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Infectious agents affecting fertility of bulls, and transmission risk through semen. Retrospective analysis of their sanitary status in Colombia¤

Agentes infecciosos que afectan la fertilidad del toro y su riesgo de trasmisión por el semen. Análisis retrospectivo del estado sanitario en Colombia

Agentes infecciosos que afetam a fertilidade do touro e seu risco de transmissão pelo sêmen. Análise retrospectivo do estado de saúde na Colômbia.

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Summary

The most important infectious diseases that affect fertility of the bull, and their transmission via semen are reviewed in this article. Additionally, a retrospective analysis of the diseases reported in Colombia was also addressed. In general, there is high seropositivity for IBR and BVD, two diseases that can be transmitted by semen due to viral latency and persistence, and lack official control programs in Colombia. It is necessary to move forward with the support of livestock associations and animal health institutions in order to establish true artificial Insemination centers that allow a permanent surveillance of donor´s health status, and the production of pathogen-free semen as a way to control transmission of diseases via semen.

Key words: *bull, Colombia, fertility, infectious agents*.

Resumen

Se revisa en este artículo las principales enfermedades infecciosas que afectan la fertilidad del toro y su trasmisión por semen. Se analiza a la vez, en forma retrospectiva, el estado sanitario de los reproductores a partir de algunos estudios realizados en Colombia. En general, existe una alta seropositividad para IBR y DVB,

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dos enfermedades que pueden ser trasmitidas por el semen en razón de la latencia y persistencia viral y para las cuales no existe un programa de control oficial. Se plantea la necesidad de avanzar con el apoyo de las asociaciones ganaderas y las instituciones encargadas de la sanidad Animal en el establecimiento de verdaderas centrales de inseminación artificial que permitan un monitoreo permanente del estado de salud de los donantes y la obtención de semen libre de enfermedades como una forma de controlar la trasmisión por esta vía.

Palabras clave*: agentes infecciosos, Colombia, fertilidad, toros.*

Resumo

Foi feita uma revisão das principais doenças infecciosas que afetam a fertilidade de touros e sua transmissão pelo sêmen. Também foi analisado retrospectivamente o estado sanitário de reprodutores a parir de alguns estudos realizados na Colômbia. Em geral, há uma alta soropositividade para IBR e BVD, duas doenças que podem ser transmitidas pelo sêmen por causa da latência e persistência viral e para os quais não há um programa de controle oficial. Isso indica a necessidade de avançar com o apoio de associações de animais e instituições responsáveis pela saúde animal no desenvolvimento de verdadeiras estações de inseminação artificial que permitem a monitorização contínua da saúde dos doadores e obtenção de sêmen livre de doença como uma forma de controlar a transmissão por esta via.

Palavras chave*: bovinos, doenças reprodutivas, saúde animal.*

Introduction

Bovine reproduction in the tropics continues to depend on a high percentage of natural service (mounting). It has been estimated that 85% of calves born in the tropics come from natural service programmes (Galina and Arthur, 1991). Selecting a bull thus becomes a critical element leading to serious economic consequences if a particular bull has problems regarding infertility or is a disease transmitter (Galina *et al*., 2007). Natural service continues to prevail in Colombia, especially in beef cattle, since artificial insemination (AI) has been slow in developing (Sabogal and Obando, 2000). This has meant that the situation has become worsened as semen is usually chosen without regard for technical criteria supported by genetic improvement, production or profitability impact studies (Giraldo, 2007).

Traditional bull selection has been based on morphological characteristics and their growth performance at determined ages more than an evaluation of their semen production and reproductive potential (Moraes, 1995). Specific exams are made on rare occasions for infectious diseases affecting the reproductive organs and which are vehiculated by semen.

Just as a cow's fertility maybe affected by a large number of infectious agents, the bull is exposed to the very same specific agents and many others directly affecting reproductive activity. This article was aimed at reviewing current information concerning the main infectious agents affecting cattle fertility and their potential transmission through semen. It sought to analyse bulls' state of health from studies carried out in Colombia and propose new research prospects tending to improve national cattle-raising fertility levels.

