UNIVERSITY OF PARMA

PhD in Animal Health

XXIV cycle

"Serological survey of bovine infectious causes of reproductive disorders in Colombia"

PhD Coordinator:
Prof. Sandro Cavirani

Tutor:
Prof. Sandro Cavirani

Assistant supervisor:
Dr. Simone Taddei

Doctoral candidate: Giovanni Moreno Figueredo

Parma, 2009-2011

INDEX

ABSTRACT

The presence of reproductive diseases of infectious origin (bacterial, viral and parasitic) in herds affects the reproductive performance of females and hence the productivity. Within these diseases are bovine viral diarrhea (BVD), leptospirosis, infectious bovine rhinotracheitis (IBR), chlamydiosis, neosporosis and the diseased induced by bovine herpes virus type IV. Some of them have been identified and relatively studied in Colombia, but the current situation of other remains unknown. In the dairy cord of Boyacá milk production is high, constituting an economic activity of high impact for the region and the country, however the epidemiological situation of many of the reproductive diseases that may affect bovine females is unknown. Therefore the health plans of prevention and integral management of these diseases are uncertain. The aim of this work was to determine the seropositivity of bovine females of different ages in some municipalities of the departments of Boyacá and Cundinamarca. We extracted serum samples of 959 animals (calves, heifers and cows), which were processed in the laboratory of infectious diseases of the Faculty of Veterinary Medicine of the University of Parma. Neutralization was used for the diagnosis of BVD and IBR, immunofluorescence for bovine herpesvirus type IV, and Neospora spp., ELISA for Clamydia spp. and microagglutination for Leptospira interrogans. In studied animals we found 29.8% of the animals positive to BVD; 25.2% to the different serovars of Leptospira, being greater the seroprevalence for *L. hardjo*; 8.7% to *N. caninum*; 15.4% to BoHV-1 (IBR); 95.4% to BoHV-4 and 63.2% to *Chlamydia psittaci*. It was not possible to establish a relationship between the presence of these diseases and the presence of abortions. It was concluded that in the study area the pathogens with higher seropositivity were BoHV-4 and *Clamydia psittaci*. Under the established conditions we

found no clear relationship between the seroprevalences to the different pathogens and the age of the animals.

INTRODUCTION

In bovine medicine and reproduction, reproductive diseases are those diseases which make fertilization, the maintenance of a complete gestation or the obtaining of an offspring with possibilities of life and health impossible or difficult, in other words those diseases which affect the reproductive parameters specific to the production system used (Anderson, 2007). The consequences, of producing infertility, are increase of open days and interval between births, although it also produces embryonic death, abortion, fetal malformations and birth of weak calves. In parallel, some of this diseases lead to clinical pictures involving organs or systems different from the reproductive, capable to affect males and females of any age, leading in some cases to death. These alterations do not only affect the health of the herd, they have also influence directly on the production, having in some cases zoonotic importance (Anderson, 2007).

These diseases have been associated with infectious, viral, bacterial and parasitic agents, such as the viruses of bovine viral diarrhea, bovine infectious rhinotracheitis and bovine herpesvirus type 4; bacteria like *Leptospira* spp*, Chlamydia psittaci, Campylobacter* spp, *Brucella abortus,* and parasites such as *Neospora caninum and Cryptosporidium* spp*,* are among the most prominent (Anderson, 2007).

As aforementioned, these agents besides affecting reproductive function are also responsible for functional and structural alterations in the digestive, respiratory and nervous systems, mainly. Therefore, the presence of these diseases in the Colombia dairy herds constitutes in some cases, a risk for human health and in others, a decrease of the productive capacity of the animals, which impacts on the productivity of country's economy. (Vargas *et al*., 2009).

According to the regulations in force, the Instituto Colombiano Agropecuario, ICA, believes that from the diseases previously mentioned, brucellosis, the foot and mouth disease and leptospirosis have to be reported. Therefore, the diagnosis of the other diseases, it is not a routine process in the herds, and unlike what is found in other countries, there is no clarity about their presence in the different regions of the country, in each of the existing production systems.

2. PRESENTATION OF THE PROBLEM

In Colombia livestock farming is mainly distributed in meat and milk production, which, by requiring different production systems, and using specific races for each one, their distribution varies from one region to another, identifying the country milk-producing areas generally located in the Andean region (Madrena, 2010). Specifically, the Department of Boyacá throughout its history has been characterized by being a dairy region with small and medium-sized producers, and some large producers. However, although it is an important sector for the economy of the country, detailed monitoring of the diseases that affect health and herd production it is not always done. Thus, although in Colombia the existence of the majority of the reproductive diseases previously mentioned has been reported, the epidemiological distribution of them, in each of the regions of the country, is unknown.

The area known as the dairy cord of Boyacá does not differ from the picture raised previously, and is for this reason that the presence of bovine viral diarrhea (BVD), infectious bovine rhinotracheitis (IBR), bovine herpesvirus type 4 (BoHV-4), leptospirosis, chlamydiosis and neosporosis is unknown in the municipalities which constitute this region of Colombia, one of the most important for milk production.

Due to the absence of information on the prevalence of these diseases, control methods applied by professionals and owners are not specific and do not obey to health plans which emerged from an epidemiological study of the property or the region, which can result in ineffective controls or unnecessary use of vaccines.

The necessity of knowing the epidemiological situation of diseases is based on that, within the plans of control of the diseases and even more of those zoonotic in nature, is imperative to have the sufficient knowledge of the problem, in every region of the country in a dynamic and almost permanent way. This is how every program for the prevention of diseases and control of risk factors, must be designed based on the results obtained from studies of epidemiological characterization of diseases.

Accordingly, for the Boyacá dairy cord, as well as for the Savannah of Bogotá, being important regions for milk production in the country, research on some of the diseases that can affect the health of the herd becomes necessary, in order to start their follow-up and achieve to give appropriate guidance to the owners for the benefit of animal health and production and even public health.

3. OBJECTIVES

Considering the aforementioned situation and the fact that in Colombia, farmers start to be aware about the reproductive diseases that affect cattle, we investigated, through a significant sampling in the area of interest, the presence of six causal agents of important reproductive alterations in the bovine species. It should be noted that while brucellosis is one of the most important reproductive and zoonotic diseases in livestock, in all visited sites, vaccination is made and many of them are free of this disease, therefore it was not considered for this study. It the same way we determined to assess the prevalence of *Neospora caninum*, bovine viral diarrhea virus, *Leptospira interrogans, Clamydia psittaci*, infectious bovine rhinotracheitis virus and bovine herpesvirus 4.

Due to the fact that samples were taken from different sites, we did not only wanted to know the total prevalence of these diseases, but also to determine the presence of each one of them at each study site, to establish their geographical distribution, and thereby determine in which herds or areas could be appropriated to start control measures.

On the other hand, it is important to know the distribution of these diseases in the age groups handled in the area, known as calves, heifers and cows, which constituted one of the objectives of the work. Along with the aforementioned, we attempted to identify the presence of the six diseases in each age group, globally on all premises and discriminated in each sampled property.

Finally, being the influence of the diseases mentioned on the reproductive performance of herds within the context of this work we wanted to determine the relationship between the seroprevalences to the different pathogens and the presence of abortions for each herd. The time of abortion was not taken into account, because many of the herds tested have production systems of medium or low modernization and do not collect records which could allow the availability of accurate information.

4. CONCEPTUAL FRAMEWORK

In cattle production, of meat or milk, one of the most important aspects corresponds to the reproductive performance of the herd, so any pathology which interferes with this is a situation to resolve. While the factors that affect reproduction are of different origins, infectious etiologies are the most common and within them, many produce abortion at different stages of gestation. In this regard, it is considered that bovine abortion affects the herds in the world, leading to major economic losses, with the drawback that in many cases it is difficult to diagnose the causative agent. It should be noted that abortion of infectious origin, can be produced mainly by viruses, fungi or bacteria (Anderson, 2007), whose prevalence and importance, vary from one region to another. Each of the diseases analyzed in this paper will be briefly described below.

4.1 Bovine Viral Diarrhea

Bovine viral diarrhea (BVD) is caused by a pestivirus of the *Flaviviridae* family, from which two biotypes are recognized, the cytopathic and the noncytopathic; according to their behavior versus cell cultures. The first produces cytoplasmic vacuolation by induction of apoptosis and shows high affinity for epithelial cells. For its part, the second does not affect the cell culture and shows affinity for lymphocyte cells. From the genetic point of view, there are two types, bovine viral diarrhea virus type I (BVDV-I) and the type II (BVDV-II), which according to the new classification is called pestivirus type 1 and type 4 (Rondon, 2007). The virus affects animals with split hoof like pigs, cows, goats and sheep. (Rondon, 2007).

The virus is transmitted vertically and horizontally, from an infected animal to a susceptible one or through fomites and from the mother to the fetus. In the first case, after the virus contact with the mucous membranes of the nasal and oral cavities, it adheres to the cell membrane and penetrates through endocytosis, spreading through different tissues, mainly in the respiratory and digestive systems; in the blood it runs free or attached to white blood cells (primarily lymphocytes, monocytes, and cells precursors of macrophages (Scott *et al*., 2006)). Inside the cells, it alters the tubulinas, alpha and beta, generating alterations of cell division and therefore decreases the expression of genes, which can affect the decrease of the synthesis of certain proteins (Rondon, 2006).

In those cases where the infection occurs in females in gestation between 42 and 125 days, the fetus may becomes a persistently infected animal (PI), because in that period the immune system is forming, so the fetus accepts the virus as its own and despite possessing it, does not generate antibodies against this (immunotolerant animals). Therefore, these animals will be spreaders of the viral agent but they are asymptomatic (Fulton *et al*., 2009).

In March 1964 in Ithaca, (State of New York), was discovered the first outbreak of BVD. The sick animal was a 4 year old cow imported two years before from England, with classic signs of winter diarrhea (liquid diarrhea, anorexia, decrease in milk production and hyperthermia) the outcome of which was fatal. In the days after this episode of diarrhea, five animals of the same farm died, manifesting the same symptoms; after these cases, other five farms located in a perimeter of 15 km from where the first outbreak happened were quickly overtaken by infection (Cavirani, 2002).

With regard to the prevalence of the disease, it has been evaluated in different parts of the world and it there has been tried to identify risk factors or predisposing factors, enabling greater epidemiological knowledge of it. In the United States there has been reported a prevalence of 29% of the BVDV1a and 1b and 32% in calves, but it is interpreted as maternal antibodies. Subsequently, the same author found in bovine females, a prevalence of 16.7%, with 0.55-3.1% of PI calves data obtained in samples taken from 4530 animal (Fulton *et al.*, 2009). In Canada the prevalence of infection is 27% in adult females and 9.1-12.7% in PI animals (Booker *et al.*, 2008). In the State of Alberta in the same country, a seroprevalence is reported for BVDV1 and BVDV2 of 28.45 and 8.9%, respectively (Scott *et al.,* 2006). In Japan a study was made between the years 2006-2007 which found a prevalence of 2.6% with a frequency of 21 persistently infected animals from 107800 evaluated in 2006 and 13 of 107800 in 2007 (Kadohira & Tajima, 2010).

In Italy the current prevalence varies widely from one region to another, being between 20 and 90%, being higher in dairy herds without vaccination (Valla, 2008).

For its part, in Latin America, in Lonja, a Chilean region of high transit of livestock, reports a prevalence of 16.07%, higher than that found in other regions of the country (Jara, 2008). In Colombia, studies in the department of Cordoba indicate antibody titers in 5.6% of animals sampled between 1980 and 1984, without finding any difference, among animals *Bos indicus* and *Bos taurus*. More recently, a similar study developed in the same department indicates prevalence of 22.5 to 36.2%, additionally the results are consistent with other researches, with regard to the lack of correlation of the prevalence with race or the region evaluated, but they do indicate higher prevalence in extensive herds and males (Betancour *et al*., 2007). The previous prevalence is widely surpassed by the reported by Vargas *et al*., (2009), who suggest that in the Savannah de Bogotá in 1994, the prevalence rate was 89%.

The disease manifests itself through three pathological pictures, bovine viral diarrhea (BVD) which corresponds to the primary postnatal infection, persistent infection and the disease of the mucous membranes (MD). In the BVD, manifestations are respiratory, digestive, or reproductive, animals showing diarrhea, decreased production and body weight and in some cases death occurs (Kadohira Tajima, 2010). Infection at the start of the pregnancy, leads to embryonic death and when it occurs in more advanced stages of pregnancy, causes abortion or birth of weak calf or malformations (Fulton *et al*., 2009). There is also reported pneumonia after delivery and metritis (Schefers *et al*., 2009). On the other hand, the virus is part of the bovine respiratory complex generating pneumonia associated with other viral or bacterial infectious agents.

In mucosal disease, is observed pyrexia, anorexia, acute diarrhea and ulcers in the mouth, with low morbidity but high mortality (Vargas & Jaime, 2009; Scott *et al*., 2006).

This is why considering it as a reproductive disease, is included among the possible causes of abortion and increased open days, in problem farms from a reproductive point of view.

4.2 Leptospirosis

Leptospirosis is a zoonotic infectious and contagious disease with high incidence in tropical regions, caused by various species of *Leptospira*, bacteria of the family *Leptospiraceae*, obligated aerobias, bacteria bound, spiral and Gram negative, capable of staying alive for months, in wet and warm media (Retrepo & Agudelo, 2007). The bacterium is transmitted horizontally by direct or indirect contact with water, food or contaminated soil or from urine of infected animals. There is also transmission through the placenta, of post-abortion tissues or direct mounting, although the latter is less frequent. The main reservoirs are rodents, cattle, dogs, swine, horses, skunks, goats, lagomorphs and bats, being the first two, the most important due to the alkaline pH of their urine, which constitutes an enabling environment for the bacteria (Agudelo & Restrepo, 2007).

The bacteria can penetrate through the skin intact or eroded or mucous membranes, having a 7-12 days incubation time. Once the leptospiremia occurs, the bacterium is distributed through various tissues including the cerebrospinal fluid, and it multiplies in the blood and tissues. In its permanence in the blood, it induces vasculitis and within the most affected organs are the liver and the kidney. The organism responds to the agent with a high production of antibodies, which are able to eliminate the agent of all the organs where it is, except for the eyes, brain, and proximal convoluted tubules in which persists for several weeks or several months (Rodriguez, 2000).

With regard to the seroprevalence, being a disease affecting many species of animals and the ability to spread between them, it is important to know some data in different species. In Italy it is reported between 1995 and 2001 a seroprevalence of 6.8% in animals, being higher in central and northern areas of the country (Monno *et al*., 2009). In Ontario is shown

39% of positive herds being greater prevalence of *L.hardjo* (Prescott & Miller 1988).

In 2007 the prevalence in horses reported in Korea, was 25%, with differences in the seasons, being greater in autumn and summer with values of 34.7% and 36.7%, respectively (Jung *et al*., 2010). In canines there is also a seasonal pattern with greater presentation in urban than rural zones (Gillian *et al*., 2009).

In Latin America there are reports in Venezuela, state of Zulia between 1998 and 2001 with 38% of seropositivity for any of the types. They also reported 56% in not vaccinated animals and 26% in vaccinated and increased presentation frequency of *L. interrogans* serovar icterohaemorragica and Hardjo. In Colombia, in the north of Antioquia was found a prevalence of 60.9% in production cattle and 10.3 and 25.7% in pigs for fattening and breeding, respectively (Ochoa, 2000). A work developed in Villavicencio (Colombia), reported a prevalence in humans between 5.2 and 48% with direct relation with exposure to animals, for work reasons, possession of canine and feline and mainly in rural areas (Góngora *et al*., 2008).

The disease manifests itself in an acute or chronic form not only in domestic but also in wild animals; it is distributed worldwide but it is important its high incidence in tropical and subtropical countries. Although it is certain predilection of some serovars for each species of animals, is considered that a species can be affected by various serovars (Rodriguez, 2000).

In sick cattle is noted abortion, decrease in fertility and decline in the production of milk (Agudelo & Restrepo, 2007), but as stated earlier, the infection is often asymptomatic. Similarly as indicated to the BVD, on farms which reported reproductive problems it is necessary to consider it within the possible infectious etiologies.

4.3 Neosporosis

Neosporosis is a disease caused by the *Neospora caninum*, included within the Philum Apicomplexa, class Sporozoea, subclass Coccidium, order Eucoccidia, suborder Eimerina and family *Sarcocystidae*, along with the genera Toxoplasma, Sarcocystis, Hammondia, and Besnoitia (Ellis *et al*., 1994; Dubey *et al*., 1988a). The members of the *Sarcocystidae* family are characterized by having heteroxen biological cycles and the formation of cysts in the intermediate host. They all have in common intermediary hosts such as the different species of herbivores and as definitive hosts, carnivorous species which remove the sporulated oocysts in the stool (Dubey *et al*., 2002).

This Protozoan was described by Bejerkas *et al*., 1991, in Norway, after the observation and diagnosis of fatal encephalopathy in dogs which seemed to be associated with *Toxoplasma gondii*, due to the presentation of encephalomyelitis, polymyositis, and formation of cysts. It was in 1988 that Dubey *et al*., described it, after obtaining the first isolated from samples of brain and muscle of canine origin inoculated in mice and rats that subsequently analyzed through the development of the test of indirect immunofluorecence for serologic diagnosis. In 1989, Dubey along with Lindsay managed to develop an immunohistochemical test for the detection of *N. caninum* in tissue. In turn, Thilsted and Dubey diagnosed it as the etiologic agent of abortions in cattle (McAllister *et al*., 1998).

Today the scientific community has devoted to study biology, pathogenicity and diagnosis of the protozoan, due to its high impact and for being responsible for large economic losses worldwide due to damage caused to the cattle.

Thanks to a large number of studies that seeks to know exactly the behavior of *N. caninum*, they managed to identify three different stages such as tachyzoítes, bradyzoites and sporozoítes. The tachyzoítes constitute the first phase known as acute, in which the parasite being obligated intracellular needs no more than 5 minutes to invade the host cell and initiate replication. The tachyzoítes have a size that fluctuates between 3-7 µm in length and 1-5 µm in width, being dependent with the morphology of the division stage; they have been described various shapes among them is the ovoid, Moon or globular (Dubey *et al*., 1996).

