

A Review of Infectious Pathogens Causes of Reproductive Failures in Bull Sperm

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Abstract

The objective of this paper is to review scientific evidence regarding infectious pathogens transmitted sexually in bulls with a focus on prevalence and diagnosis. Bull semen quality is one of the important keys to the success of artificial insemination (AI). According to the world organization of animal health (OIE), there are several pathogens that should be excluded in semen examination. The OIE International Animal Health Code, published in 1992, describes the accreditation of artificial insemination centers for export purposes, including the risks of infectious pathogens transmitted through semen. The pathogens have been classified as viral, bacterial and protozoal agents. Some of these agents can affect reproduction performance for instance, bull fertility and reproductive failures in cows inseminated. Hence the diagnosis of these pathogens is crucial factor to prevent infectious male infertility and diseases transmission.

Keywords: Bull semen, disease transmission, fertility, reproductive failure

Introduction

Beef demand in Indonesia is increasing every year. There are several factors that boosting the demand of beef product in developing countries including the growth of population, urbanization, economic refinement and consumer preferences shift (Delgado *et al.*, 2001). It has been a government concern to enhance cattle population since 2001 through the meat self-sufficiency program (PSDS) followed by a special effort required pregnant cows (UPSUS SIWAB) program (Ditjen PKH, 2017). Unfortunately, up to now Indonesia has not been able to meet the necessity. There has always been a gap between supply and demand of beef whereas beef production only satisfied 45% of national demand (Kementerian Pertanian, 2017). The inadequacy is attained from imported products in form of feed fattening cattle and frozen beef (Agus & Widi, 2018). In furtherance of improvement of beef cattle population, conception rate and offspring performance as a target for meat sufficiency in 2026, the implementation of Artificial Insemination (AI) assistance can be done (Kementerian Pertanian, 2016). A single selected bull can reproduce qualified progenies and

prevents sexual-transmitted diseases (Rusdiana & Soeharsono, 2017). Therefore, fertility and healthy reproductive system are of upmost in cattle breeding through AI (Sannat *et al.*, 2015). The success of AI depends on semen quality and AI practices (Koziola *et al.*, 2022). In Indonesia, 6 million of cryopreserved bovine semen are produced from BBIB Singosari, BIB Lembang, BIBD Banjarbaru and BIBD Ungaran and distributed for UPSUS SIWAB each year (Handayani *et al.*, 2021). Preventing the dissemination of pathogen is an obligatory for the AI industry (Givens, 2021). Improvement of reproduction not only enhancing cattle population but also the economy and living standards of many rural communities throughout the world.

Some viral, bacterial and protozoal may infects bull reproduction system and contaminates semen. The infection may lead into an outbreak if it caused by pathogen transmitted sexually. The DNA or RNA agents detected in semen might not presence in blood testing to result in transmission to cows either by natural mating or through artificial insemination (Givens, 2018). Pathogens that contaminate sperm at the time of storage from equipment and work can

endanger the quality of the collected semen so that it can contaminate the female reproductive tract. Pathogenic microorganisms can damage sperm quality in two ways, namely directly affecting sperm quality and indirectly affecting sperm production. Most infecting pathogens show mild symptoms in their hosts but have a fatal impact during reproduction, causing fetal development failure (Reichel *et al.*, 2018). To reduce the risk of diseases transmission, not only blood samples examination should be done but also clear and detailed standard examination of semen from bull. Routine and continually monitoring of hygiene status of semen production must be done regarding vitality of spermatozoa, fertility of inseminated cows and diseases transmission prevention (Mitra *et al.*, 2016).

There are various conditions required of the breeding bulls and their semen depends on the country. A detailed standard semen collection from donor bulls have been established to reduce semen borne pathogen transmission. According to Ministry of Agriculture Rules Number 10/PERMENTAN/PK.210/3/2016, Indonesia set free-12-diseases frozen semen from viral, bacterial and protozoal agents. Diseases from viral pathogens including bovine viral diarrhea (BVD), infectious bovine rhinotracheitis (IBR), enzootic bovine leucosis (EBL) and Jembrana for Bali cattle. Several bacterial agents causing anthrax, brucellosis, septicaemia epizootical, bovine tuberculosis, paratuberculosis, leptospirosis and campylobacteriosis were examined from semen production. Other examination is should be done for

Trichomoniasis, a sexually transmitted disease from protozoal agent (BBIB Singosari, 2022). The purpose of this paper is to describe the different type of infectious pathogen transmitted sexually or transmitted via bovine semen which affecting the reproductive performance and infertility of bulls. The epidemiology of diseases and diagnostic test prescribed are reviewed.