The pathogenesis of reproductive infections in bulls

Saprophytic microflora and other pathogens are found in bulls' preputial sac. Other infectious agents may be acquired through venereal, respiratory or digestive infection from infected animals.

Bull fertility may be temporally or permanently affected, depending on the type of infectious agent (virus, fungi, bacteria, and protozoan) and the lesions produced on reproductive tract organs.

A large number of microorganisms have been isolated from semen and the prepuce. Twenty-seven different types of bacteria, fungi and blastomycetes were identified in 337 semen samples in a 1985 study and almost identical flora in 139 preputial washing liquids (Flastscher and Holzmann, 1985); this means that there is controversy concerning the true effects of such agents on freezing, fertilising power and the appearance of inflammatory processes (Parez, 1984).

The direct effect of pathogenous agents has been mainly focused on the testicles and glands forming part of the reproductive tract. Infection could be limited to a single organ (seminal vesicles) or spread extensively to other organs such as the epididymis, seminiferous ampoules, prostate, bulbourethral glands and urethra; in other cases they could reach the urinary bladder, urethra and kidneys (McCauley, 1980).

The inflammatory processes producing these infections are complex and difficult to differentiate amongst the affected organs; they have thus been brought together under the term seminal vesiculitis syndrome (McCauley, 1980). Vesiculitis has ranged from 0.85 - 10% in studies evaluating the potential of young bulls' reproductive health (Cavalieri and Van Camp, 1997); however, greater incidence has been found in slaughterhouses (49%) (Ball *et al*., 1968), significantly increasing their rejection rate.

The pathogenesis of vesiculitis has not been clearly defined, even though some factors make it predisposed such as septicaemia, pneumonia, omphalophlebitis and homosexual behaviour amongst young bulls (McCauley, 1980). Another factor has been related to nutrition, especially high energy diets causing ruminal acidosis, ruminitis followed by bacteremia (Rovay *et al*., 2008). This leads to stressing bulls' nutritional management in Colombia, especially when they are being prepared for participating in showground events as this could have adverse medium- and long-term effects on fertility; such situation has not been evaluated to date.

Before starting to deal with specific pathogens, it should be mentioned that the World Animal Health Organization (formerly known as the International Epizootias Office - IEO) has listed several diseases as having proven importance in transmission through semen. Such diseases have been grouped into two groups according to whether their transmission through semen has been demonstrated.

Diseases whose presence and transmission through semen has already been demonstrated

1. Foot and mouth disease.
2. Vesicular Stomatitis.
3. Infectious Bovine Rhinotracheitis (IBR).
4. Bovine Virus Diarrhoea (BVD).
5. Papillomatosis.
6. Leptospirosis.
7. Tuberculosis.
8. Paratuberculosis.
9. *Mycoplasma*.
10. Anaplasmosis.
11. Brucellosis.
12. Campylobacteriosis.
13. Trichomoniasis.

Diseases whose presence through semen has been demonstrated but not their transmission

1. Babesiosis.
2. Leucosis (when there is contamination with blood).
3. Trypanosomiasis.

The most studied agents have been brucellosis and tuberculosis, possibly because they have been involved in eradication programmes (Thibier and Guerin, 2000).

Pathogens associated with bull infertility

Bacterial agents

Brucellosis. Brucellosis is produced by a facultative intracellular, gram-negative coccobacillary bacteria; it does not form a capsule or spores and is not mobile (Seleem *et al*., 2010). Ten species from the genus *Brucella* have been identified to date (Tiller *et al*., 2010). *B. abortus* is the specie affecting cattle; 7 biovarieties are known (Seleem *et al*., 2010). The lesions produced by *B. abortus* directly affect the testicular parenchyma where it could become cultured; genital tract cells produce erythritol promoting this pathogen's growth and are thus its preferred localization (Givens and Marley, 2008). It is an important cause of vesiculitis in regions having a high disease incidence (McCauley, 1980). Pathological lesions are caused by ampullitis (unilateral orchitis and epididymitis) and are accompanied by fibrosis of the vaginal tunic and the presence of abscesses (Nicoleti, 1980).