The bradyzoites are approximately 5 to 8 µm long and 1 to 2 µm in width and have the same organelles as the tachyzoítes (Dubey *et al*., 2003). However, inside it its nuclei is in subterminal position, it has less rhoptry and more granules of amylopectin. This stadium is located within the tissue cysts formed intracitoplasmatic space of a single cell and they are usually found in nervous tissue (Barr *et al*., 1991). A tissue cyst can reach up to 100 µm in diameter, with a thick, smooth cystic wall on its external face, of up to 4 µm of thickness (Dubey *et al*., 2002). The wall consists of two layers, an outer cytoplasmic membrane and a thick internal sheet consisting of numerous vesicles and granules attached to the membrane (Bjerkas *et al*., 1991).

Oocysts are the phase of elimination by the definitive host via the feces allowing the output into the environment. This stadium is spherical or sub spherical and is 11.7 µm in length and 11.3 µm in width, approximately. The Oocyst wall is between 0.6 and 0.8 µm thick and contains two sporochystes of 8.4 µm in length by 6.1 µm in width. Each one of these contain four sporozoítes each, being elongated and with a size of 6.5 µm in length by 2,0 µm width (McAllister *et al.*, 1998; Lindsay *et al.*, 1999).

In Spain, a study was conducted to a high prevalence of *N. caninum* in dogs of rural origin, which quickly led to relate the presence of those with seropositive cows, indicating that the canine near farms, constitute a risk factor. There were found 120 positive canine between 275 evaluated (43.63), with higher prevalence in farm dogs (47.51%) than in strays (39.55%) (Vázquez, 2007). On the other hand, in Venezuela there was found that 4000 crossbred Holstein cows, with reports of abortions, had a prevalence of 44%, in the sampled animals, which indicated the existence of correlation between the disease and the presence of abortion (Obando *et al.*, 2010).

In Colombia a number has been made in different regions, showing the presence of the parasite. In the Amazon region there was a prevalence of 40.4% in Brown Swiss cattle (Quevedo *et al.*, 2003); in Fredonia (Antioquia), was from 34.6 to 39.2%, in Holstein and Angus-bred animals, respectively (López *et al.*, 2007) and in the municipality of Montería (Córdoba) Oviedo *et al.*, in 2007, found 10.2% positive cows who have in addition report on abortion and mummification.

Bovine animals which suffer from neosporosis, manifest increase open days, prolonged jealousy, abortions between 5 to 6 month of gestation, embryonic reabsorption and fetal mummification. Another type of manifestations is seen in calves with less than 4 months of age, in which nervous symptoms dominate (Dubey *et al.*, 2006).

4.4. Infections caused by Herpes Viruses

The Herpes viruses are a group of DNA viruses of the family *herpesviridae* that affect different species of animals producing several disease pictures. So far, worldwide, there have been isolated eight types from naturally infected animals, all with a common denominator, symmetrical icosahedral capsule morphology (Muylkens *et al.*, 2007). Within them, the more important and therefore more studied in veterinary medicine are type 1 and type 5, the first, characterized by affect respiratory and reproductive systems and the second triggers neurological problems. Type 4 is also identified as the cause of infertility and therefore is of great importance in cattle production; however it has not been studied as the previous ones.

*****Bovine Herpes Virus Type 1 (BoHV-1)

This type of virus belongs to the subfamily alpha *herpesvirinae* and this has two sub-types, subtype 1, which corresponds to strains that cause the infectious bovine rhinotracheitis (IBR) and subtype 2, which causes the infectious pustular vulvovaginitis (IPV) and the infectious Balanoposthitis (IBP) (Ackermann & Engels 2006; Radostits *et al.*, 2000). The virus can remain in the nerve ganglia neurons. Transmission is direct through aerosols or by contact with respiratory, eye, or reproductive secretions of infected animals, or indirectly through individuals or equipment. It can also be transmitted through semen by natural mounting or artificial insemination (Wiedmann 1993; Van Oirschot, 1995) and even during embryo transfer (Wentink *et al*., 1993; Engels & Ackermann, 1996). On the other hand, the virus remains in a latent state and thus persists for long periods of time, being able to reactivate periodically, as a consequence of physiologic stress of the animal or by corticoid treatment (Lemaire 2000; Whetstone *et al.*, 1989).

The presence of the virus has been shown worldwide in different mammals, including bovine animals. In Europe, there are reported prevalences of 70%, 60%, 50% and 25% in the Netherlands, Italy, United Kingdom and Spain, respectively; disease-free countries as Austria, Finland, Sweden and Switzerland, also exist (Valla, 2009).

Recent reports regarding Colombia cannot be found in literature, however a 51.7% seroprevalence in bovine females in the Caribbean region, 21.5% in the Andean region and 20.6% in the Eastern Plains foothills are reported (Griffiths 1982). Bulls of the Savannah of Bogotá met 15.3% of positive reactors; however, few are the viral isolates (Góngora *et al*., 1995). In a study conducted in the departments of Córdoba and Sucre, between the years 1980-1984, was found a prevalence of the 29.6% in serum samples from 2,295 cows (Otte *et al*., 1985). The study showed that there were no significant differences between dairy cattle and beef and the prevalence increases progressively as it increases the age of the animals (Otte *et al*., 1985).

The VPI-BPI is a sexually transmitted infection, characterized by temporary infertility (Babiuk *et al.*, 1987; Miller *et al.*, 1991). Other presentations include dermatitis, mastitis and metritis (Bwagamoi & Kaminjolo 1971; Lomba *et al.*, 1976) and the encephalic kind described as a deadly disease in calves (George 1991). In lactating cows, it produces decreased production (Straub 1990) and infertility (Trapp 2003).

***** Bovine Herpes Virus Type 4**. (BoHV-4).**

It belongs to the subfamily *Gammaherpesvirinae* and to the genera *Rhadinovirus* (Zimmermann *et al.*, 2001). The first isolation was described by Bartha *et al.*, 1966 in Europe in animals with respiratory and eye disease. The pathogenesis remains unclear (Gur & Dogan 2010).

Like other herpes virus, BoHV-4 establishes a latent infection in cattle after natural infection, which also happens after experimental inoculation in cattle and rabbits (Thiry *et al.*, 1989; Naeem, *et al.*, 1990; Castrucci *et al.*, 1991; Donofrio *et al.*, 2007). The endothelial cells of bovine animals are susceptible to infection with BoHV-4 (Donofrio *et al.*, 2007) and the virus also infects persistently the cells of the marginal zone of the spleen. An experimental study detected the presence of viral DNA in the thoracic aorta suggesting a pathogenic role of infection by BoHV-4 in the atherosclerosis. (Donofrio *et al.*, 2007) Therefore, it would be interesting to investigate how the infection and reactivation of latent viruses affect the process of atereogenesis.

Although the majority of the herpes virus replication is limited to the host species, BoHV-4 is able to replicate in a wide range of animal species as well as cell culture (Egyed 1998).

International reports show the presence of the virus in cattle from Italy, Belgium, India, Spain, Serbia and USA, among others. Unlike what happens with other reproductive diseases, this one has no serological reports that reveal the prevalence of this disease globally (Donofrio *et al*., 2009), situation that is also reflected in Colombia.

In all cases, their presence is associated with reproductive alterations such as abortion, metritis postpartum, vaginitis and chronic infertility, although it also shows diarrhea enteritis, respiratory diseases, mastitis, tumors of the urinary bladder and rumen, interdigital dermatitis and lesions on the skin in different species of domestic and wild animals (Goyal & Naeem 1992). It is still unknown if virus plays a primary role in the initiation of the disease or if it acts as a secondary pathogen in the later stages of the infection initiated by other pathogens.

4.5 Chlamydiosis .

This disease is caused by *Chlamydia psittaci* (now reported from many authors also as *Chlamidophila psittaci*), a Gram negative bacterium, which makes part of a group of atypical organisms, for being an obligate intracellular parasite of eukaryotic cells, given its inability to synthesize ATP and possessing typical replication cycles (Jara, *et al*. 2001). Birds are the main reservoir, as well as several mammalian species, such as cattle, goats, sheep and cats, which can become infected and present systemic and debilitating disease (Gasque. 2008). There is an infectious form that is outside the cells, called elementary body, resistant to desiccation and which can remain viable for months if it is protected by organic detritus. It has been reported that this form of the bacterium can survive up to 2 months in the food of birds, 15 days in glass and 20 days in hay. Mosquitoes, mites, and lice may be involved in its mechanical transmission.

Depending on factors such as the virulence of the agent, the environment, as well as age, sex and animal physiological state of the animal, or states of stress, chlamydia infections may manifest as pneumonia, enteritis, conjunctivitis, polyarthritis, encephalomyelitis, abortion and infertility (Jara, *et al*., 2001). It has been linked with abortion, in horses and cattle, while canine identifies various syndromes. *Chlamydophila psittaci* genotype C, possibly acquired from a domestic bird, was isolated from a group of dogs with respiratory and reproductive recurring problems, as well as with severe episodes respiratory and keratoconjunctivitis (OIE 2009).

With regard to the prevalence, the first reported case of chlamidiosis was a European outbreak in poultry imported from the Republic of Argentina. Later in 1929 there was another epidemic with over 100 cases in Cordoba, Argentina moving then to Europe, where more than 800 cases were diagnosed with a total of 143 dead animals.

Epidemiological studies in cattle are limited, so the prevalence of the disease in many countries is unknown. On the other hand Cavirani and coll. did a study in the Valley of the Po River and in the provinces of the north of Italy between the years 1998 and 2000 making a serological sampling only in cows that had aborted, finding 290 positive to *C. psittaci* from 671 analyzed sera (Cavirani, *et al*., 2001). In Colombia a case-control study was conducted in 1995, in 80 farms in the eastern Llanos, with a prevalence of 33.3% (Otte, *et al*. 1995).

For its control it is necessary to take several steps in order to reduce the risk of infection, so far there are no reports of available vaccines. Accordingly preventive measures are applied such as the control of wild birds and rodents that act as mechanical vectors (Jara, *et al*. 2001). Being a bacterial agent, the treatment of infected animals is done with tetracyclines, changing the route and dose according to the type of animal. However, there is the possibility that in those cases in which the drug does not reach the required plasma concentration, for the time necessary, there remain carrier animals, which end up spreading agent (Wheelhouse, *et al*. 2010).

5. METHODOLGY

This study was a cross-sectional observational type because it was developed over a defined period of time, whose sampling units were female cattle at different ages, from dairy herds of an area of Boyacá.

5.1 Geographical characterization of the country

Colombia is a country located between the tropics of cancer and Capricorn, north of South America. It is divided by the Equator being part in the northern hemisphere and the rest in the southern hemisphere. It is characterized by diversity of climates and ecosystems, due to its topography, as the country is crossed from South to North by the cordillera of the Andes. While the climate of each region changes in the different times of the year, there are no seasons, therefore there are not extreme temperatures (IGAC; Zambrano, 1998).

The country has been divided into five eco-regions, these are: (Cárdenas, 2006).

- Andean region (30% of the national territory), houses 75% of the national population human, in addition, this is where most of the dairy productions are in the country. It is the area of highest altitude in the country and has notable landforms. It includes the valleys of the rivers Cauca and Magdalena (ICAG). The existing vegetation corresponds to different types of forest (wet, dry and rain), with temperatures of 3^{o}C in Paramo areas, up to 24^{o}C in the so called coffee zone (Jiménez, 2006).

- Caribbean Region. Corresponds to the coastal plains on the Atlantic Ocean, predominantly flat, but it also has mountainous areas outside the Andes Mountains (IGAC). It has different vegetation types and climates ranging from 0°C in the Sierra Nevada de Santa Marta to 30°C in Guajira (Bernal, 1988).

- Pacific Region. It consists of coastal valleys on the Pacific Ocean, with some higher areas (Jiménez, 2006). The vegetation type is forest (humid, wet and rain), with temperatures above 24°C. Along with the Amazonia represent the most humid regions of the country (Bernal, 1998).

- Orinoquia. Located east of the country and is characterized for being flat and having low altitude. It has the Orinoco River basin and is part, along with the Amazonia of the so-called Llanos Orientales (IGAC). The vegetation type corresponds to dry forest and humid tropical, with temperatures above 24^{o}C (Bernal, 1988).

- Amazonia. Just like Orinoquia is flat and has low altitude, is located in the south-east of the country, it includes the Amazon River basin and the jungle of the same name (IGAC). The forest vegetation type corresponds to (humid, wet and rain) with temperatures above 24^{o}C, although in some areas is 12^{o}C (Bernal, 1988).

-Insular. It is formed by a group of low altitude islands on the Caribbean Sea. They are considered tourist regions.

Cold climate regions in Colombia are between 2000-3000 meters above sea level (masl), and have a flat to slightly raised level with slopes of 0-3%, up to steep and very steep with a slope greater than 50%. For the most part acid soils with low available phosphorus, high in organic matter and saturation of aluminum, with potassium, calcium and magnesium levels from low to medium and deficient levels of sulfur, boron, zinc and molybdenum (Cárdenas, 2006).

The Federación de Ganaderos de Colombia has identified regions of higher milk production, which are located between 1,800 and 3,200 meters above sea level, which are found in the northern highlands of Antioquia Department, Boyacá dairy cord and the Savannah of Bogotá (Cárdenas, 2006).

5.2 Geographical location of the region subject of the study

Boyacá department lies in the Centre of Colombia, in the East mountain range of the Andes (Figure1), between the 04°39'10" and 07°03'17" North latitude and 71°57'49" and 74°41'35" West longitude. It has an area of 23.189 km2 representing the 2.03% of the national territory. It is bordered by the North with the departments of Santander and Norte de Santander, on the East with the departments of Arauca and Casanare, to the South with Meta and Cundinamarca, and the West to Cundinamarca and Antioquia (IGAC, 2008).

The department has a total population of 1.413.064 inhabitants. It is divided into 123 municipalities, numerous villages and populated places.

The relief belongs to the Andean system. The East mountain range occupies most of the departmental territory with altitudes up to 5,380 m, in the Cocuy snowcapped mountain, which corresponds to the only snowcapped mountain of this mountain range.

Figure1. Location of the Boyacá department
(http://boyacajuntos.blogspot.com/)

The altiplano, place where we can find one of the more affluent and densely populated areas of the country, also knowned as the Altiplano

Cundiboyacense, which extends from the páramo of Sumapaz to the foundations of the Sierra Nevada del Cocuy, Boyacá does not constitute a continuous plateau nor it manifests the uniformity of the Savannah of Bogotá, but rather is characterized by a series of valleys interspersed with great fertility that alternate with more or less high mountains. Three rainfall areas stand out: the central highlands of lesser rain (annual average of 1.000 mm), and the high slopes on both flanks of the Central range (annual average of less than 2.500 mm) and the central highlands with bimodal rainfall regime. Due to its varied relief every thermal floor is shown from snow to warm (IGAC, 2008).

Sampling area for this study is included within the so-called altiplano cundiboyacense and corresponded to the municipalities of Ventaquemada Oicatá, Tunja, Tuta, Sotaquira, Paipa, Soracá, Igüaque and Sopó.

Ventaquemada is located to the southwest of the Department of Boyacá 2.630 meters above sea level, with temperatures that are between 8 and 14^{o}C. The most important economic activity of the municipality is the production of milk and potato cultivation.

Oicatá municipality is located in the center province of the Boyacá department, in the altiplano cundiboyacense. Located at 2,815 meters above sea level is between the cold and Paramo thermal floors with temperatures ranging from 10 to 14^{o}C. Within the economic activity of the municipality it is emphasized the cultivation of potatoes and corn, as well as the breeding of milk (POT, Oicatá, 2000).

Tunja is the capital of the Department of Boyacá with an area of 118 Km2, of which 87% corresponds to the rural area and 13% to the urban area. It is located between 2,700 and 3,150 meters above sea level on the highest part (POT, Tunja, 2001).

Tuta is a predominantly mountainous terrain which is why the altitude determines the climate, which is between 11 and 14^{o}C; having two wet and two dry periods a year.

Sotaquira, located in the center province of the Department located at 5º46'52" of north latitude, 73°15' of west longitude from the Greenwich Meridian. It lays 2.860 masl with an average temperature 14ºC. Economic activities include livestock, agriculture, agro-forestry and mining (Sotaquira, 2010).

The municipality of Paipa is located at 2,577 masl, with average temperature of 14^{o}C, despite having as main activity the tourism, the breeding of milk is an important line.

Soracá, is a municipality located in the Boyacá department with an area of 57 km^2. It belongs to the central mountain range of the Andes; is 2542 meters above sea level and the temperature ranges from 7 to 13°C; it has cold and Paramo (Andean tundra ecosystem found between 2900-5000 masl) climates. Agriculture is the most prominent economic activity, followed by livestock breeding (Soracá, 2010).

In the dairy region of the Boyacá Department and specifically in the study area, grazing systems are predominant with rotation of paddocks. Meadows have mostly as grass, *Penisetum clandestinum* (grass kikuyo), alternating with *trifolium pratense* and *T. repens* (red and white clover). In the 90% of the sites studied, farmers supplement the animals with raw potatoes, on a daily basis at the time of milking and this replaces the use of concentrate. This allow rotational grazing where there is a temporary occupation of the pastures, equitably alternating periods of occupation with rest periods (Figure 2).

Figure 2 Dairy herds in the municipality of Oicata, Boyacá

5.3 Sample Population

5.3.1. Property Description

The selection of the farms was made by convenience because was based on ease of access and cooperation from the owner and the personnel and the existence of the required information. For this we approached the type of study and its benefits. The process began, delimiting the source population, which was made up of small and medium dairy producer farms located in Boyacá (Figure 3). To determine the relationship between the diseases subject of the study with the presentation of reproductive problems the registration of the reproductive behaviour of animals was taken into account.

Figure 3. Manual milking in the study

The sites selected for carrying out the sampling was identified numerically like so:

Farm Number 1: Located in the municipality of Soracá, has low level of modernization, animals graze with rotation of kikuyu paddocks, manual milking is done, animals do not receive any supplementation; the cattle is vaccinated against foot and mouth disease and brucellosis. Samples were taken from 23 adult bovine females. Abortions are reported.