Semen-Borne Pathogens

The infectious microorganisms affecting the reproduction system and fertility in several ways, such as direct effect on the reproductive system and the quality of semen. Regardless, other system that might affected are decreasing semen quantity and quality, prevent libido occurrence, mating ability and prolonged reaction time (Perumal *et al.*, 2013). The World Organization for Animal Health and The International Embryo Technology Society listed pathogens that should be free for bovine semen production (Table 1).

Different countries have spesific requirements for SPF semen certification. Agricultural authorities and veterinarian in Indonesia require a minimum of viral, bacterial and protozoal includes Bovine viral diarrhea virus (BVDV), Bovine herpesvirus 1 (BoHV-1), Bovine leukemia virus (BLV), *Brucella abortus*, *Pasteurella multocida*, *Mycobacterium bovis*, *Mycobacterium avium* subsp. *Paratuberculosis*, *Leptospira* sp., *Campylobacter fetus* subsp. *Venerealis*, and *Trichomonas foetus*.

Table 1. List of Spesific Pathogen Free (SPF) in bovine semen

Bacterial Diseases	Viral Diseases	Protozoal Diseases
Brucella abortus (Brucellosis)	Bovine Viral Diarrhea Virus (BVDV)	<i>Trichomonas foetus</i> (Trichomoniasis)
Campylobacter fetus subsp venerealis (Campylobacteriosis)	Infectious Bovine Rhinotracheitis Virus (IBR, BHV-1)	
Leptospira spp (Leptospirosis)	Bluetongue Virus Schmallenberg Virus	

Viral Pathogens

There are three important viral pathogens transmitted through semen that continually evaluated for donor bulls in Indonesia. The presence of this viral agents may lead reproduction disturbance and infertility. OIE guidelines to maintain health of donor bulls in Artificial Insemination Center is a requirement for international distribution of semen with

negligible risk of infecting people or animals with pathogens. Major viral pathogen diseases that could transmitted through frozen semen are reviewed.

Bovine Viral Diarrhea

The disease is classified as an important disease regarding animal health and economic impact. BVD distributed in cattle population worldwide. The virus replicates in the seminal

vesicles, prostate gland, and epididymis of the bull (Kirkland *et al.*, 1991). It belongs to the genus Pestivirus of the family Flaviviridae. BVD antigen has also been detected in the epithelial cells of the ductus epididymis, accessory glands, urethra, Sertoli cells, and spermatogonia (Borel *et al.*, 2007). Detection of virus in testicular tissue might result prolonged testicular infection with detection time for 7 month following an acute infection (Givens *et al.*, 2003). Eventhough the pathogen can be found in bull semen, it may not always affect semen quality, particularly those in acute or persistent infections (Fray *et al.*, 2001). Artificial insemination with contaminated semen from an acutely or persistently infected bull may induce an infection in female cattle (Kirkland *et al.*, 1997). This syndrome lead fertility problems in heifer. The clinical signs that might occurs are reduced conception rates, production of persistently infected calves, or birth of clinically normal offspring (Givens & Waldrop, 2004). Various effects on fertility in cattle may be due to strain differences of BVD virus (Evans *et al.*, 2019). Characterization of the agent and surveillance system of pestivirus globally should be carried out, especially the country whereas BVD poorly investigated and those that may have a major influence on other parts of the world, for example, due to export of foetal bovine serum (FBS) or semen (Wrathal *et al.*, 2006).

Infectious Bovine Rhinotracheitis

Infectious bovine rhinotracheitis is caused by bovine herpesvirus 1. The virus can cause clinical disease of the male reproductive tract which is called infectious balanoposthitis (Oirschot *et al.*, 2000). The bovine herpesvirus 1 colonizes and replicates in the mucosa of the prepuce, penis, and distal urethra, and can be present in the seminal plasma of infected bulls (Tanghe *et al.*, 2005). Semen quality from infected bull may not affect motility or acrosomal status. The virus can inhibit sperm-zona binding by interacting with spermatozoa (Oirschot *et al.*, 1995). Bovine herpesvirus 1 is a sexually transmitted disease and contaminated semen may lead a result in infection, reduced conception rates, endometritis, abortion, and infertility. Infected bulls developed persistent infection and may carried the virus throughout life (Eaglesome *et al.*, 1997). In the opposite, there are indications that seropositive bulls managed in a low stress environment showed free of virus for prolonged intervals (Hiraoka *et al.*, 2022).