Even though no comparative studies of susceptibility have been carried out according to gender, it is thought that bulls are more resistant to infection (Nicoleti, 1980). Individualised management of bulls in dairy-cattle farming has led to factors concerning the risk of contracting the infection becoming reduced (Gallego, 1988).

Infection in bulls could lead to reduced libido and lower semen quality and infertility (Givens and Marley, 2008). Contaminated semen could transmit infection when AI is used, even though the risk due to natural service is less frequent (Hare, 1982).

Two *Brucella abortus* seropositive bulls were followed-up in a case report involving several tests in which they presented vesiculitis and, later, orchitis; however, the bacteria could not be isolated from the semen (Plant *et al*., 1976). Such type of studies has indicated that the transmission of this disease through semen may not be the most important route.

Brucellosis prevalence in the Caldas Department was 0.6% in one study and orchitis was observed in 5% (12 farms) of the 235 farms surveyed (Aricapa *et al*., 2008).

Campylobacteriosis. This is a venereal disease affecting both animals and humans; it is produced by curved, gram-negative microaerophilic bacteria (Skirrow, 1994). Two subspecies (*C. fetus ssp. venerealis* and *C. fetus ssp. Fetus*) are known which are highly related at genome level; however, they differ regarding the disease which they produce, the habitats they occupy and their biochemical characteristics (Brooks *et al*., 2004). Infection with *C. fetus ssp. venerealis* in cows is characterised by infertility, embryo death and abortion. The bacteria become located in the epithelium of a bull's penis, prepuce and urethra where chronic infection, lacking any characteristic sign, becomes established (Eaglesome and García, 1992).

Diagnosis represents a problem when studying campylobacteriosis since the culture requires a means of transport, selective culture mediums and a special atmosphere, as well as time (Brooks *et al*., 2004).

This disease, together with trichomoniasis, has the greatest importance in the transmission of disease through semen (Rovay *et al*., 2008). Bulls marked for AI must be declared free of such diseases even though adding antibiotics to semen leads to this pathogen being easily controlled (Thibier and Guerin, 2000).

Leptospirosis. The aetiological agent is a spirochete (literally: spiral, hair) measuring 01µm in diameter and having 6-20 µm length. The genus *Leptospira* includes two species: pathogenic and saprophytic. The pathogenic leptospires include 13 species and more than 260 serovars (Adler and Moctezuma, 2010). The leptospires affecting cattle are mainly caused by the *serovar hardjo* making cattle a maintenance host for this serovar; in turn, two serologically indistinguishable but genetically different genospecies belong to it: *Leptospira interrogans serovar hardjo* (type *hardjo-prajitno*) and *Leptospira borgpetersenii serovar hardjo* (type *hardjo-bovis*). The serovar type *hardjo bovis* is the most common in cattle around the world, whilst the *hardjo-prajitno* type has mainly been isolated from cattle in the United Kingdom (Grooms, 2006).

A bull may present orchitis during the acute phase of leptospirosis, even though persistent infections are not very frequent and do not lead to the elimination of leptospires in semen (Ellis *et al*., 1986). By contrast, other researchers include leptospires within the group of infectious agents vehiculated by semen as they survive at freezing and cryoconservation temperatures (Eaglesome and García, 1997).

Diagnosis is difficult as samples easily become contaminated with bacteria, thereby hampering isolation, and serological measurements do not always give a positive reflection of an animal's status regarding infection. Detection methods have thus been sought using the semen; the polymerase chain reaction (PCR) seems to be the most suitable of them (Heinemann *et al*., 2000). Fortunately, this pathogen can be easily controlled by adding antibiotics to the semen (Thibier and Guerin, 2000).