Farm Number 2: Located in the municipality of Ventaquemada, has medium modernization, animals graze with rotation of kikuyo, Ryegrass and clover paddocks, mechanical milking with portable equipment is performed; milking cows are supplemented with potato, carrot and concentrate. The cattle are vaccinated against foot and mouth disease, brucellosis, and Clostridiosis Samples were taken from 13 adult bovine females. Abortions are reported (Figure 4).

Figure 4. Aborted fetus in a sampled Farm

Farm Number 3: Located in the municipality of Ventaquemada, has medium modernization, animals graze with rotation of kikuyo, Ryegrass and clover paddocks, mechanical milking with portable equipment is performed; milking cows are supplemented with potato, carrot and concentrate. The cattle are vaccinated against foot and mouth disease, brucellosis, and Clostridiosis coal. Samples were taken from 22 animals of which 17 were adult bovine females and 5 were heifers. No reported abortions.

Farm Number 4: Located in the municipality of Ventaquemada, has medium modernization, animals graze with rotation of kikuyo and clover paddocks, mechanical milking with fixed equipment is performed but without a milking room; milking cows are supplemented with potato. The cattle is vaccinated against foot and mouth disease, brucellosis, and Clostridiosis . Samples were taken from 27 adult bovine females. Abortions are not reported.

Farm Number 5: Located in the municipality of Ventaquemada, has medium modernization, animals graze with rotation of kikuyo and clover paddocks, mechanical milking with portable equipment is performed; milking cows are supplemented with potato. The cattle is vaccinated against foot and mouth disease, brucellosis, and Clostridiosis. Samples were taken from 19 adult bovine females. Abortions are not reported.

Farm Number 6: Located in Oicatá, has good modernization for the region, animals graze with rotation of kikuyo, Ryegrass and clover paddocks, mechanical milking with portable equipment is perfomed; milking cows are supplemented with concentrate. The cattle is vaccinated against foot and mouth disease, brucellosis, and Clostridiosis. Samples were taken from 31 adult bovine females. Abortions are not reported.

Farm Number 7: Located in Oicatá, has moderate modernization, animals graze with rotation of kikuyu paddocks, mechanical milking with portable equipment is performed; milking cows are supplemented with concentrate and potato. Samples were taken from 31 adult bovine females.

Farm Number 8 (Figure 5): Located in Oicatá, has moderate modernization, animals graze with rotation of kikuyo and ryegrass paddocks, mechanical milking is performed in milking rooms, milking cows are supplemented with concentrate, potato, carrot, corn and Vicia *Sativa*. The cattle is vaccinated against foot and mouth disease, brucellosis, and Clostridiosis. Samples were taken from 31 adult bovine females. Abortions are reported.

Figure 5. Use of nutritional supplement for cows in a farm

Farm Number 9: Located in Oicatá, has good modernization, animals graze with rotation of kikuyo and ryegrass paddocks, mechanical milking with portable equipment is performed; milking cows are supplemented with concentrate and potato. The cattle is vaccinated against foot and mouth disease, brucellosis, Clostridiosis, IBR, BVD. Samples were taken from 62 animals, of which 52 were adult bovine females and 10 were heifers. Abortions are reported.

Farm Number 10: Located in Oicatá, has good modernization, animals graze with rotation of kikuyo and ryegrass paddocks, mechanical milking with portable equipment is performed; milking cows are supplemented with concentrate and potato. The cattle is vaccinated against foot and mouth disease, brucellosis, Clostridiosis, IBR, BVD. Samples were taken from 31 animals; of which 9 were adult bovine female and 22 were heifers. Abortions are reported.

Farm Number 11 (Figure 6): Located in Sopó, Cundinamarca, has high modernization, production animals are confined, the rest of the animals graze with rotation of kikuyo and ryegrass paddocks, mechanical milking is performed in milking rooms, milking cows are supplemented with concentrate. The cattle is vaccinated against foot and mouth disease, brucellosis, Clostridiosis, IBR, BVD and Leptospira. Samples were taken from 398 animals, of which 270 were adult bovine females, 2 were males, 82 were heifers and 44 were calves. A high number of abortions are reported.


Figura 6. Sample collection from cattle.

Farm Number 12: Is located in Iguaque, has low modernization, animals graze with rotation of kikuyu paddocks, manual milking is performed; milking cows are supplemented with concentrate, the cattle is vaccinated against foot and mouth disease and brucellosis. Samples were taken from 64 adult bovine females. Abortions are not reported.

Farm Number 13: Located in Oicatá, has low modernization, animals graze with rotation of kikuyo and clover paddocks, manual milking is performed; milking cows are supplemented with concentrate and potato. Samples were taken from 41 adult bovine females. Abortions are reported.

Farm Number 14: Located in Oicatá, has moderate modernization, animals graze with rotation of kikuyu paddocks, mechanical milking with portable equipment is performed; milking cows are supplemented with concentrate. Samples were taken from 11 adult bovine females. Abortions were reported.

Farm Number 15: Located in Oicatá, has moderate modernization, animals graze with rotation of kikuyu paddocks, mechanical milking with portable equipment is performed; milking cows are supplemented with concentrate. Samples were taken from 11 adult bovine females. Abortions were reported.

Farm Number 16: It is located in Paipa has low modernization animals graze with rotation of kikuyu paddocks, manual milking is performed, milking cows are supplemented with potato. Cattle are vaccinated against foot and mouth disease and brucellosis. Samples were taken from 21 adult bovine females. Abortions are not reported.

Farm Number 17: It is located in Paipa, has medium modernization, animals graze with rotation of kikuyu paddocks, mobile mechanical milking is performed; milking cows are supplemented with concentrate. Cattle are vaccinated against foot and mouth disease and brucellosis. Samples were taken from 25 animals of which 18 were adult females and 7 heifers. Abortions are reported.

Farm Number 18: It is located in Sotaquira, has good modernization, animals graze with rotation of kikuyo and clover paddocks, mechanical milking with portable equipment is performed; milking cows are supplemented with concentrate. The farm is brucellosis and foot and mouth disease free. Samples were taken from 22 animals of which 8 were adult bovine females and 14 heifers. Abortions are reported.

Farm Number 19: It is located in Tuta, has half modernization, animals graze with rotation of kikuyu paddocks. Cattle are vaccinated against foot and mouth disease and brucellosis. Samples were taken from 23 heifers.

Farm Number 20: Located in Oicatá, has moderate modernization, animals graze with rotation of kikuyo, Ryegrass and clover paddocks, manual milking is performed; milking cows are supplemented with concentrate. Samples were taken from 17 adult females.

Farm Number 21 (Figure 7): Located in Oicatá, has moderate modernization, animals graze with rotation of kikuyu paddocks, mechanical milking with portable equipment is performed; milking cows are supplemented with concentrate, carrot and potato. Samples were taken from 25 adult females.

5.3.2 Description of the Sampled Animals

The sampled animals were Bos Taurus bovines, of the breeds Red Holstein, Black Holstein, Normando and crosses between them. Inclusion criteria were not determined since the interest of the work was to assess the presence of the diseases in the existing population in the visited sites. In

Figure 7. Handling characteristics

some cases samples were taken from all animals of the farm and elsewhere this did not happen, depending on the availability and willingness of the owner. In all dairy farms sampled only female animals were found, due to the use of artificial insemination. Bulls or steers, which were also sampled, were found only in some sites. The animals were distributed in three age groups as such:

*Calves, females between 1 – 6 months

*Heifers, females between 7 – 24 months or to the moment of their first delivery

*Adults, females in production or in dry periods, older than 24 months or those who already had their first abortion.

Despite the fact that animals used for the study were in different farms, the handling characteristics are very similar because the production system is similar throughout the region (Figure 8). With regard to vaccine plans, in all cases the cattle was vaccinated against brucellosis and foot and mouth disease and in the case of greater modernization farms, which in our

Figure 8. Portable Mechanical milking

case were three sites, it is common practice also to vaccinate against parainfluenza, IBR, BVD and leptospirosis.

Clinical evaluation of animals or the determination of disease status was not included within the objectives of the work, however during the sampling in no cases was detected any clear clinical picture and neither was reported by the owner or Manager.

5.4 Taking and Sending of Samples

The sampling was made through puncture into the flow or coccygeal vein until obtaining 8 - 10 ml of blood (Figure 9). The blood was taken in Vacutainer and switched to red tubes (without anticoagulant), to be immediately centrifuged at 2500 rpm for five minutes. Subsequently the

Figura 9. Sample taking from the vein

serum was separated and was packaged in Eppendorf tubes that resist freezing, in order to keep them in a freezer at a temperature of - 20°C until the time of the dispatch to the laboratory, where they were processed (Figure 10). Samples were transported under the appropriate indications and fulfilling all the requirements of mobilization of biological material indicated by the ICA and the Ministry of Health of the Italian Government. The processing of the samples was carried out in the laboratory of infectious diseases of the Faculty of Veterinary Medicine of the University of Parma (Italy).

Figura 10 Blood and será obtained

5.5. Procedures

5.5.1 Diagnosis of BVD.

It was made through the test of seroneutralization (SN), which consists on identifying and assess the ability of serum antibodies to neutralize the cytopathic effect (CPE) of a BVDV strain. Neutralizing antibodies are responsible for the protective effect of serum and are directed against determinant specific antigens of the surface of the virus (FAO, 1985).

The test of SN was performed by serially diluting sera 1:2; 1:4; 1:8; 1:16; 1:32; 1:64; 1:128 and 1:256, in a final volume of 25 µl. Positive serum control was included as well as negative serum control and cell control wells., Twenty-five µl containing 100 tissue culture infectious dose 50% (TCID50) of a NADL strain of the virus BVD were added to the diluted sera and incubated for 90 minutes at room temperature. After incubation, 50 μl of MEM medium containing 10% of radiated fetal bovine serum (FBS) and $2x10^5$ cells/ml of Madin Darby Bovine Kidney (MDBK) were added (Figure 11). Finally the micro-plates were incubated in a cultivation stove with wet camera at 37°C and with 5% of CO2 for 72 hours. Expression of viral infectivity and serum neutralizing activity through CPE were detected by phase contrast microscopy (Reinhard, Carrasco, Tadich, 2001).

Figure 11. Seroneutralization for diagnosis of BVD and IBR in the Laboratory of infectious diseases of the University of Parma.

The neutralization antibody titres were expressed as the reciprocal of the final dilution of serum that completely inhibited viral infectivity. For the interpretation of the results, we considered positive animals, those with an antibody titer at least equal to 2. In the case of vaccinated animals, they were considered positive when the antibody titer was equal or greater than 128 (Reinhard, Carrasco, Tadich, 2001).

5.5.2 Diagnosis of IBR.

The diagnosis of this disease used the same technique of seroneutralization, described above, but using a strain New York of BoHV-1 as virus (Figure 12). For reading and interpretation we considered positive sera those with titers higher or equal to 2 for unvaccinated animals and, titers higher or equal to 32 for vaccinated animals (Valla, 2009).

Figura 12. Shaking process of the samples for seroneutralization test

5.5.3 Diagnosis of *L. Interrogans.*

Anti-leptospira serum antibodies were detected by the microscopic agglutination test (MAT). Suspensions of 7 strain of leptospira (serovars *hardjo, pomona, bratislava, copenhageni, grippotyphosa, canicola, tarassovi*) were used as antigens. Leptospira strains were cultured in EMJH medium (Becton Dickinson, Maryland, USA) for 3-4 days at 30°C and diluted 1:2 in sterile saline for the test. One hundred-fifty µl of the diluted leptospira were added to 25 µl of each serum previously diluted in sterile saline and incubated at 37°C for 3-4 hours. After incubation 8-10 μl of the suspension were transferred to a slide and observed by a dark field microscope (Nikon, Eclipse 50i) at 100X magnification. All sera were first screened at a 1:100 dilution and those who gave a positive reaction were further titrated in serial two-fold dilutions to titre end-point. Antibody titres were expressed as the reciprocal of the final dilution of serum that, because of specific agglutination, gave 50% or more of reduction of free leptospires in the suspension. Any agglutination of all the serovar tested by the same serum was considered an specific effect. A titre ≥ 100 was deemed positive, i.e. indicating exposure to leptospira. For vaccinated animals, titres > 800 were deemed as caused by infection and not only by vaccination. None of the sera reacted with all the strains of leptospira tested, indicating that all the positive reactions can be considered specific and not due to an aspecific effect. (Van Balen *et al* 2009).

5.5.4 Diagnosis of *Neospora caninum*

It was done by indirect immuno-fluorescence (IFI), for which 10 μl of each serum were placed in a well of a multiwells slide containing the antigen. After the distribution of the samples, the slides were incubated at 37°C for one hour in a humid chamber. Then the slides were washed by gently submerging for 30 times in PBS and then for 30 times with distilled water. After washing, an anti-bovine IgG secondary antibody and Evans blue were added to each well. After adding the aforementioned, it was incubated for one hour at 37°C in the wet chamber. After this the slides were washed with PBS again (30 times) and then with distilled water (30 times). Slides were left to dry at room temperature (RT) and then readed with a fluorescent microscope. (Dubey, 1988) (Figure 13).

Figura 13. Fluorescent microscope for sample lecture

5.5.5 Diagnosis of BoHV-4

The diagnosis of this disease used the same technique of indirect immuno-fluorescence (IFI), described above for Neospora.

5.5.6 Diagnosis of *Chlamydia psittaci*

Anti-Chlamydia psittaci serum antibodies were detected by ELISA (Civitest Bovis Chlamydia PS, Laboratorios Hipra S.A, Amer, Spain) following manufacturer's instructions.

6. STATISTICAL ANALYSIS

In line with the objectives of the work, the results of each of the diseases were entered in the matrixes as positive and negative identifying the number of the animal, the property and the age group, to which each belonged. Subsequently, we determined the total prevalence for each disease in the dairy cord and then the prevalence for each age group and each sampled property. In order to determine if there were significant differences between the prevalence of diseases of study in each age group and on every farm an analysis of variance was performed. Subsequently, the relationship between the presence of each disease in each site and town was determined, by an X^2 (chi square) test of goodness of fit in order to determine if there are differences between the number of positive and negative individuals and assessed properties, as well as in each of the municipalities of study. The same statistical test was conducted to establish the difference between the prevalence found in each age group. As a result, the following assumptions were made:

Null hypothesis (OH): number of positive individuals is statistically equal to the number of negative individuals ($p \geq 0.05$)

Research Hypothesis (RH): number of positive individuals is statistically different to the number of negative individuals ($p<0.05$).

7. RESULTS AND DISCUSSION

7.1 General Prevalence

7.1.1 Bovine Viral Diarrhea

Of the total of sampled animals (N=959), 377 were seropositive to this disease, which is considered important, but it is a relatively low number when compared with other regions of the world (Figure 14).

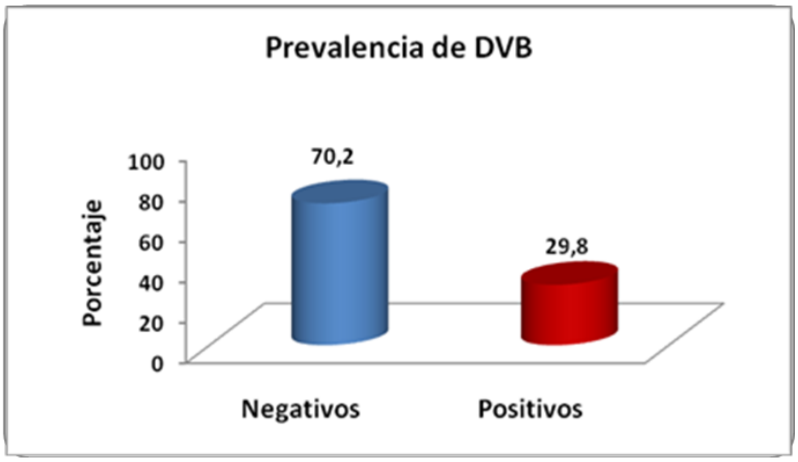

Figure 14. Prevalence of BVD on sampled animals

With the purpose of determining if there is a relation between the numbers of positive and negative animals towards the virus we stated:

* Research Hypothesis (RH): number of positive individuals is statistically equal to the number of negative individuals ($p \geq 0.05$)

* Null hypothesis (OH): number of positive individuals is statistically different to the number of negative individuals ($p<0.05$).

The evidence indicates that there is difference between the number of positive and negative individuals to BVD (p=3, 6E-11; $p<0.05$; $chi^2=43.82$); being lower the amount of positive individuals found (figure 14).

This study differs from that found in Peru, where prevalence exceeding 50% in dairy production was reported (Quispe *et al.*, 2008). In other regions of the world there are prevalence below those found in this work like some provinces of Ecuador reporting 16.06% (Jara, 2008) or others like Lithuania with broad ranges of seroprevalence (11-100%) (Mockeliunienė *et al.*, 2004). The prevalence found here, was similar to that found in the department of Córdoba (Colombia) where it was 29.4% (Betancur *et al.*, 2007), and to that found in Alberta, Canada in milk bovine females, where were reported 28.5% of positive animals (Morgan *et al.*, 2008); despite the percentage similarities, the handling conditions are different from one region to another.

The presence of infected animals in a herd can be explained by the presence of PI animals which constitute one of the main diffuser factors of the disease (Valla, 2008), however this study was not designed to identify the presence of PI animals. Other factors that explain the presence of the virus in the herds is the mobilization of infected animals or the introduction of them in disease-free farms (Valla, 2008).

It is important to know that with a high of antibodies seroprevalence an area, there is a greater likelihood of having an active infection in animals, evident symptoms and presence of PI animals, which requires the implementation of control and biosecurity measures to reduce this infection.

7.1.2. Leptospirosis

Unlike other diseases studied in this work for leptospirosis the presented results are partial, because so far the processing of the samples has not been completed. Thus, there are 624 samples results for seven serovars.