Enzootic Bovine Leucosis

Bovine Leukimia Virus is the causative agent of Enzootic Bovine Leucosis. The Deltaretrovirus produces a benign persistent lymphocytosis. BLV is found in lymphocytes and in some adult cattle, the infection developed lymphatic tumors (Givens, 2018) but there are only small number of BLV-infected cattle develop EBL and present with B-cell lymphosarcoma (OIE, 2017). Infected animals can transmittes the virus horizontally via secretions contact, iatrogenically via contaminated instruments such as tattoo pliers and dehorning gouges, and vertically via transplacental exposure (Givens, 2021). There has been one study that reported the pres. A seropositive status is considered evidence for ongoing infection. The virus is rarely found in semen though possibly present in association with virus-infected lymphocytes in the genital tract (Van Oirschot *et al.*, 2000). While substantial evidence demonstrates that BLV is rarely – if ever – transmitted by artificial insemination, transmission was detected when infected lymphocytes were inoculated into the uterus of seronegative cows (Van Oirschot *et al.*, 2000). To ensure that semen is free of BLV, Chapter 11.6 of the OIE terrestrial animal health code recommends that semen donor bulls be residents of BLV-free herds and come from a serologically negative dam (if less than 2 years of age), or that blood samples from the bull test negative for antibodies against BLV on two occasions, the first being at least 30 days before and the second at least 90 days after collection of semen (Prihandani *et al.*, 2011).

Bacterial Pathogens

Bacterial agent examination from bulls is focused on anthrax, brucellosis, septicemia epizootical, bovine tuberculosis, paratuberculosis, leptospirosis and campylobacteriosis. Anthrax is excluded from the discussion since it not sexually transmitted bacterial agent.

Brucellosis

Brucella abortus is an organism that can also localize in the reproductive tract of the bull. The cells of the genital tract contain high concentrations of erythritol which enhances the growth of this zoonotic pathogen. Infection can lead to orchitis, epididymitis, seminal vesiculitis, ampullitis, decreased libido and infertility. The

organism can also be present in collected semen (Hiraoka *et al.*, 2022).

Septicaemia epizootical

This disease is caused by the bacterium *Pasteurella multocida*. *Pasteurella multocida* is a pathogenic bacterium that usually lives in the upper respiratory tract of the host. This attack of the respiratory tract can cause high fever, swelling in the neck area that extends upwards and into the chest area until death. This condition occurs endemic and fatal to livestock so that it can interfere both economically and environmental health. This pathogen is the causative agent of Haemorrhagic septicemia in cattle and has an impact on reducing farmers' income. Hemorrhagic septicemia is also known as pneumonic pasteurellosis, and is popularly known as 'snoring' disease or septicemia epizootica in Indonesia. *Pasteurella multocida* isolates were categorized mainly into five groups (A: hyaluronic acid, B, D: heparin, E, and F: chondroitin). Based on the capsule antigen and based on the lipopolysaccharide antigen (LPS) it is classified into 16 serotypes. Each of the above serogroups indicates host and causative disease specificity and susceptibility. For example, serotype A is the causative agent of chicken cholera in poultry, and has even been isolated from pigs with pneumonia in Korea. *Pasteurella multocida* B and E are *P. multocida* promoters of hemorrhagic septicemia in cattle and buffalo. *Pasteurella multocida* D and F are the causative agents of persistent atrophic rhinitis (PAR) and pneumonia in pigs (Srinivasan *et al.*, 2018).

Bovine tuberculosis

Bovine tuberculosis (BTB) is a chronic disease that attacks livestock. Have a negative impact on productivity and environmental health. In addition, it can result in considerable economic losses as well as zoonotic risks. This disease is considered endemic. BTB is caused by *Mycobacterium bovis* (*M. bovis*) is a member of the *Mycobacterium tuberculosis* group (Lilenbaum & Martins, 2014).

Paratuberculosis

Paratuberculosis is a chronic granulomatous infection that occurs in the intestinal tract of cattle and other domestic and wild ruminants. This disease is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). These organisms are acid fast, gram positive, facultative intracellular pathogens that require iron for

growth and have the ability to survive in macrophages. Under ideal conditions, it can survive up to one year in the environment. MAP has also been shown to withstand pasteurization. Because of the long incubation period of MAP (between 2 and 7 years), the majority of infected cattle (about 95%) are considered to be in the subclinical stage, with less than 5% of infected cattle showing clinical signs of disease. The term Johne's disease usually refers to the clinical syndrome of diarrhea and weight loss due to advanced MAP infection.