Bulls persistently infected with the *serovar hardjo* type *hardjo-bovis* do not usually respond to treatment when the bacteria reaches the seminal vesicles and the kidneys (Alt *et al*., 2001, Peter, 1997), thereby leading to animals being rejected for natural service.

Paratuberculosis. Mycobacterium avium ssp. paratuberculosis (MAP) is a gram-positive acid-alcohol-resistant bacillus and is the aetiological agent for paratuberculosis or Johne's disease in ruminants. The disease was described for the first time in Colombia in 1924 (Góngora and Villamil, 1999). Its possible relationship with Crohn's disease in humans has been discussed recently (Sanderson *et al*.; 1992; Reddacliff *et al*., 2010). The main transmission route is oral-faecal; however, MAP has been isolated from sub-clinically infected (BonDurant, 2005) donor bulls' semen and reproductive organs (Tunkl and Aleraj, 1965; Larsen and Kopecky, 1970; Larsen *et al*., 1981). The animals usually present symptoms between 3 to 6 years of age.

It has been found that the elimination of the bacillus through semen occurred intermittently in a clinically infected bull, since it was only isolated in three samples out of the eight obtained during a 9 month interval (Larsen *et al*., 1981), whilst isolation was achieved in a single semen sample from the 100 obtained in a sub-clinically infected bull (Ayele *et al*., 2004). It was isolated in 100% of the seminal samples taken from a clinically infected bull during a period lasting a little over a year in a more recent study. Semen quality was notably affected in this study, although possibly more due to the emaciated state of the bull than the effect of the pathogen *per se* (Khol *et al*., 2010).

This bacillus has also survived the action of antibiotics and cryopreservation (Givens and Marley, 2008).

No prevalence studies regarding the main Colombian cattle-raising regions are known and official control programmes are lacking (Zapata *et al*., 2008), even though a combination of molecular techniques and culturing has emerged recently for identifying positive animals, thereby allowing the disease to be controlled on dairy-cattle farms (Zapata *et al*., 2010). The foregoing situation contrasts with national programmes begun in other countries a long time ago, i.e. the USA (USDA, 2005), Australia (Perry *et al*., 2006; Animal Health Australia, 2010), Japan (NIAH, 2011) and the Netherlands (Muskensa *et al*., 2000).

Arcanobacteriun pyogenes. This gram-negative anaerobic bacterium has been mentioned as being an important pathogen in the presentation of suppurative vesiculitis in areas where brucellosis has been well controlled (McCauley, 1980; Dargatz *et al*., 1987). *Arcanobacterium pyogenes* was the most frequently occurring isolate in a study of 14 bulls suffering from vesiculitis in Canada. The treatment for this pathology was not effective, except for an experimental treatment involving injecting ceftiofur and penicillin directly into the gland (Martínez *et al*., 2008).

Histophilus somnus. Infection with this bacterium causes the disease known as thromboembolic meningoencephalitis**.** It has been isolated from bulls producing purulent ejaculate (Hare, 1982). It can also be isolated from apparently normal bulls' reproductive tracts and semen (Humphrey *et al*., 1982).

Mycoplasma. M. bovigenitalium from the genus *Mycoplasma* occurs most often in bulls' genital tracts*.* Its presence in the prepuce and preputial orifice does not cause lesions; on the contrary, if it reaches the testicles and nearby glands it may cause lesions leading to low spermatic motility and reduced resistance to freezing and unfreezing (Kirkbride, 1987).

A study of 45 donor bulls investigated the source of semen contamination, establishing that *Mycoplasma* was located in the prepuce and the distal part of the urethra, leading to suggesting that the aforementioned sites should be washed and disinfected before taking semen, thereby forming part of the control strategies (Fish *et al*., 1985). Contamination of semen with *Mycoplasma* also originates from using diluters containing egg yolk or milk (Bielanski, 2007), even though the use of diluters containing such substances is becoming increasingly infrequent (Bielanski, 2007). Cows infected with these pathogens present severe salpingo-oophoritis. It is also considered an important pathogen which could affect embryo production *in vitro* through semen. One study found that this pathogen came from contaminated semen in more than 50% of embryos adhered to the pellucid area (Bielanski *et al*., 2000).