The tests showed that 128 animals were positive for *L. hardjo bovis*, 52 to *L. canicola*, 27 to *L. pomona* 14 to *L. copenhageni*, 13 to *L.grippothyphosa*, 10 to *L. tarassovi* and 5 to *L. bratislava* (Table 1). It should be noted that some animals were positive to more than one serovar.

Table 1. Number of positive animals and prevalences of *Leptospira* spp in some study farms

serovar	hardjo	pomona	Bratislava	copenhageni	grippothyphosa	canicola	tarassovi
n. sieropos.	128	27	5	14	13	52	10
sieroprevalenza	0,1334724	0,02815433	0,00521376	0,01459854	0,0135558	0,054223	0,010428
sieroprevalenza %	13,347237	2,81543274	0,52137643	1,45985401	1,3555787	5,422315	1,042753

The seroprevalence found here is lower than the one reported by Otte et al. (1995), which for *L hardjo bovis* found 62.2%, *L canicola* 8.9%, *L Pomona* 4.4% and for L *grippothyphosa* 4.4% in the Llanos orientales. But being a zoonotic disease the prevalence found in the Boyacá dairy cord is considered important. Considering the results it is important to note that on the farms studied showed the presence of stray dogs and contact with cattle, like rodents, which could be a source of infection.

7.1.3. Neosporosis

In this case the test showed that 775 negative, 110 dubious and 74 positive, giving a lower prevalence (figure 15).

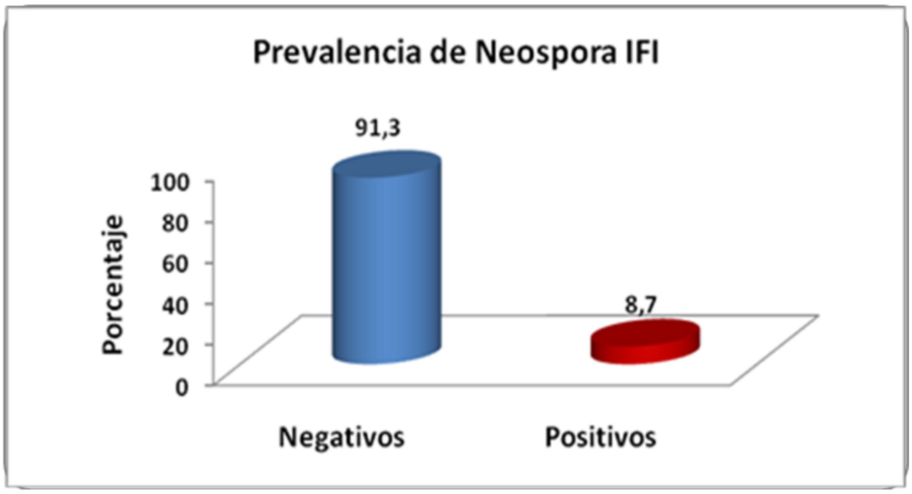

Figure 15 . Prevalence de *N.caninum* en los sampled animals

With the purpose of determining if there is a relation between the numbers of positive and negative animals towards the parasite we stated the following hypothesis:

* Research Hypothesis (RH): number of positive individuals to *N.caninum* is statistically equal to the number of negative individuals ($p \geq 0.05$)

* Null hypothesis (OH): number of positive individuals to *N.caninum* is statistically different to the number of negative individuals ($p<0.05$).

The evidence indicates that there is a significant difference (p=6.8E-128; $p<0.05$; $chi^2=578.80$), between the group negative of and positive animals to the parasite, the first one being the biggest.

This study found a low-prevalence to the parasite, in contrast to that found in Fredonia (Antioquia) where 34.6% of animals are positive towards it (López *et al.*, 2007). The reported prevalence in Montería (10.2%), (Oviedo *et al.*, 2007), was similar to that found in this work (8.7%). In Italy is reported a seroprevalence equal to the ones found in this study, in herds in the South of the country and higher towards the North (16%) (Otranto *et al*., 2003). In Catalonia, Spain, there are wide ranges of seroprevalence between 3.5-34.3%, and this is clearly related, to reproductive problems in females (Pabón, 2007).

The seropositivity to *N. caninum* in cattle is associated with high population densities and the presence of dogs and birds on the farms (Otranto *et al*., 2003). In the first case, in summer, because of the lack of food or in intense winter, where excess rainfall leads to flooding of pastures, situations of stress and overcrowding are created which can lead to the spread of the parasite. On the other hand, we managed to see in 90% of evaluated properties in this work that, because of socio-cultural characteristics of the area and sampled exploitations, there are loose dogs in the farms, in direct contact with bovine animals; also workers who live on farms, usually have chickens and hens for their own consumption, which are kept loose and into contact with bovine animals, constituting a risk factor. (Figure 16).

Figura 16. Presence of birds in contact with cattle

7.1.4. Infectious Bovine Rhinotracheitis.

The BoHV type 1, cause of the IBR was found in the sampled farms in low proportion, being positive 148 animals of the 959 sampled, which correspond to those not vaccinated and only 15 females of the total population of study were seropositive being vaccinated against the virus (figure 17).

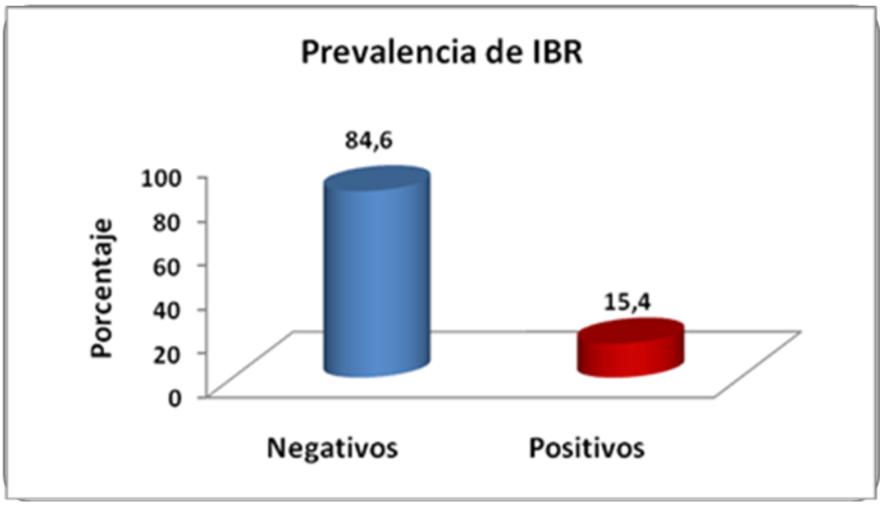

Figure 17 . Prevalence of IBR on sampled animals

With the purpose of determining if there is a relation between the numbers of positive and negative animals towards Herpes virus type 1 we stated the following hypothesis:

* Research Hypothesis (RH): number of positive individuals to IBR is statistically equal to the number of negative individuals ($p \geq 0.05$)

* Null hypothesis (OH): number of positive individuals to IBR is statistically different to the number of negative individuals ($p<0.05$).

The evidence indicates that there is a significant difference (p=1.1E-101; $p<0.05$; $chi^2=43.82$), between the positive and negative animals, the second one being the biggest.

In Colombia, in some municipalities of Magdalena Medio a prevalence of 55.5% was found (Piedrahita *et al*., 2010); more than 20 years ago in the Urabá Antioquia were found 67.59% seropositive bulls (Zúñiga *et al*., 1978); in the South of England there are reports of 42.5% of positive cows and 43.1% of heifers (Woodbine *et al*., 2009) and a similar prevalence to the previous one (54.4%) was reported in Yucatán (Solís *et al*., 2003) all surpassing notoriously the one found in this work which was 15.4%.

Although in the region of study BoHV-1 vaccination is practically non-existent, the frequency of presentation was low; also were no reports of history of symptoms compatible with the disease. Some works indicate that the prevalence of this disease varies from one year to another in a notorious way, possibly by the factor of stress generated by the strong climatic changes, finding variations up to 50% from a time to another. One of the major risk factors, although not the only one, is the genital transmission by direct mounting (Bolmann *et al*., 1997), which is excluded for the animals in this study, since the sampled sites used artificial insemination and breeding bulls are used only in very rare cases.

According to the results found, we can affirm that this disease does not constitute a problem for the region, however a follow-up of it must be done because in areas of low prevalence, where cows are not vaccinated, practices of biosafety are not carried out, which makes the reintroduction of the virus possible. Despite the aforementioned, it is taken into account that the seronegativity can also be due to the presence of animals with latent infection, which show no antibodies unless the infection is reactivated. Therefore, we reiterate the importance of tracking these animals in future studies.

7.1.5. Infection with Bovine Herpes Virus Type 4

The number of positive animals was high (N=617) (figure 18).

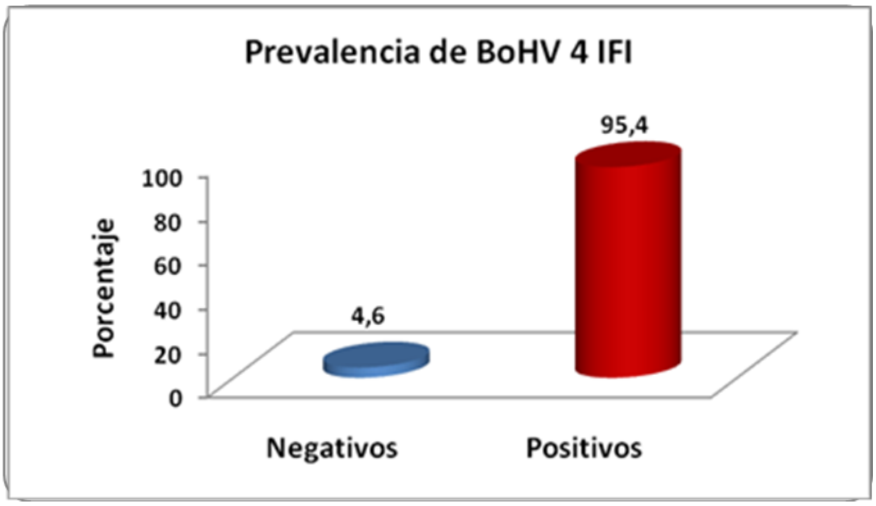

Figure 18 . Prevalence of BoHV-4 in sampled animals

With the purpose of determining if there is a relation between the numbers of positive and negative animals towards Herpes virus type 4 we stated the following hypothesis:

* Research Hypothesis (RH): number of positive individuals to BoHV 4 is statistically equal to the number of negative individuals ($p \geq 0.05$)

* Null hypothesis (OH): number of positive individuals to BoHV 4 is statistically different to the number of negative individuals ($p < 0.05$).

The evidence indicates that there is a significant difference (p=7.8E-118; $p<0.05$; $chi^2=532.56$); between the negative and positive animals, the second one being the biggest.

In this study, the greatest seroprevalence found corresponded to this virus (95.4%), similar to that found in other countries. It is difficult to compare the prevalence found here with the one found in other works due to the lack of global reports of the presence of antibodies for this virus. In Colombia this work is the first report on BoHV-4 seroprevalence. BoHV-4 infection affects the endometrial mucous leading to post-partum metritis (Frazier *et al*., 2002), pathology that is often reported in Colombia, producing high economic loses, though the triggering agent is not always defined. Research done in other countries, indicate that in dairy farms in the area of Belgrade, there is a seroprevalence of 84.37% (Nikolin *et al*., 2007) and in Holstein bovine females in some regions of Turkey 69% are seropositive and manifest reproductive problems (Gür & Dogan, 2010).

This viral disease, whose causative agent has been isolated and identified genotypically in several countries, is a pathology of interest that must be studied carefully in Colombia, even more so with the high prevalence reported here.

7.1.6. Chlamydiosis

Of the sampled animals 566 were positive for the bacteria 329 were negative (figure 19).

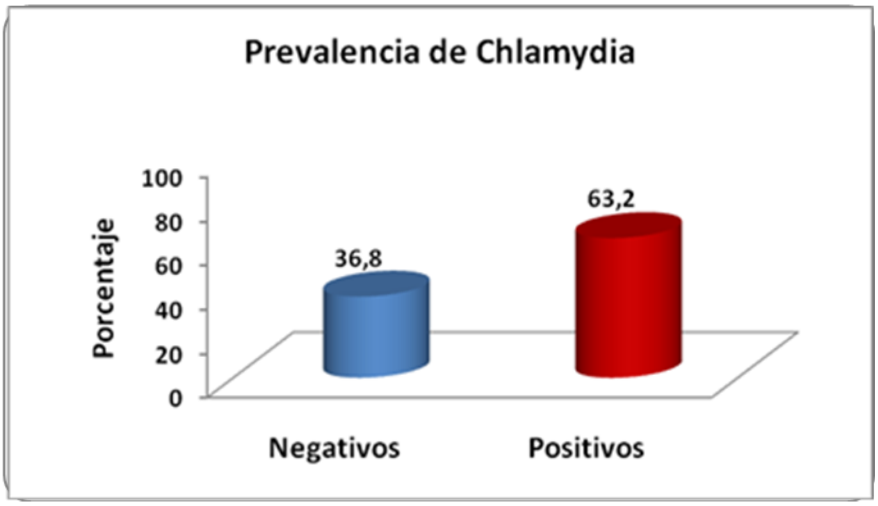

Figure 19. Prevalence of *C. psittaci* in sampled animals

With the purpose of determining if there is a relation between the numbers of positive and negative animals towards *C.psitacci* we stated the following hypothesis:

* Research Hypothesis (RH): number of positive individuals to *C. psittaci*, is statistically equal to the number of negative individuals ($p \geq 0.05$)

* Null hypothesis (OH): number of positive individuals to *C. psittaci* is statistically different to the number of negative individuals ($p < 0.05$).

The evidence indicates that there is a significant difference (p=2.34E-15; $p < 0.05$; $chi^2=62.76$), between both groups, being lower the number of negative samples.

The prevalence found in this study (63.2%) was greater than that found in the province of Parma, Po Valley (Italy) which was 45% (Cavirani *et al*., 2001); while it was similar to that found in bulls from five federal States of Germany (50.6%) (Kauffold *et al*., 2007).

This bacterium is considered of genital transmission because it is found in the preputial mucosal and is excreted in semen; however it is done through fecal matter as well (Kauffold *et al*., 2007). Therefore, the spread of it is constant, which may explain the high prevalence found, mainly where natural mounting is done. As mentioned above, on the studied farms reproductive management is made by artificial insemination, which decreases the possibility of transmission to animals. However, the main reservoirs for the bacteria are birds, which eliminate it through fecal matter, contaminating food for cattle and their environment in general (European Commission, 2002). As a result, the presence of birds (pigeons, chickens and hens) that was evident in the areas of sampling, may explain the presence of this bacteria in cattle, because they are in direct contact with them, as well as troughs and grazing pasture. As this bacteria has a zoonotic character, it is necessary to take control and biosecurity measures to reduce the presence of it in livestock farming, which should focus on avoiding contact of natural reservoirs with production animals.

Other animals which can be infected and spread Clamydia *psittaci* are sheep and goats. However for the studied sites, this is not a risk factor because, even though in Boyacá sheep owning is very common, this animal species were not found in the sampled farms (Pérez & Storz, 1987).

7.2 Prevalence According to Age Group

7.2.1 Bovine Viral Diarrhea

With the purpose of determining if there are differences between the seroprevalence to the virus in the age groups, we stated the following hypothesis:

* Research Hypothesis (RH): the prevalence of BVD is statistically the same in every age group ($p \geq 0.05$)

* Null hypothesis (OH): The prevalence of BVD is statistically different in at least one of the age groups ($p<0.05$).

The test indicates that for BVD there is a statistically significant difference ($p<0.05$) between the prevalences of age groups (p3.38E-06; $p<0.05$; $chi^2=25.20$). The higher prevalences are presented in cows (42%:309ind) and heifers (36.6%:64ind) and the lowest in calves (6.8%: 3ind) (figure 20).

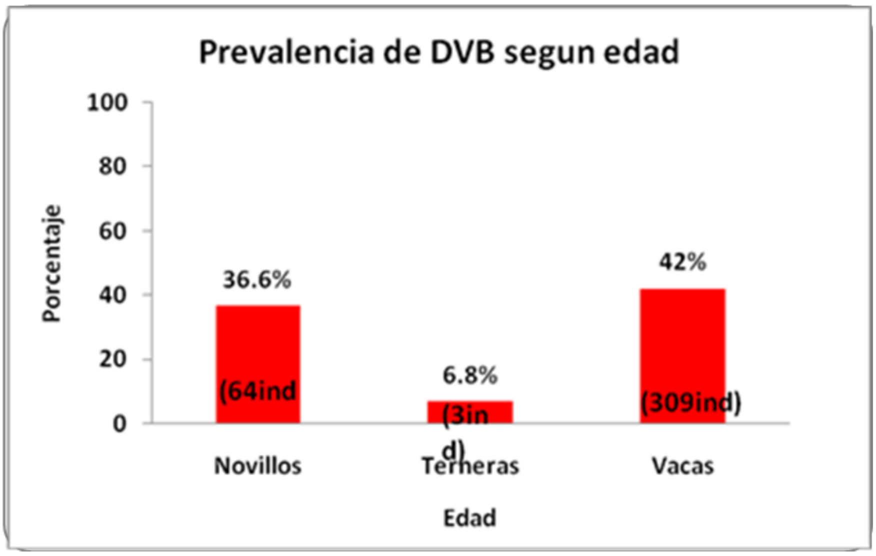

Figure 20 . Seroprevalence to BVDV in age groups.

According to the results found in this study, it is evident, a similarity with the work of Betancur *et al* (2007), who indicates increased seroprevalence in adult animals and within them, the older ones, because they are animals that have been more likely to suffer states of immunosuppressive. Another study also shows higher prevalence in adult animals than young, indicating that BVD seropositivity increases with age, although in infants high prevalence is found possibly because of the presence of colostral antibodies, which were not evident in this work (Mockeliuniene *et al* (2004).

7.2.2. Neosporosis

With the purpose of determining if there are differences between the seroprevalence to the bacterium in the age groups, we stated the following hypothesis:

* Research Hypothesis (RH): the prevalence of *N. caninum* is statistically the same in every age group ($p \geq 0.05$)

* Null hypothesis (oH): The prevalence of *N. caninum* is statistically different in at least one of the age groups ($p<0.05$).

