blood	IHC, Virus isolation, FAT
Bluetongue virus	Low	Variable	Rare	Fetus – autolyzed, hydrocephaly, arthrogryposis, dwarfism, excessive gingival tissue	Placenta, fetus (brain, spleen), maternal blood	PCR, Virus isolation

Fungal							
Infectious Agent	Abortion Rate	Abortion Time	Recurrence	Lesions	Samples	Diagnostics	
Aspergillus Mucor Rhizopus	Sporadic, up to 5-10%	4 months to term	Possible	Placenta – severe necrotizing placentitis, enlarged cotyledons, leathery thickened intercotyledonary areas Fetus – autolyzed, gray round lesion on neck and head	Placenta, fetus (abomasal contents, lungs, skin,)	Fungal culture, H&E, KOH	

Rickettsial							
Infectious Agent	Abortion Bate	Abortion Time	Recurrence	Lesions	Samples	Diagnostics	
Anaplasma marginale	Low	Variable	Possible	Fetus – splenic enlargement, lung and liver petechiation	Blood, Fetus	Organism identified on blood smear, PCR	

*ELISA=enzyme-linked immunosorbent assay *FAT=fluorescent antibody test *IHC=immunohistochemistry *KOH=potassium hydroxide test *PCR=polymerase light chain

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