It is considered that this is an important contamination route regarding cows' reproductive tract and that antibiotics should control its proliferation in semen; however, another more recent study has shown that the antibiotics most used in semen (gentamycine, tylosin, lincomycine and spectinomycine) did not control its presence or growth in cultures made from semen samples taken from AI-destined bulls (Visser *et al*., 1999).

Ureaplasma diversum. The prepuce and the urethra provide the normal habitat for this microorganism; it has rarely been isolated from the testicles and accessory glands. It does not cause lesions in the reproductive tract which might lead to infertility (Kirkbride, 1987). It has been reported as being the cause of balanopostitis, vesiculitis and alterations in seminal morphology, even though bulls are asymptomatic in most cases. It has been implicated as causing abortion and infertility in cows. As in the previous case, antibiotics used in semen have not been effective in controlling *Ureaplasma* and it is a pathogen which is frequently found in the semen of bulls used for AI. More than 50% of the samples obtained from 35 bulls at a collection centre in a study carried out in Brazil were positive by culture and PCR. This study again discusses antibiotics' efficacy in controlling certain pathogens such as *Ureaplasma (*Marques *et al., 2009).*

Acholeplasma ssp. This has been isolated from 32% of preputial washes and 12% of semen samples; however, few indications have related the microorganism's presence to lesions leading to infertility (Fish *et al*., 1985).

Other bacteria. Infections caused by *Clamidia* may be localised in bulls' reproductive tracts. It has been isolated from the testicles, epididymis and semen of bulls suffering from seminal vesiculitis (Storz *et al*., 1968); it has also been able to survive cryoconservation (Teankum *et al*., 2007). *Clamidia* was found in 9.2% of semen samples, 10.7% of preputial washes and 18% of faecal samples in an investigation carried out on 120 bulls in 6 German federal states, thereby confirming the risk of sexual transmission (Kauffold *et al*., 2007). Other microorganisms which can be transmitted by semen include *Coxiella burnetii*, *Mycobacterium bovis* and *Mycoplasma mycoides ssp. mycoides* (Kruszewska and Tylewska-Wierzbanowska, 1997; Wentink *et al*., 2000); however, no studies dealing with their presence in Colombia are known.

Viral agents

Several viral agents have been isolated from bovine semen; some may be freely found in seminal plasma and others become firmly adhered to the sperm head and thus may not just infect cows through seminal plasma but also lead to the possibility of direct infection of oocytes. The difficulty regarding viral agents reported in bovine semen lies in the fact that a good number of such agents produce chronic or persistent infection. Several studies have been carried in an attempt to eliminate viruses from semen, but with poor results. Some studies have reported certain effectiveness with washing semen through procedures such as Percoll or Swim-up which lead to producing embryos *in vitro*, even though there are no conclusive studies on the matter (Bielanski, 2007).

Infectious bovine rhinotracheitis (IBR). This is a respiratory disease which is produced by bovine herpesvirus, type 1 (BHV-1), belonging to the Herpesviridae family. According to genomic and antigenic analysis, BHV-1 is divided into BHV-1.1 and BHV-1.2, in turn being subdivided into subtype BVH-1.2 and BHV-1.2b (Barr and BonDurant, 2000). When BHV-1 affects the genital tract of cattle it causes infectious pustular vulvovaginitis / infectious pustular balanopostitis (Fauquet *et al*., 2004). BHV-1 may also cause conjunctivitis, reproductive disorders and neonatal mortality (Straub; 1990, Takiuchi *et al*., 2005).

IBR was recognized for the first time in Colombia by investigators from the International Centre for Tropical Agriculture (CIAT) using a zebu bull which had genital lesions; 3 virus isolates were obtained (CIAT, 1972, 1973, 1974, 1975).