The test indicates that there is a statistically significant difference ($p<0.05$) between the prevalences of age groups ($p=0.0083$; $p<0.05$; $chi^2=9.58$); higher prevalence was found in calves (T=21.6%:8ind) and lower in heifers (8.1%:12ind) and cows (8.2%:54ind) (Figure 21).

However, most of the consulted literature indicates that there is no significant difference between the prevalence of the disease and the age groups (López, *et al*., 2007; Oviedo *et al*, 2006; Otranto *et al*.; 2003).

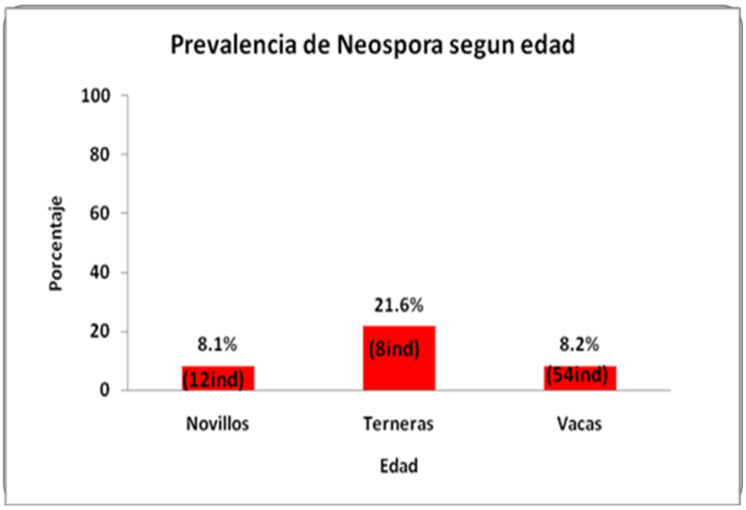

Figure 21 . Seroprevalence to *N.caninum* in age groups

7.2.3. Infectious Bovine Rhinotracheitis

With the purpose of determining if there are differences between the prevalence to the virus in the age groups, we stated the following hypothesis:

* Research Hypothesis (RH): the prevalence of IBR is statistically the same in every age group ($p \geq 0.05$)

* Null hypothesis (OH): The prevalence of IBR is statistically different in at least one of the age groups ($p<0.05$).

The test indicates that for IBR there is no statistically significant difference ($p \geq 0.05$) between the prevalences of age groups ($p=0.0657$; $p \geq 0.05$; $chi^2=3.39$), beign the same in heifers (8.6%:15ind) and cows (18.1%:133ind), although for this ones was higher (Figure 22).

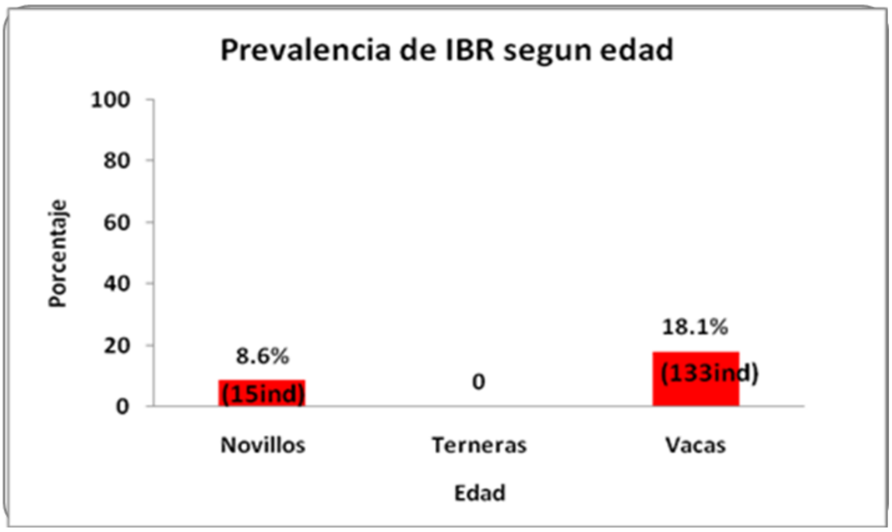

Figure 22 . Seroprevalence to BoHV-1 in age groups

Some studies indicate that there is no significant difference in age groups, in a manner similar to that found in this study (Piedrahita, *et al.*, 2010; Woodbine *et al.*, 2009; Betancur *et al* 2006). Some authors suggest that infants may have high levels of antibodies, but these could be of colostral origin and will decline with age until the animal is exposed to the virus, moment in which it will produce the seroconversion (Piedrahita, *et al.*, 2010). However such situation was not observed in the calves sampled group, although this can be explained by the low prevalence observed in sampled farms.

7.2.5. Infection with Bovine Herpes Virus Type 4

With the purpose of determining if there are differences between the prevalence to the virus in the age groups, we stated the following hypothesis:

* Research Hypothesis (RH): the prevalence of BoHV-4 is statistically the same in every age group ($p \geq 0.05$)

* Null hypothesis (OH): The prevalence of BoHV-4 is statistically different in at least one of the age groups (p<0.05).

The test indicates that for BoHV-4 there is no statistically significant difference ($p \geq 0.05$) between the prevalences in age groups ($p=0.6171$; $p \geq 0.05$; $chi^2=0.97$). There is however a not significant mayor prevalence of BoHV-4 in calves (100%:30ind), and cows (96.7%:497ind), and minor in heifers (87%:87ind) (Figure 23).

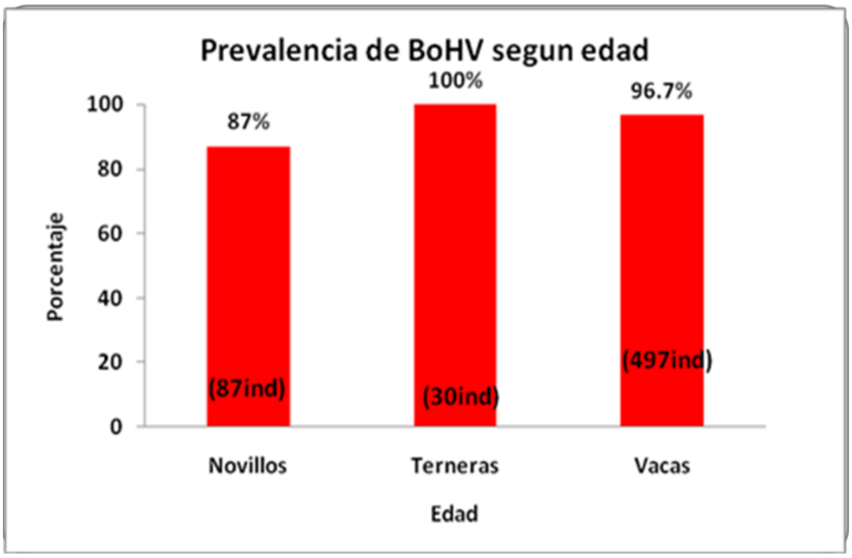

Figure 23. Seroprevalence to BoHV-4 in age groups

Because there is not enough literature on the epidemiological characteristics of this virus, there is no specific information about potential serological differences in different ages of cattle. Nevertheless, noticeable changes in the titers of antibodies during the lifetime of the animal have been studied, leading to different levels of seroconversion in a herd. In pregnant females that were seronegative a couple of weeks before the delivery, an increase of antibody titres can result after delivery, possibly by re-infection or initial exposure to the virus (Frazier *et al*. 2002). This is why it is still unknown what factors can generate seroconversion but it is stated that this occurs at any time when the individual is faced with the virus. Therefore, it can be inferred that as noted in this study, the age is not a determining factor.

7.2.6. Chlamydiosis

With the purpose of determining if there are differences between the seroprevalence to *C. psittaci* in the age groups, we stated the following hypothesis:

* Research Hypothesis (RH): the prevalence of *C. psittaci* is statistically the same in every age group ($p \geq 0.05$)

* Null hypothesis (oH): The prevalence of *C. psittaci* is statistically different in at least one of the age groups ($p<0.05$).

The test indicates that for *C. psittaci* there is a statistically significant difference ($p<0.05$) between the prevalences in age groups (p=5.37E-07; $p<0.05$; $chi^2=28.87$. In calves the prevalence was lower (T=18.2%:8ind); while in cows (66.6%:455ind) and heifers (61%:100ind) was higher (Figure 24). Was not found significant diference between calves and cows.

Based on the type of transmission of *C. psittaci*, it is expected that the seroprevalence in calves is lower than that observed in adult animals, when they are kept in confinement with reduced possibilities of contact. with other species of animals as dogs and birds or with adults of the same species (Corsaro & Greub, 2006; Longbottom & Coulter, 2003).

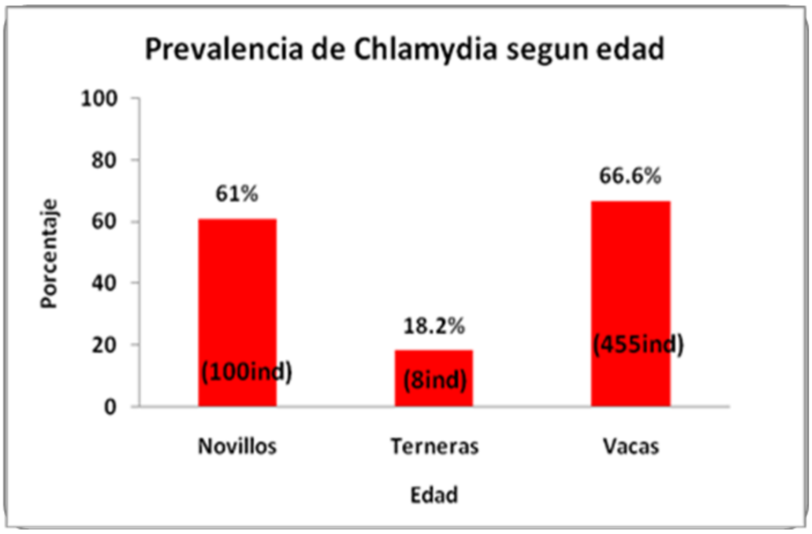

Figure 24. Seroprevalence to *C. psittaci* in age groups

Such a situation was evident on the farm where calves were sampled, because their handling conditions determine low possibilities of contact with other animals, although workers handling them move around the farm.

7.3 Prevalence by farm

7.3.1 Bovine Viral Diarrhea

We found that in five of the 21 evaluated farms, the prevalence was 100%, while in seven farms was below 50% (Figure 25).

With the purpose of determining if there are differences between the prevalence of each one of the evaluated properties, we stated the following hypothesis:

* Research Hypothesis (RH): the number of animals positive to BVD is statistically the same to the animals negative to this disease ($p \geq 0.05$)

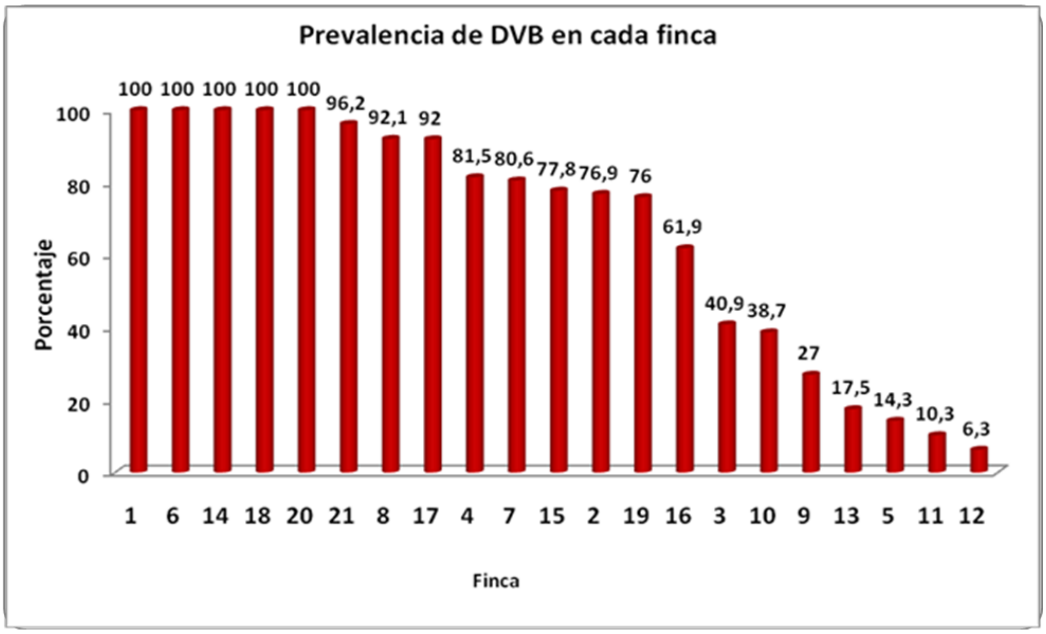

Figure 25 . Prevalence of BVD in the evaluated properties

* Null hypothesis (oH): the number of animals positive to BVD is statistically different to the animals negative to this disease (p<0.05).

The test indicates that there is a significant difference (p<0.05), between the positive and negative animals in the properties 1, 18, 6, 14, 20, 21, 8, 9, 13, 17, 4, 5, 7, 19, 11 and 12; finding the higher prevalence in the farms 1 (100%:23ind), 18 (100%:22ind), 6 (100%:31ind), 14 (100%:11ind), 20 (100%:18ind), 21 (96.2%:25ind), 8 (92.1%:35ind), 15 (77.8%:7ind), 17 (92%:23ind), 16 (61.9%:13ind), 4 (81.5%:22ind), 2 (76.9%:10ind), 7 (80.6%:25ind) and 19 (76%:19ind); lower prevalence in the farms 10 (38.7%:12ind), 9 (27%:17ind), 13 (17.5%:17ind), 3 (40.9%:9ind), 5 (14.3%:3ind), 11 (10.3%:41ind), and 12 (6.3%:4ind). The test indicates that the number of positive and negative animals is statistically the same in the farms 15 (77.8%:7ind), 10 (38.7%:12ind), 16 (61.9%:13ind), 2 (76.9%:10ind), 3 (40.9%:9ind) (Figure 25) (Table 2).

Table 2 . Seroprevalence to BVDV by farm, Chi^2 and Pvalue.

MUNICIPALITY	FARM	PREVALENCE	CHI^2	VALOR P	P
Soraca	1	100	23.00	1.62E-06	< 0.05
Sotaquira	18	100	22.00	2.73E-06	< 0.05
Oicata	6	100	31.00	2.58E-08	< 0.05
Oicata	14	100	11.00	0.000911	< 0.05
Oicata	20	100	18.00	2.21E-05	< 0.05
Oicata	21	96.2	22.15	2.52E-06	< 0.05
Oicata	8	92.1	26.95	2.09E-07	< 0.05
Oicata	15	77.8	2.78	0.095581	≥ 0.05
Oicata	10	38.7	1.58	0.208668	≥ 0.05
Oicata	9	27.0	13.35	0.000259	< 0.05
Oicata	13	17.5	16.90	3.94E-05	< 0.05
Paipa	17	92	17.64	2.67E-05	< 0.05
Paipa	16	61.9	1.19	0.275234	≥ 0.05
Ventaquemada	4	81.5	10.70	0.001069	< 0.05
Ventaquemada	2	76.9	3.77	0.052204	≥ 0.05
Ventaquemada	3	40.9	0.73	0.393769	≥ 0.05
Ventaquemada	5	14.3	10.71	0.001063	< 0.05
Tuta	7	80.6	11.65	0.000644	< 0.05
Tuta	19	76	6.76	0.009322	< 0.05
Sopo	11	10.3	250.8 9	1.66E-56	< 0.05
Iguaque	12	6.3	49.00	2.56E-12	< 0.05

Considering the average prevalence in the propierties of each assessed municipality we found the higher prevalences were in the municipalities of Soracá and Sotaquira (figure 26).

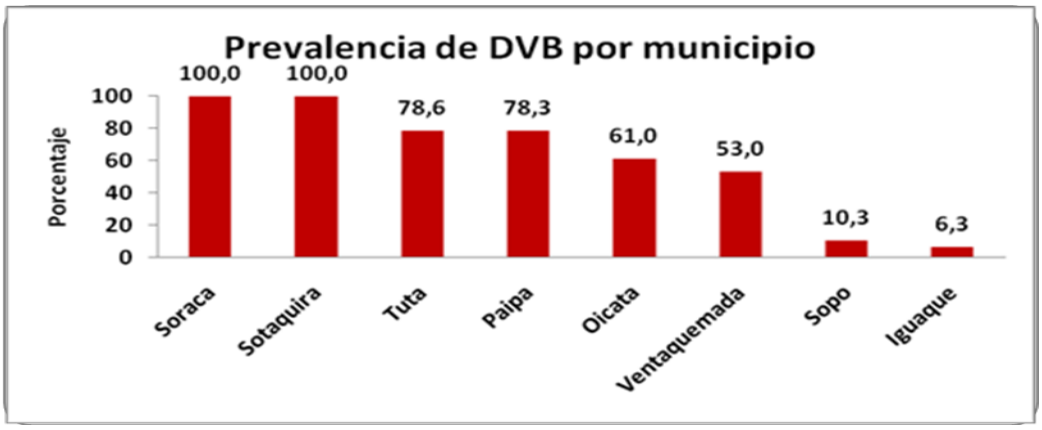

Figure 26 . Seroprevalence to BVDV in each farm of the assessed municipalities

Therefore, it was determined if there is a significant difference between the prevalences found in each of the municipalities studied. The tests indicate that there is a statistically significant difference for BVD ($p<0.05$), between the prevalences found in the municipalities ($p=1.33E-29$; $p<0.05$; $chi^2=152.3$); there were two municipalities with high prevalence (Soraca and Sotaquira), and two municipalities with low prevalences (Sopo and Iguaque). The municipalities with the highest prevalence of BVD are: Soraca (100%:23ind), Sotaquira (100%:22ind), Tuta (78.6%:44ind), Paipa (78.3%:36ind), Oicata (61%:163ind), Ventaquemada (53%:44ind); the municipalities with the lowest prevalence of BVD are: Sopo (10.3%:41ind), Iguaque (6.3%:4ind) (Figure 26).