Leptospirosis

This disease is one of the major influences on the development of ruminant reproductive health, such as infertility. Leptospirosis is a zoonotic disease that is influenced by spirochaete pathogens, belonging to the genus *Leptospira*. These microorganisms spread in the proximal renal tubules of ruminants and are excreted in the urine. Transmission of leptospirosis is mainly by exposure to water or soil contaminated with the urine of infected animals or through direct contact with infected animals. Infectious conditions are divided into 2 groups, namely adaptive strains and host carriers. This condition causes subclinical infection. The second group is strains that exist in the environment. This group often becomes plague (Campos *et al.*, 2017). Various ways have been done to find out techniques for prevention and treatment of this strain. Cases of acute leptospirosis are very rare. Control of leptospirosis in cattle is carried out in order to develop identification and treatment in subclinical cases, quarantine is carried out on infected animals by treatment with antibiotics and systematic immunization with vaccines. Several methods have been used to detect them, including through the association of serological tests as agglutination microscopic test (MAT) (BonDurant, 2005) for screening/detection of seroactive in livestock groups and analysis of urine through the Polymerase Chain Reaction (PCR) method, an individual approach was carried out. It is considered suitable for detecting kidney disease carrying leptospira in cattle.

Campylobacteriosis

Campylobacteriosis is caused by *Campylobacter fetus* subsp. *venerealis* (Cfv) which is a microaerophilic Gram-negative bacterium. The organisms reside on the penile and preputial mucosa of infected bulls, causing

no clinical signs in the male. Occurrence of CfV is restricted to the genital tract of cattle and is furthermore responsible for bovine genital campylobacteriosis (BGC) (Felleisen, 1999). BGC characterized by infertility and abortion in cattle (Campero *et al.*, 2017).

Protozoal Pathogens

Trichomoniasis

Bovine trichomoniasis is a parasitic disease caused by *Trichomonas foetus*. There are no specific clinical signs of infected bull except a preputial discharge associated with small nodules on the preputial and penile membranes in the early stage of infection (Felleisen, 1999). To the contrary, the flagellate transmitted to female cattle might develop vaginitis, endometritis, premature abortions, pyometra and infertility (Yao, 2021). The infected animals may be carriers for their whole life. The parasite resists in the diluted semen and to freezing. Therefore the donors have to be tested frequently either through a microscopic examination or through a cultural test in order to exclude any infection.

Diagnosis and Control

Detection of semen-borne pathogen can be done in several ways. The most popular is detecting and identifying bacteria in clinical specimens by genotype instead of phenotype (Relman, 1998). PCR amplification with primers to conserved regions results in PCR amplicons of the species-specific regions, which can be sequenced and compared with reference microbial gene banks for identification is the most accurate in specificity and sensitivity (Kiessling *et al.*, 2008). This approach can be used for various clinical samples such as blood and semen. Control of pathogen of sexually transmitted diseases can be done continually during blood and semen collection. Table 2 shows the diagnostic methods for pathogens in semen.

Minimizing the risk of sexually transmitted diseases distribution is important. Good health and physical status of bulls should be maintained properly. Biosecurity protocols become necessary to reduce the probability of introduction diseases. Biosecurity and standard operating practice manuals should be present and frequently updated and followed to reduce the potential for introduction of disease (Givens, 2021).

Table 2. Diagnostic methods for pathogens in semen according WOA (The World Organisation for Animal Health)

Disease	Pathogen	Diagnostic Method
Bovine Viral Diarrhea (BVD)	Bovine viral diarrhea virus (BVDV)	Virus isolation or antigen detection tests on blood samples; serological testing to determine antibody presence
Infectious Bovine Rhinotracheitis (IBR)	Bovine herpesvirus 1 (BoHV-1)	Serological testing and virus isolation from semen samples.
Enzootic Bovine Leukosis	Bovine leukemia virus (BLV)	Serological testing to detect antibodies against BLV.
Brucellosis	<i>Brucella abortus</i>	Serological tests such as the Rose Bengal test or complement fixation test.
Septicemia Epizootica (Hemorrhagic Septicemia)	<i>Pasteurella multocida</i>	Bacterial culture and isolation from blood or tissue samples.
Bovine Tuberculosis	<i>Mycobacterium bovis</i>	Intradermal tuberculin testing; culture and PCR for confirmation.
Paratuberculosis (Johne's Disease)	<i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i>	Fecal culture, PCR, and serological tests.
Leptospirosis	<i>Leptospira</i> spp.	Microscopic agglutination test (MAT) and PCR for detection in urine or semen.
Campylobacteriosis	<i>Campylobacter fetus</i> subsp. <i>venerealis</i>	Culture and PCR testing of preputial samples.
Trichomoniasis	<i>Trichomonas foetus</i>	Culture and PCR

Conclusion

Semen quality examination is crucial step in selection of bull for the purpose of artificial insemination (AI). This technique increasing the productivity and profitability through better conception rate and better generation performance. This review help to describe sexually transmitted pathogen, how to diagnose and control the diseases so that the productivity and the profitability of a particular commercial herd can be enhanced.

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