This is one of the most important viral diseases as the state of viral latency implies that infected animals become carries for life and frequent viral reactivation is caused by stress factors.

Bulls affected during an outbreak of the disease which occurred in an AI centre in Belgium presented brief pyrexia, uni- or bilateral orchitis and azoospermia. Mononuclear infiltration of the connective tissue, without neutrophils and degeneration of the germinal epithelium was found in one of the testicles examined by histopathology; the attempt at isolation led to positive results (Thiry *et al*., 1981). IBR-infected bulls eliminate the virus in semen during their whole lives (Van Oirschot, 1995), even though it has been thought that the virus cannot be eliminated from seropositive bulls if they are managed with low levels of stress (Eaglesome and García, 1997).

The presence of the virus was detected in the post-nuclear region of the sperms' cephalic hood in a bull from a farm having fertility problems (Elashary *et al*., 1980).

The pertinent worldwide literature is abundant regarding recognising this virus' transmission through semen or embryos (Bitsh, 1973; Kahrs, 1980; Kahrs and Littell, 1980). Sanitary legislation thus establishes sever restrictions on importing biological material from countries where the disease is prevalent (Hare, 1982). More recently, the World Organization for Animal Health (OIE) has included sanitary policy regarding this virus (as well as other pathogens) in its guidelines concerning the taking of bovine semen, its treatment and recollecting and manipulating cattle embryos (World Organization for Animal Health, 2009).

A new type of virus, bovine herpes virus type 5 (BHV-5), having 85% genetic homology with BHV-1 (Chowdhury, 1995), is responsible for neurological problems in calves, having a high rate of lethality; it has been isolated from semen (Gomes *et al*., 2003). No sero-epidemiological studies concerning this virus are known to have been carried out in Colombia.

Bovine viral diarrhoea. The term bovine viral diarrhoea refers to a group of RNA virus classified within the pestivirus genus, 2 species being known: BVDV1 and BVDV2 (Ridpath, 2010). The presence of BVDV2 is currently unknown in Colombia (Vargas *et al*., 2009).

BVD was reported for the first time in Colombia from a batch of 800 young heifers imported from Holland in 1975. The necropsy findings and serological tests confirmed this case by revealing the presence of "the disease of the mucosa" in infected animals (Borda, 1975).

BVD virus can replicate itself in the prostate, seminal vesicles and epididymis (Kirkland *et al*., 1991). The antigen has also been detected in epithelial cells from the epididymis, accessory glands, urethra, Sertoli (nurse) cells and spermatogonia (Borel *et al*., 2007).

A marked effect on spermatic quality has been observed in experimentally infected bulls, consisting of low concentration, low motility and an increase in the frequency of primary spermatic abnormalities (diadem effect) (Paton *et al*., 1989). Following initial infection, the virus stays in the testes for up to 7 months (Givens *et al*., 2003). Another study (even though having been considered an exceptional case) presented the elimination of the virus from semen during an 11-month period in the presence of active antibodies (Voges *et al*., 1998).

The BVD virus may be present in the semen of animals suffering acute infection or in persistently-infected animals. Persistently-infected animals (even though very few become breeding animals) are those representing the highest risk regarding BVD transmission through semen since viral elimination from semen is much higher ($10^{7.6}$ $CCID_{50}$/mL) than from acute infections (5–75 DICC50/mL); (Bielanski, 2007; Gard *et al*., 2010).

Protozoa

Trichomoniasis. The protozoan parasite *Tritricomona foetus* is the aetiological agent of this venereal disease; three varieties have been described to date: Belfast, Brisbane and Manley

(Skirrow and Bondurant, 1988). The infection may occur asymptomatically; however, some reports have associated this condition with transient balanopostitis (Jubb *et al*., 1985).