7.3.2. Neosporosis

Among the 21 sampled farms, 15 had animals which were positive to the parasite and unlike BVD no farm had a prevalence of 100% (Figure 27).

With the purpose of determining if there are differences between the prevalence of each one of the evaluated farms, we stated the following hypothesis:

* Research Hypothesis (RH): the number of animals positive to *N. caninum* is statistically the same to the animals negative to this disease ($p\geq0.05$)

* Null hypothesis (OH): the number of animals positive to *N. caninum* is statistically different to the animals negative to this disease ($p<0.05$).

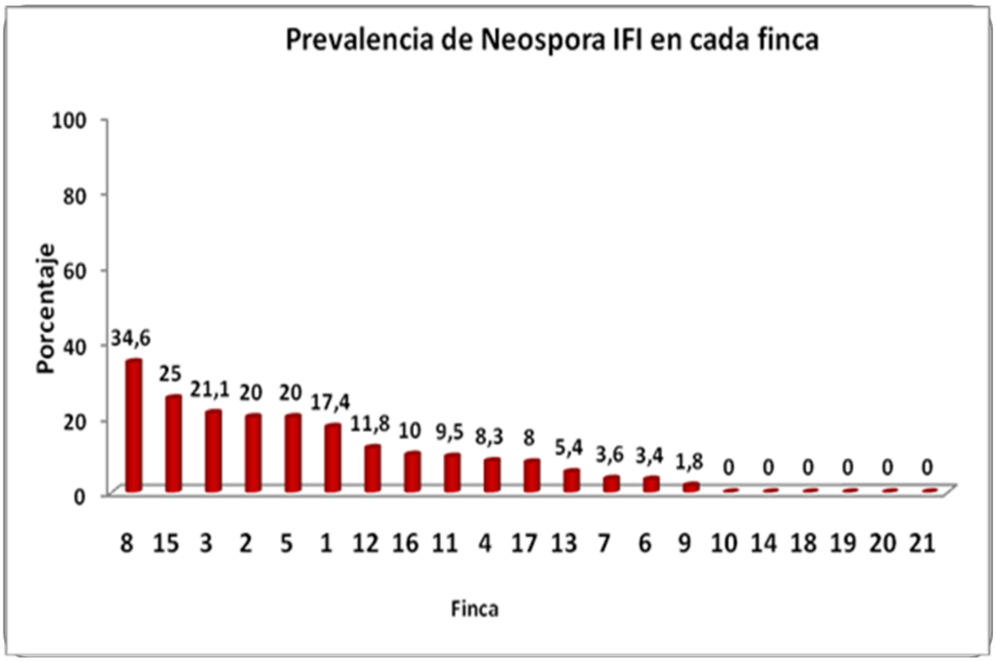

Figure 27. Seroprevalence to *N. caninum* in each assessed farm

The test indicates that for *N. caninum* there is a statistically significant difference ($p<0.05$) between the number of positive and negative animals in the farms 13, 6, 9, 3, 5, 4, 1, 12, 16, 17, 11, 7, 19 and 18. The highest prevalences of *N. caninum* are in the farms 8 (34.6%:9ind), 15 (25%:2ind), 3 (21.1%:4ind), 2 (20%:2ind), 5 (20%:3ind) and 1 (17.4%:4ind); lower prevalences of *N. caninum* in farms 13 (5.4%:2ind), 6 (3.4%:1ind), 9 (1.8%:1ind), 4 (8.3%:2ind), 12 (11.8%:6ind), 16 (10%:2ind), 17 (8%:2ind), 11 (9.5%:33ind), 7 (3.6%:1ind); there is no *N. caninum* in the farms 10, 14, 20, 21, 19 and 18. The test indicates that the number of positive and negative animals is statistically the same in farms 8 (34.6%:9ind), 15 (25%:2ind), and 2 (20%:2ind) (Figure 27) (Table 3).

The highest prevalences were found in Soracá and Ventaquemada whilst in Sotaquirá was 0% (Figure 28).

Table 3. Seroprevalence to *Neospora caninum* by farm, Chi^2 and Pvalue.

MUNICIPALITY	FARM	PREVALENCE	CHI^2	VALOR P	P
Oicata	8	34.6	2.46	0.11666 5	≥ 0.05
Oicata	15	25	2.00	0.15729 9	≥ 0.05
Oicata	13	5.4	29.43	5.79E-08	< 0.05
Oicata	6	3.4	25.14	5.34E-07	< 0.05
Oicata	9	1.8	52.07	5.35E-13	< 0.05
Oicata	10	0	30.00	4.32E-08	< 0.05
Oicata	14	0	11.00	0.00091 1	< 0.05
Oicata	20	0	18.00	2.21E-05	< 0.05
Oicata	21	0	26.00	3.41E-07	< 0.05
Ventaque.	3	21.1	6.37	0.01161 7	< 0.05
Ventaque.	2	20	3.60	0.05778	≥ 0.05
Ventaque.	5	20	5.40	0.02013 7	< 0.05
Ventaque.	4	8.3	16.67	4.46E-05	< 0.05
Soraca	1	17.4	9.78	0.00176 2	< 0.05
Iguaque	12	11.8	29.82	4.73E-08	< 0.05
Paipa	16	10	12.80	0.00034 7	< 0.05
Paipa	17	8	17.64	2.67E-05	< 0.05
Sopo	11	9.5	227.55	2.04E-51	< 0.05
Tuta	7	3.6	24.14	8.94E-07	< 0.05
Tuta	19	0	25.00	5.73E-07	< 0.05
Sotaquirq	18	0	21.00	4.59E-06	< 0.05

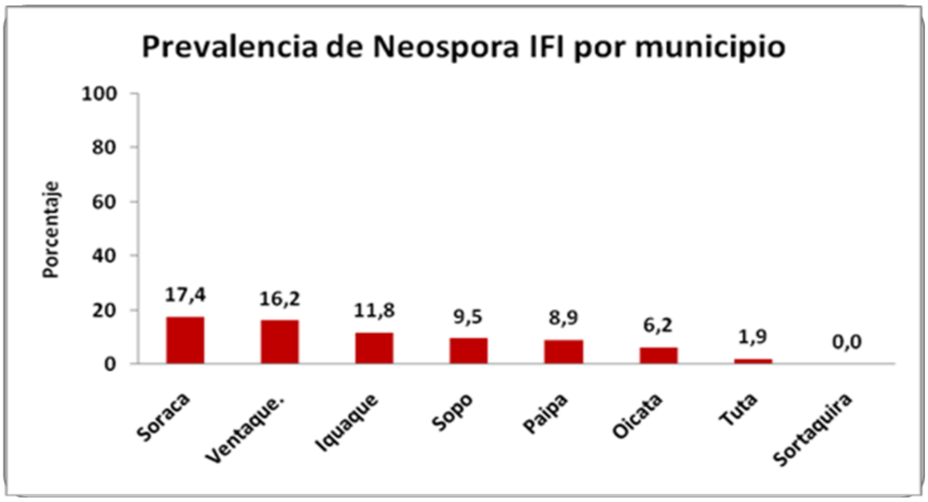

Figure 28 . Seroprevalence to *N. caninum* in sampled farms of each municipality

Therefore, it was determined that there is a significant difference between the prevalences of *N. caninum* found in each of the municipalities of study. The results evidence indicates that there is a statistically significant difference for *N. caninum* ($p<0.05$) among the prevalences of the municipalities ($p=0.0038$; $p<0.05$; $chi^2=20.99$); there are two municipalities with low prevalences (Oicata and Tuta). The municipalities with highest prevalences of Neospora are: Soracá (17.4%:4ind), Ventaquemada (16.2%:11ind), Iguaque (11.8%:6ind), Sopo (9.5%:33ind), Paipa (8.9%:4ind); the municipalities with lowest prevalences of Neospora are: Oicata (6.2%:15ind) and Tuta (1.9%:1ind); Sotaquira did not present seropositivity to *N. caninum* in this study (Figure 28).

7.3.3. Infectious Bovine Rhinotracheitis

In six of the 21 assessed farms there were no animal positive to the virus that causes IBR and the highest prevalences found were between 65 and 82% (Figure 29).

With the purpose of determining if there are differences between the prevalence of each one of the evaluated properties, we stated the following hypothesis:

* Research Hypothesis (RH): the number of animals positive to IBR is statistically the same to the animals negative to this disease ($p\geq0.\ 05$)

* Null hypothesis (OH): the number of animals positive to IBR is statistically different to the animals negative to this disease ($p<0.05$).

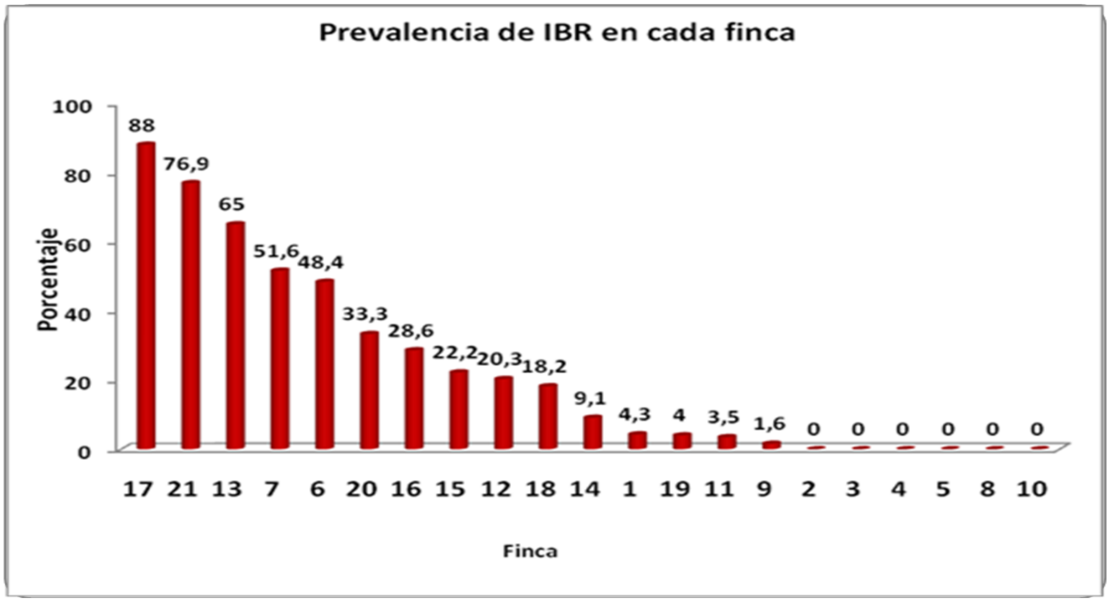

Figure 29. Seroprevalence to IBR in the assessed farms

The test indicates that there is a significant difference ($p<0.05$) between the number of positive and negative animals in the 17, 16, 21, 14, 9, 8, 10, 19, 12, 18, 1, 11, 2, 3, 4 and 5. The highest prevalence of IBR were in the farms17 (88%:22ind), 21 (76.9%:20ind), 13 (65%:26ind) and 7 (51.6%:16ind); lower prevalences of IBR in the farms 16 (28.6%:15ind), 6 (48.6%:16ind), 20 (33.%:6ind), 15 (22.2%:6ind), 14 (9.1%:1ind), 9 (1.6%:1ind), 19 (4%:1ind), 12 (20.3%:13ind), 18 (18.2%:4ind), 1 (4.3%:1ind), 11 (3,5%:5ind); there was not any IBR in the farms 8, 10, 2, 3, 4 and 5. The test indicated the number of positive and negative animals is statistically the same in the farms 13 (65%:26ind), 6 (48.4%:15ind), 20 (33.3%:6ind), 15 (22.2%:2ind), 7 (51.6%:16ind) (Figure 29) (Table 4).

Table 4. Seroprevalence to IBR by farm, Chi^2 and P value.

MUNICIPALITY	FARM	PREVALENCE	CHI^2	VALOR P	P
Paipa	17	88	14.44	0.000145	< 0.05
Paipa	16	28.6	3.86	0.049535	< 0.05
Oicata	21	76.9	7.54	0.00604	< 0.05
Oicata	13	65	3.60	0.05778	≥ 0.05
Oicata	6	48.4	0.03	0.857462	≥ 0.05
Oicata	20	33.3	2.00	0.157299	≥ 0.05
Oicata	15	22.2	2.78	0.095581	≥ 0.05
Oicata	14	9.1	7.36	0.006656	< 0.05
Oicata	9	1.6	59.06	1.53E-14	< 0.05
Oicata	8	0	38.00	7.07E-10	< 0.05
Oicata	10	0	31.00	2.58E-08	< 0.05
Tuta	7	51.6	0.03	0.857462	≥ 0.05
Tuta	19	4	21.16	4.22E-06	< 0.05
Iguaq.	12	20.3	22.56	2.03E-06	< 0.05
Sotaq.	18	18.2	8.91	0.002838	< 0.05
Sorac.	1	4.3	19.17	1.19E-05	< 0.05
Sopo	11	3.5	343.97	8.72E-77	< 0.05
Ventaque.	2	0	13.00	0.000311	< 0.05
Ventaque.	3	0	22.00	2.73E-06	< 0.05
Ventaque.	4	0	27.00	2.03E-07	< 0.05
Ventaque.	5	0	21.00	4.59E-06	< 0.05

The highest prevalence was found in the municipalities of Paipa and Tuta and in Ventaquemada. Therefore, we determined if there was a significant difference between prevalences of IBR found in each of the municipalities studied. (Figure 30).

The test indicates that for IBR there is a statistically significative difference ($p<0.05$), between the prevalences of the municipalities ($p=2.25E-21$; $p<0.05$; $chi^2=112.9$); there was a municipality with high prevalence (Paipa), and two municipalites with low prevalences (Soracá and Sopó).

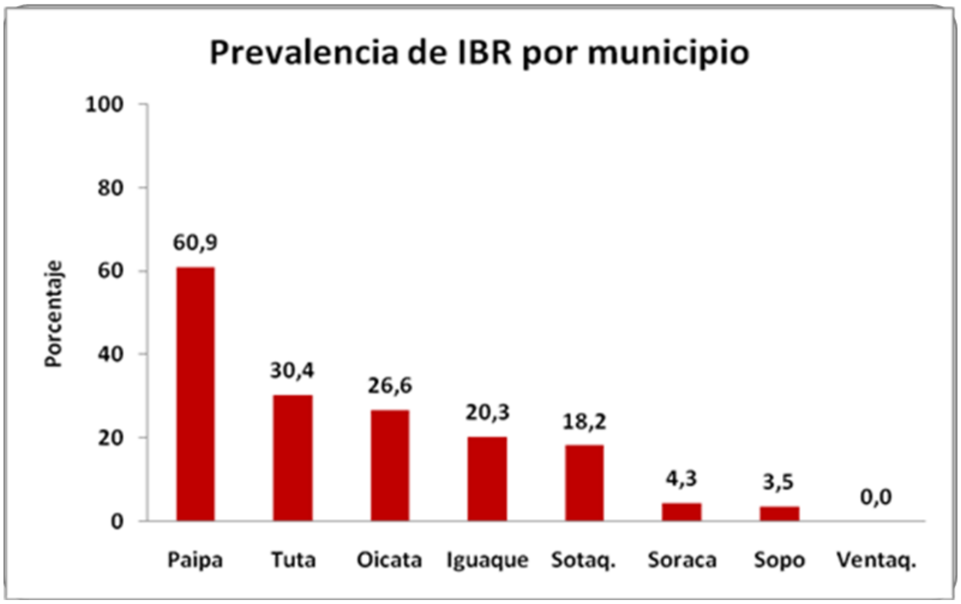

Figure 30. Seroprevalence to IBR in the different sampled farms

The municipality with highest prevalence of IBR is: Paipa (60.9:28ind); the municipalities with lowest prevalences are Tuta (30.4%:17ind), Oicatá (26.6%:71ind), Iguaque (20.3%:13ind), Sotaquirá (18.2%:4ind), Soracá (4.3%:1ind), Sopó (3.5%:141ind); there was no seropositivity to IBR in Ventaquemada (Figure 30).

7.3.4. Infection with Bovine Herpes Virus Type 4

Of all the evaluated diseases, the one that showed highest prevalence by farm was BoHV-4, with 15 of the 21 farms with prevalence of 100%, nevertheless it should be kept in mind that in the study are included farms vaccinated against the virus (Figure 31).

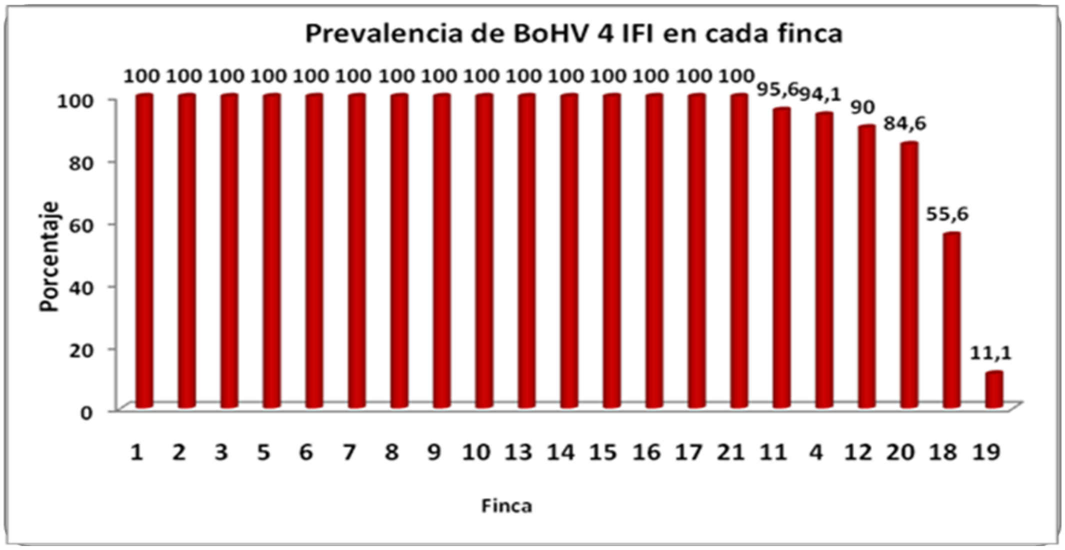

Figure 31. Seroprevalence to BoHV-4 in evaluated farms

With the purpose of determining if there are differences between the prevalence of each one of the evaluated properties, we stated the following hypothesis:

* Research Hypothesis (RH): the number of animals positive to BoVH-4 is statistically the same to the animals negative to this disease ($p \geq 0.05$)

* Null hypothesis (oH): the number of animals positive to BoVH-4 is statistically different to the animals negative to this disease ($p < 0.05$).

The test indicates that for BoHV-4, there is a statistically significant difference ($p < 0.05$) between the number of positive and negative animals in the farms 6, 8, 9, 10, 13, 14, 15, 21, 20, 16, 17, 1, 7, 19, 2, 3, 5, 4, 11 and 12.The test indicates that the number of positive and negative animals is statistically the same in the farm 18 (55.6%:5ind). High seroprevalence to BoHV-4 were present in every farm except one: 6 (100%:27ind), 8

(100%:25ind), 9 (100%:56ind), 10 (100%:20ind), 13 (100%:34ind), 14 (100%:6ind), 15 (100%:5ind), 21 (100%:17ind), 20 (84.6%:11ind), 16 (100%:8ind), 17 (100%:15ind), 1 (100%:15ind), 7 (100%:25ind), 2 (100%:10ind), 3 (100%:12ind), 5 (100%:19ind), 4 (94.1%:16ind), 11 (95.6%:263ind), 12 (90%:27ind), 18 (55.6%:5ind) and the lowest prevalence was found in the farm 19 (11.1%:1ind) (Figure31) (Table 5).

Table 5. Seroprevalence to BoHV 4 by farm, Chi^2 and Pvalue.

MUNICIPIO	PREDIO	PREVALENCIA	CHI^2	VALOR P	P
Oicata	6	100	27.00	2.03E-07	< 0.05
Oicata	8	100	25.00	5.73E-07	< 0.05
Oicata	9	100	56.00	7.25E-14	< 0.05
Oicata	10	100	20.00	7.74E-06	< 0.05
Oicata	13	100	34.00	5.51E-09	< 0.05
Oicata	14	100	6.00	0.014306	< 0.05
Oicata	15	100	5.00	0.025347	< 0.05
Oicata	21	100	17.00	3.74E-05	< 0.05
Oicata	20	84.6	6.23	0.012555	< 0.05
Paipa	16	100	8.00	0.004678	< 0.05
Paipa	17	100	15.00	0.000108	< 0.05
Soraca	1	100	15.00	0.000108	< 0.05
Tuta	7	100	25.00	5.73E-07	< 0.05
Tuta	19	11.1	5.44	0.019631	< 0.05
Ventaq.	2	100	10.00	0.001565	< 0.05
Ventaq.	3	100	12.00	0.000532	< 0.05
Ventaq.	5	100	19.00	1.31E-05	< 0.05
Ventaq.	4	94.1	13.24	0.000275	< 0.05
Sopo	11	95.6	229.09	9.39E-52	< 0.05
Iguaque	12	90	19.20	1.18E-05	< 0.05
Sotaquirq	18	55.6	0.11	0.738883	≥ 0.05

With relation to the distribution of prevalence in the municipalities of study we observed that in Soracá, Ventaquemada and Oicatá were the highest prevalences of the study. Despite in some cases the prevalence was lower than

100% the presence of this disease was generalized in the study area (Figure 32).

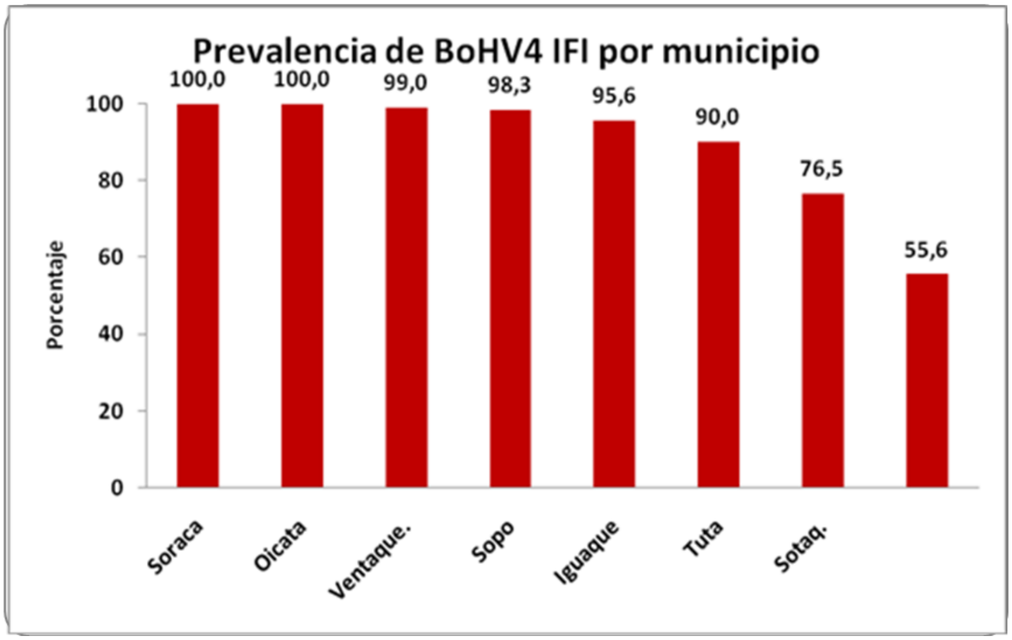

Figure 32. Seroprevalence to BoHV-4 in the evaluated farms

Therefore, we determined if there is a significant difference between the prevalences of BoHV-4 found in each of the municipalities of study. The test indicates that for BoHV-4 there are statistically significant differences ($p<0.05$) between prevalences of the studied municipaltities ($p=0.0067$; $p<0.05$; $chi^2=19.51$). The municipalities with highest prevalences are: Paipa (100%:23ind), Soracá (100%:15ind), Oicatá (99%:201ind), Ventaquemada (98.3%:57ind), Sopó (95.6%:263ind), Iguaque (90%:27ind), Tuta (76.5%:26ind); the municipality with the lowest prevalence is Sotaquirá (55.6%:5ind) (Figure 32).

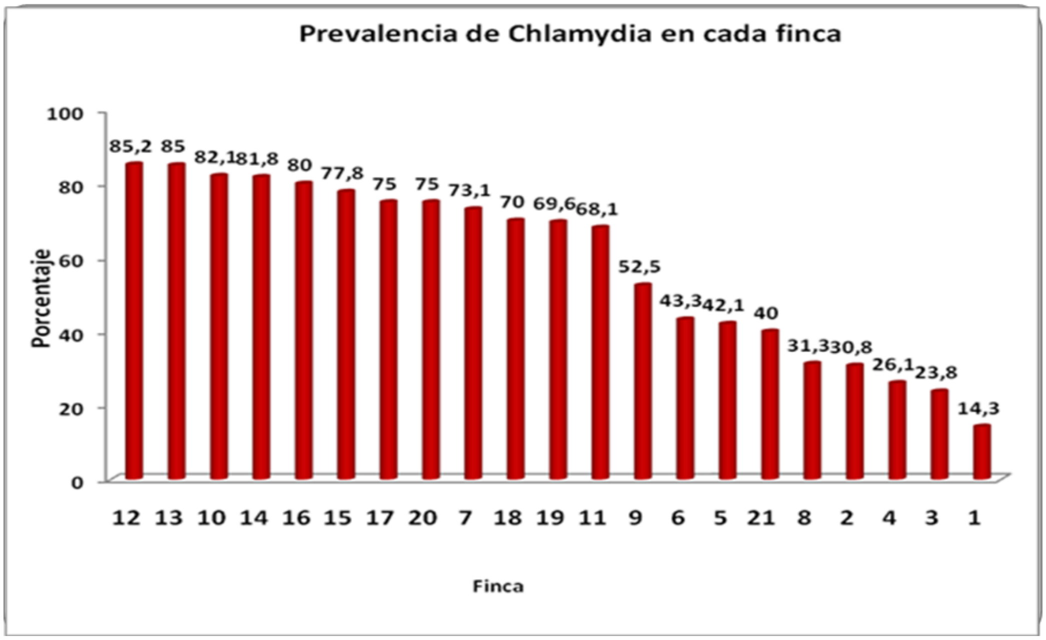

Figure 33. Seroprevalence to *C.psittaci* in the evaluated farms

7.3.6. Chlamydiosis

Seropositivity to the bacterium was present in every tested farm although the prevalence varied from one to the other. With the purpose of determining if there are differences between the prevalence of each one of the evaluated farms, we stated the following hypothesis:

* Research Hypothesis (RH): the number of animals positive to *C. psittaci* is statistically the same to the animals negative to this disease ($p \geq 0.05$)

* Null hypothesis (OH): the number of animals positive to *C.psittaci* is statistically different to the animals negative to this disease ($p < 0.05$).

The test indicates that for *C. psittaci*, there is a statistically significant difference ($p < 0.05$) between the number of positive and negative animals in the farms 12, 13, 10, 14, 20, 8, 16, 17, 7, 11, 4, 3 and 16. The highest prevalences were found in the farms 12 (85.2%:52ind), 13 (85%:34ind), 10 (82.1%:23ind), 14 (81.8%:9ind), 15 (77.8%:7ind), 20 (75%:12ind), 9 (52.5%:31ind), 16 (80%:16ind), 17 (75%:18ind), 7 (73.1%:19ind), 19 (69.6%:16ind), 18 (70%:14ind), 11 (68.1%:258ind); minor prevalences were found in farms 6 (43.3%:13ind), 21 (40%:8ind), 8 (31.3%:10ind), 5 (42.1%:8ind), 2 (30.8%:4ind), 4 (26.1%:6ind), 3 (23.8%:5ind), 1 (14.3%:3ind). The test indicates that the number of positive and negative animals is statistically the same in the farms 15 (77.8%:7ind), 9 (52.5%:31ind), 6 (43.3%:13ind), 21 (40%:8ind), 19 (69.6%:16ind), 18 (70%:14ind), 5 (42.1%:8ind) and 2 (30.8%:4ind) (Figure 33) (Table 6).

Table 6 . Seroprevalence to *C. psittaci* by farm, Chi^2 and P value.

MUNICIPALITY	FARM	PREVALENCE	CHI^2	VALOR P	P
Iguaque	12	85.2	30.31	3.68E-08	< 0.05
Oicata	13	85	19.60	9.55E-06	< 0.05
Oicata	10	82.1	11.57	0.00067	< 0.05
Oicata	14	81.8	4.45	0.034808	< 0.05
Oicata	15	77.8	2.78	0.095581	≥ 0.05
Oicata	20	75	4.00	0.0455	< 0.05
Oicata	9	52.5	0.15	0.696118	≥ 0.05
Oicata	6	43.3	0.53	0.465209	≥ 0.05
Oicata	21	40	0.80	0.371093	≥ 0.05
Oicata	8	31.3	4.50	0.033895	< 0.05
Paipa	16	80	7.20	0.00729	< 0.05
Paipa	17	75	6.00	0.014306	< 0.05
Tuta	7	73.1	5.54	0.018603	< 0.05
Tuta	19	69.6	3.52	0.060569	≥ 0.05
Sotaquira	18	70	3.20	0.073638	≥ 0.05
Sopo	11	68.1	49.52	1.96E-12	< 0.05
Ventaq.	5	42.1	0.47	0.491297	≥ 0.05
Ventaq.	2	30.8	1.92	0.165518	≥ 0.05
Ventaq.	4	26.1	5.26	0.02181	< 0.05
Ventaq.	3	23.8	5.76	0.016377	< 0.05
Soraca	1	14.3	10.71	0.001063	< 0.05

In every evaluated municipality there was seropositivity to this bacterium, being higher in the municipalities of Iguaque and Paipa and lower in Soracá (Figure 34).

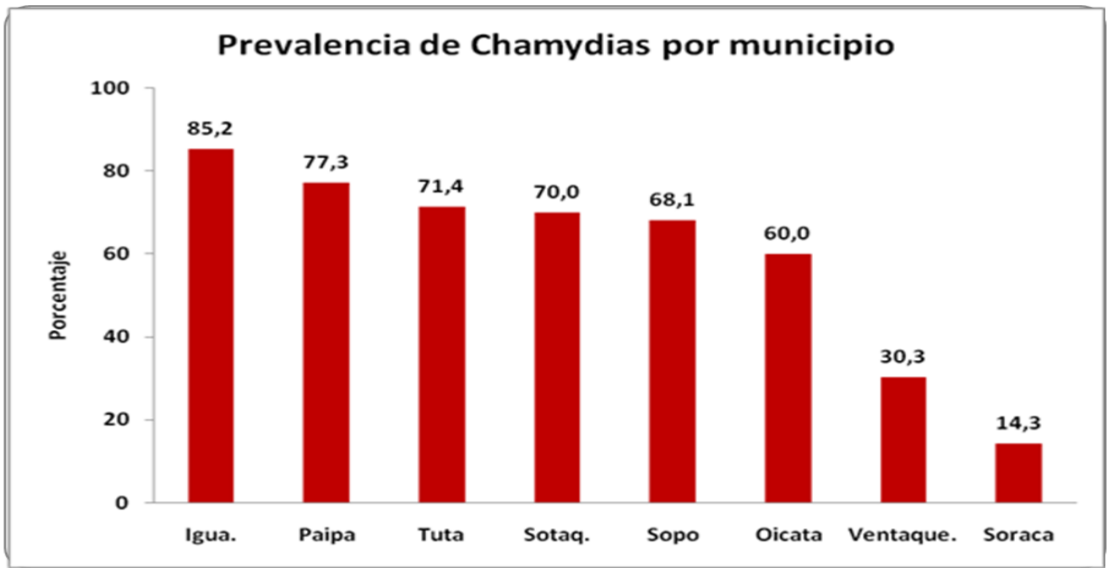

Figure 34 . Seroprevalence to *C.psittaci* in the evaluated municipalities

Therefore, we determined if there was a difference between the prevalences to *C. psittaci* found in each of the municipalities of study. The test showed that there is a statistically significant difference ($p<0.05$) between prevalences of municipalities ($p=1.13E-12$; $p<0.05$; $chi^2=70.6$); there are two municipalities with low prevalences (Ventaquemada and Soraca). The municipalities with highest prevalences to *C.psittaci* are Iguaque (85.2%:52ind), Paipa (77.3%:34ind), Tuta (71.4%:35ind), Sotaquirá (70%:14ind), Sopó (68.1%:258ind), Oicatá (60%:147ind); the municipalities with lowest prevalences to *C.psittaci* are Ventaquemada (30.3%:23ind), Soracá (14.3%:3ind) (Figure 34).

7.4 Relation between Prevalences in the farms and Age Groups

7.4.1 Bovine Viral Diarrhea

In nine out of 21 studied farms, samples were taken from heifers and three of them had prevalences higher than 50% (1, 17, 19), another three, were lower than that value (9, 3, 11) and in two farms (8, 10) it was the same or very similar to 50%. The calves were from just one farm (11) and the prevalence was lower than 50%. Adult females were sampled in every farm and can be distributed between those farms where more than 50% of the animals were positive (1, 18, 6, 14, 20, 21, 8, 15, 17, 16, 4, 2, 7) and those seven farms where the seropositivity was lower than 50% (10, 9, 13, 3, 5, 11, 12) (Figure35).

7.4.2. Neosporosis

In the case of heifers in three of the sampled farms (8, 3, 11), the amount of positive animals to the parasite was higher than 50% and in five farms did not appeared. The group of calves form the sampled farm showed a prevalence lower than 50% and cows, in fifteen farms (8, 15, 13, 6, 9, 3, 2, 5, 4, 1, 12, 16, 17, 11, 7), had a prevalence higher than 50% and in five (10, 14, 20, 21, 18), was lower (Figure 36).