The first important sign of the presence of trichomoniasis on a particular cattle farm consists of prolonged intervals between births and post-service pyometry. Furthermore, some risk factors are related to herd and management practice. Rae *et al*., (2004) have reported a greater probability of the disease becoming present on large cattle farms involving extensive management conditions; prevalence was 53.9% on cattle farms having 500 or more cows but fell to 10% on cattle farms having 100-400 cows. The increased cow-bull ratio or the increased number of bulls per mating group is a management practice favouring the presence of trichomoniasis. Breeding cattle-raising management in Colombia thus provides the conditions for the disease to become present; however, diagnosis represents a limiting factor, meaning that disease prevalence could be underestimated.

No studies are known showing the effect of this protozoan on spermatic quality. Preputial mucosal crypts being localized on the surface (not penetrating it) may be the factor reducing its pathogenicity for other organs. Moreover, the crypts' greater depth in old bulls provides a better microaerophilic environment favouring chronic infection (Peter, 1997). It has been found recently that trichomona in pseudocyst form was present in 55% of preputial smegma samples whilst pyriform forms typical of this protozoarian were only observed in 25% of the samples. This situation shows the lack of direct microscope observation's sensitivity (Pereira-Neves *et al*., 2011).

Neosporosis. Neospora caninum has been found in bovine semen (Ortega-Mora *et al*., 2003), even though its transmission in venereal form or through cow donor embryos has been questioned (Dubey and Lindsay, 2006). The effective transfer of embryos has been recommended to avoid this parasite's vertical transmission (Baillargeon *et al*., 2001).

Bacteriological and serological studies of bulls in Colombia

Few national studies have been carried out involving the breeding animal as an important source of disease transmission via the coital route or the use of contaminated semen.

Griffiths *et al*., (1984) isolated *Trichomona foetus* and *Campylobacter fetus* in 13.7% and 15% of bulls, respectively, in an investigation carried out on 103 farms in Colombia's 8 main cattle-raising regions. Eight of the 23 bulls had positive titres for *L. hardjo and L. pomona* serovars.

A sanitary evaluation of 48 bulls from the Cundinamarca department found 23.9% positivity for *Tritrichomona*, 17.3% for *Campylobacter*, 43.4% for *Salmonella,* 28.2% for *Brucella* and 52.17% for *Leptospira* (Villalobos *et al*., 1986). A 67.6% IBR prevalence has been reported in breeding bulls in animals from Urabá in the Antioquia department (Zuñiga *et al*., 1978).

A 15.3% seropositivity for IBR, 83% for BVD, 42% for bovine leucosis virus (BLV) and 92% for *Leptospira spp* was found in 11 dairy breed bulls from the savannah around Bogotá; reactors to Leptospira serovars were pomona (62%), canicola (38%), hardjo (23%), gryphotyphosa and icterohaemorraghiae (18%). The same study revealed the presence of IBR/BVD (17%), BVD/*Leptospira spp* (83%), BVD/BLV (42%), BLV/*Leptospira spp* (31%) and BVD/BLV/*Leptospira spp* coinfection (33%) (Góngora *et al*., 1995). This would mean that several infectious agents could converge on the same farm and in the same animal without the epidemiological importance and the dynamics of the different coinfections being known regarding spermatic quality.

An overall 37.4% seropositivity was found in 4,230 samples received from different Colombian departments for IBR diagnosis by ELISA test. The sera having the greatest seropositivity came from the Santander and Cesar departments (72%) whilst those having the lowest seropositivity came from the upper Magdalena valley (58.4%). An interesting observation concerned the bulls' high seropositivity (more than 60%) (Cotrino, 1977).

A 73.43% seropositivity was found for the first sampling and 75% for the second in 207 bulls from 9 municipalities in the Valle del Cauca department when two serological samplings were done for IBR, separated by a 2-month interval; seropositive animals were found in 32 of the 33 farms sampled. Greater seropositivity was observed in beef cattle, this being explained by the introduction of animals which had not been subjected to quarantine and whose sanitary state had not been evaluated (Díaz, 2000).

IBR, BVD and Leptospirosis prevalence was 90%, 33% and 5% in 60 bulls from the municipalities of Paicol, La Plata and Nátaga, respectively, in the south of the Huila department; no reactors to Brucellosis were found (Sanabria and Trujillo, 2002).

A seroepidemiological study of IBR on 150 samples from cows from 32 farms in Montería (Córdoba) and 20 bulls revealed an overall 74.4% seroprevalence, bulls' seropositivity being as high as 95% (Betancur *et al*., 2006).

A more recent study of 316 samples from 6 farms from the Antioquia and Valle del Cauca departments using the viral neutralisation test revealed 100% seroprevalence for cattle farms, whilst overall prevalence for individuals was 75.63%. The prevalence for cattle farms from Antioquia and Valle del Cauca was 85.51% and 69.84%, respectively (Ruiz *et al*., 2010).

In addition to IBR seroepidemiological studies in which high seropositivity has been observed, several field strains have been isolated by immunosuppresion on the savannah surrounding Bogotá (Góngora *et al*., 1995), the Meta department (Chaparro *et al*., 2002), Córdoba (Vera and Betancur, 2008) Antioquia and Valle del Cauca (Ruiz *et al*., 2010). Concomitantly, researchers from the Universidad Nacional have molecularly characterised some of them; the strain isolated from the savannah near Bogotá thus corresponded to BHV-1 subtype 2b which has been associated with genital clinical forms presenting low virulence (Smith *et al*., 1995), whilst an isolation made in Meta was classified as being BHV-l.1 (Vera *et al*., 2006). The pathogenicity of the strain from near Bogotá was confirmed later on (Chaparro *et al*., 2002), thereby contradicting the hypothesis that IBR strains circulating in Colombia have low pathogenicity (Zapata *et al*., 2002).

The foregoing findings reflect worrying serological reactivity in bulls coinciding with seropositivity values for the overall population. The fact that some bulls included in the studies are/were semen donors is also worrying, thereby suggesting an important source of diffusion for the disease by this route.

Very few studies have dealt with BVD. It was found that infection in cows was correlated with infection in bulls (40%) in a sample of 32 farms having no background of BVD vaccination where random serological samples were taken from 20 bulls; infected bulls would thus represent an important source of BVD transmission (Betancur *et al*., 2007).

Regarding brucellosis, no important results have been observed concerning reduced prevalence arising from ICA's eradication campaign (Resolution 1192/2008) compelling the vaccination of 3-8 month-old calves involving two cycles per years and having more than 80% coverage of reproductive-aged cows. The results of ICA's epidemiology programme have shown that 2% seropositivity was obtained in bulls in 2007 and 2008, this being greater than that for the preceding two years (Orjuela *et al*., 2009). There is thus great concern that some land which had achieved brucellosis-free status (4,934 areas of land (1.01%) out of the 484,305 recorded) is now losing such status (Colbuitria, 2010).

The following questions thus arise from the present review*: ¿Does the semen currently being produced and sold in Colombia really comply with the existing standards determined by ICA? ¿Could the relevant Colombian health authority embark on official control programmes for those diseases becoming an important obstacle for exporting semen due to their high prevalence?*

¿What is the percentage of IBR- and BVD-positive donor bulls whose semen is frozen? ¿Is there any correlation between the results of tests evaluating the potential of bulls' reproductive health with seropositivity to some of the diseases reviewed here? ¿Does Colombia have the diagnostic infrastructure allowing the presence of some of the viruses analysed in this review to be detected in semen?

The foregoing questions require an immediate response from the national scientific community aimed at creating real controls for improving national cattle-raising competitiveness and profitability; cattle-raisings associations and Fedegan could thus play a fundamental role in this**.**

Conclusions

Many infectious agents may affect bull fertility. However, transmission through semen will depend on the donors' sanitary state. The different studies analysed here concerning bulls' state of health in different regions of Colombia reflects national herds' state of health to a certain extent; advances must thus be made in controlling them if they are to become competitive internationally. It is wellknown that some countries still suffering from these diseases, and which are therefore the object of control and eradication programmes, impose severe marketing restrictions.

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