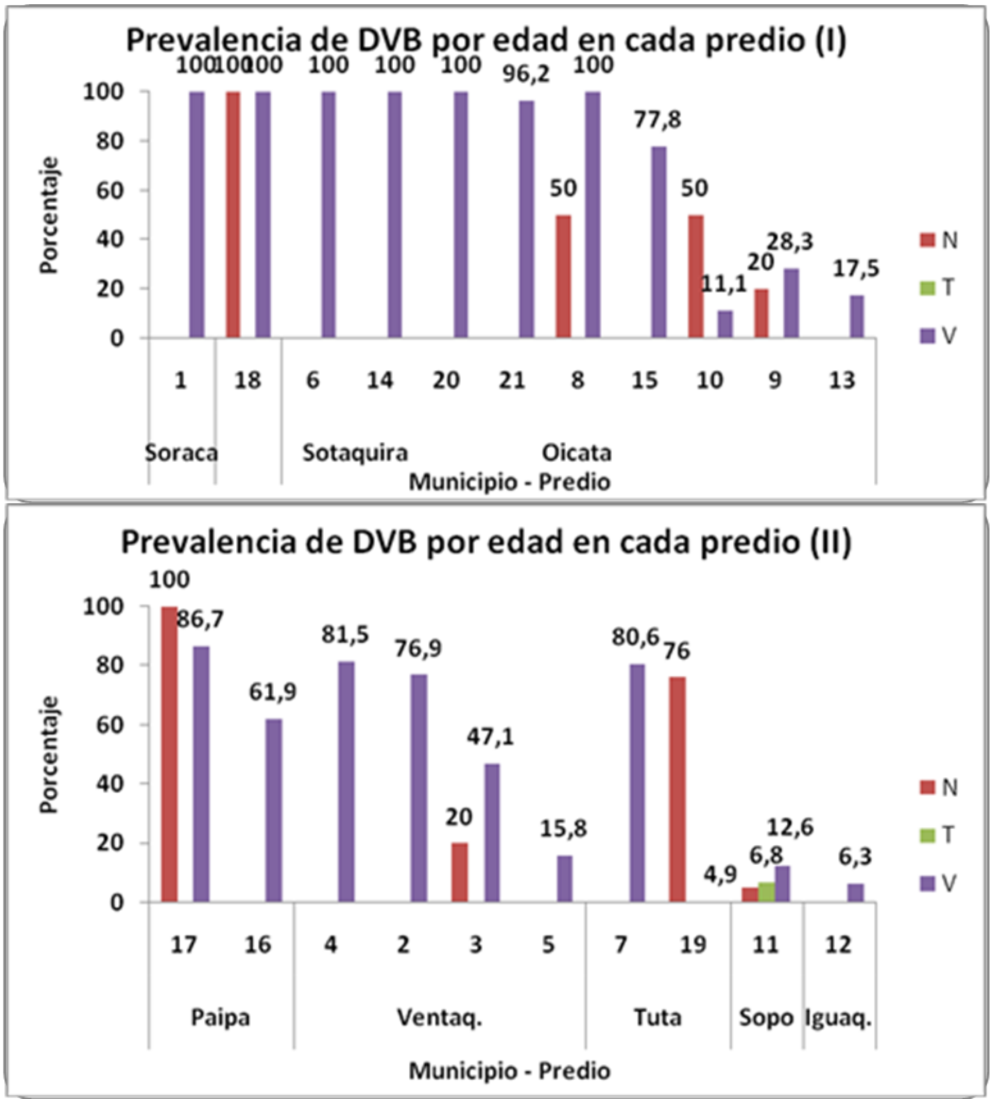

Figure 35. Relation of the seroprevalence to BVD with age group in each studied farm

7.4.3. Infectious Bovine Rhinotracheitis

In one farm (17), the heifers showed a prevalence higher than 50%, in another one (19), it was lower than this value, and in seven (9, 8, 10, 18, 11, 3, 5), it did not present, In calves there was seronegativity and in adult females, in nine farms (16, 6, 20, 15, 14, 9, 12, 1, 11), seropositivity was inferior than

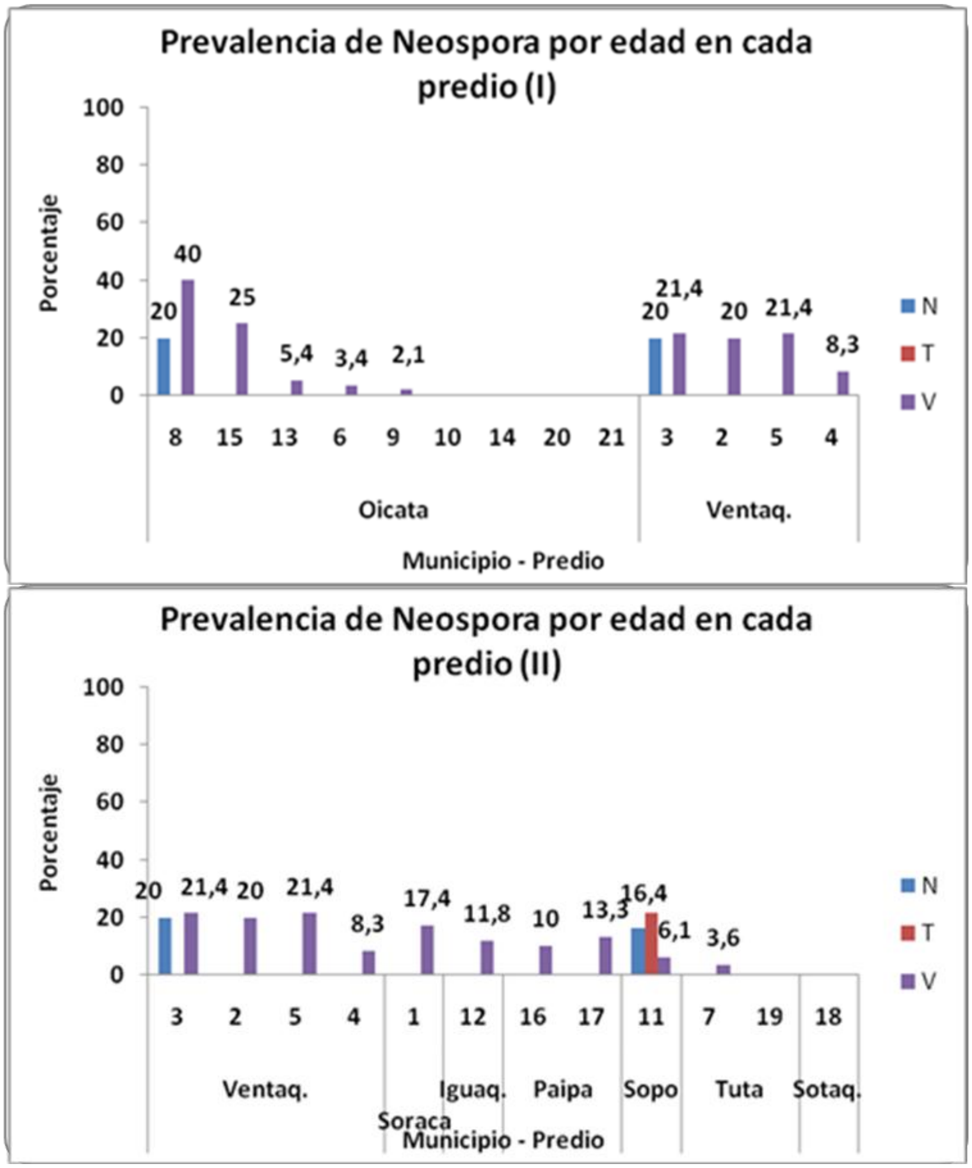

Figure 36.Relation of the seroprevalence to *N.caninum* with age group in each studied property.

50%, in four farms (9, 8, 10, 18, 11, 3, 5) and in six farms (8, 10, 2, 3, 4, 5), was negative (Figure 37).

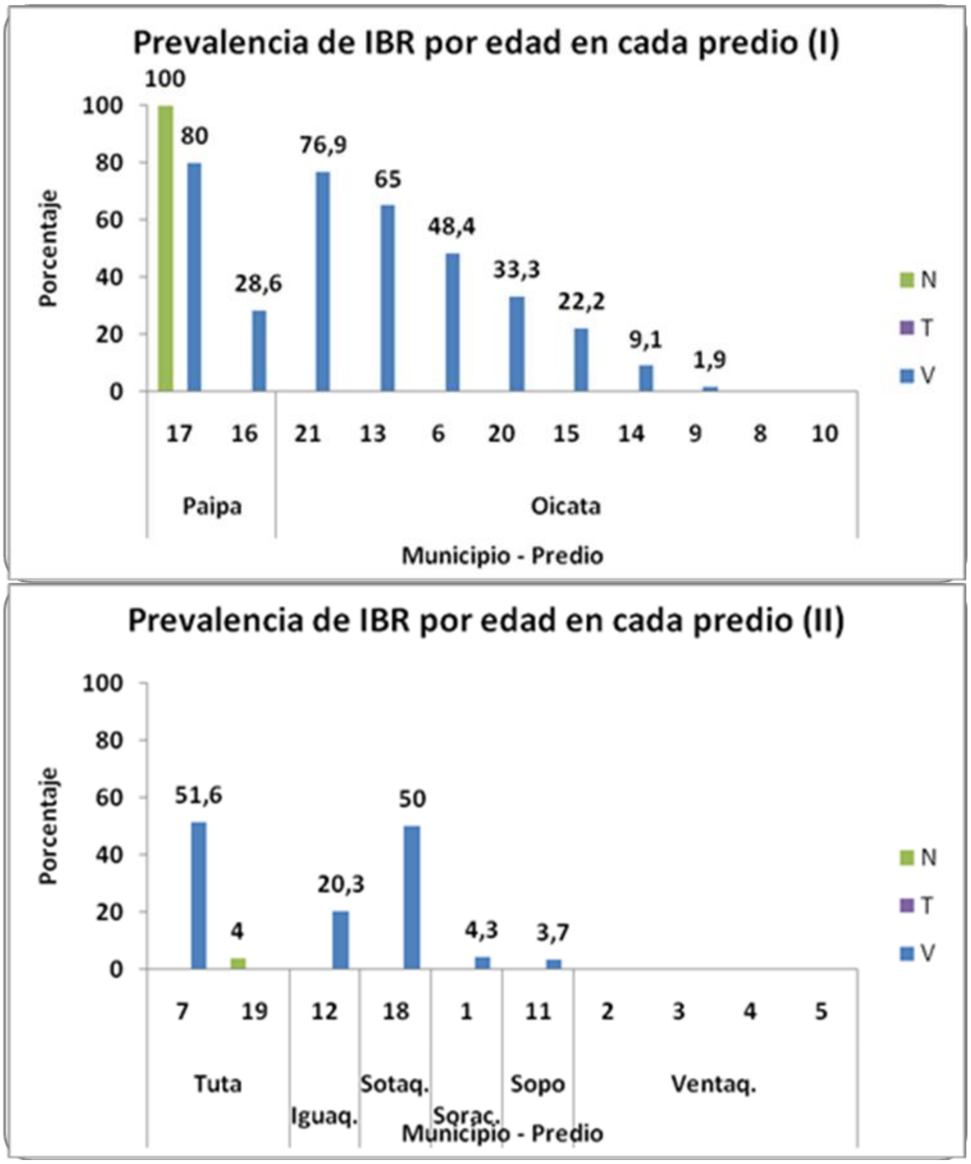

Figure 37. Relation of the seroprevalence to IBR with age group in each studied farm.

7.4.4. Infection with Bovine Herpes Virus Type 4

The group of heifers from eight farms (8, 9, 10, 17, 3, 5, 11, 18), present prevalences higher 50% and in one farm (19) it was lower than 50%. In the case of the calves their seropositivity was higher than 50%, as in 19 farms (6, 8, 9, 10, 13, 14, 15, 21, 20, 16, 17, 1, 7, 2, 3, 5, 4, 11, 12) for adult females, leaving a farm (18) with prevalence lower than 50% (Figure 38).

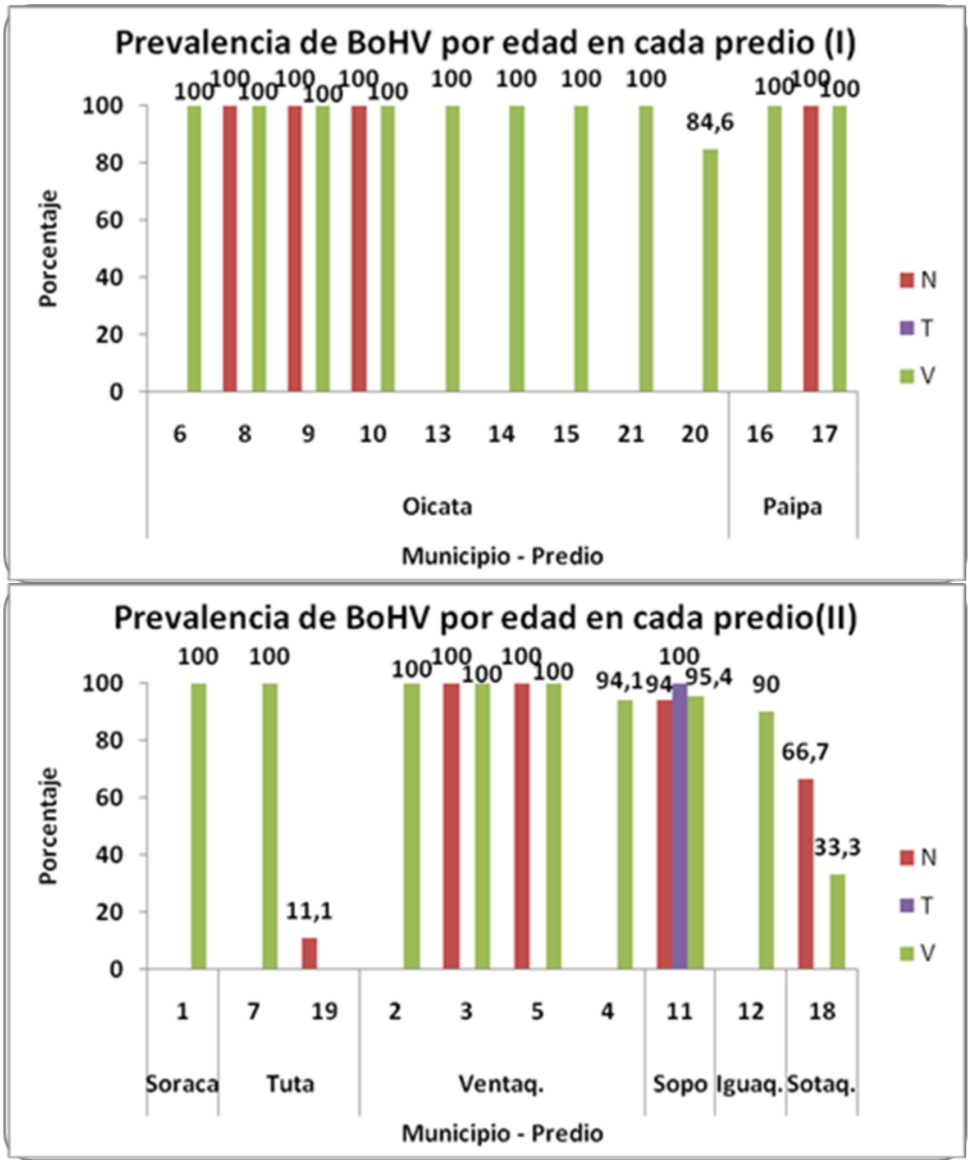

Figure 38 Relation of the seroprevalence to BoHV-4 with age group in each studied farm

7.4.6. Chlamydiosis

In seven farms (10, 9, 17, 19, 18, 11, 5) from the nine in which samples from the heifer group were taken, seropositivity was above 50%, in one (8), was lower and in three there was not any. The seropositivity in calves in the farm (11) was under 50% and adult females, in 11 farms (12, 13, 10, 14, 15, 20, 16, 17, 7, 18, 11) were over 50% seropositive, in nine farms (9, 6, 21, 8, 5, 2, 4, 3, 1) they were under that percentage (Figure 39).

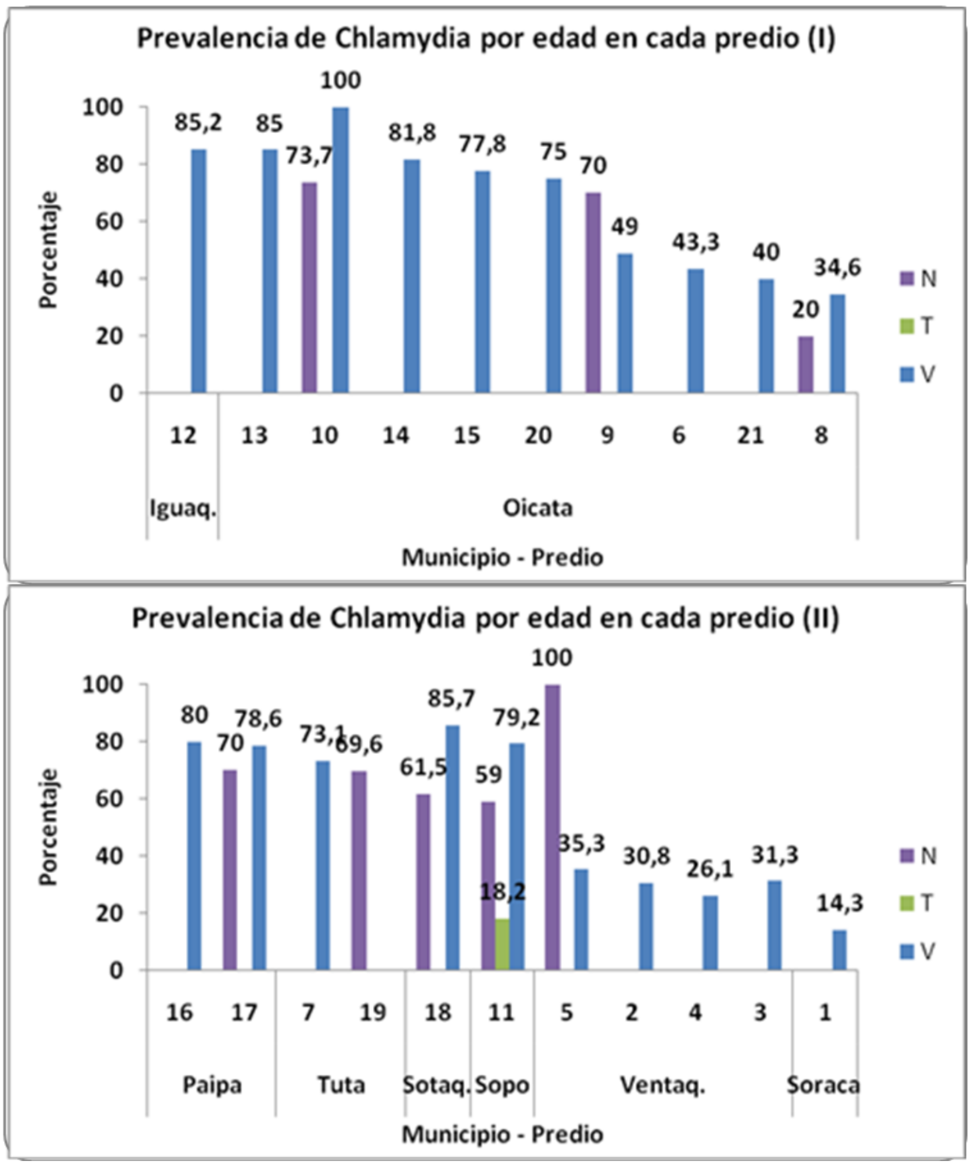

Figure 39 Relation of the seroprevalence to *Clamydia psittaci* with age group in each studied farm.

Therefore, the group of heifers, in decreasing order showed seropositivity to BoHV-4, *Chlamydia psittaci*, BVD, IBR and *Neospora caninum*. Calves showed higher seropositivity to BoHV-4, followed by *Neospora caninum*, *Chlamydia* psittaci and BVD; they did not showed seropositivity to IBR. Finally adult females showed the following results: BoHV-4 (94.9%), BVD (65%), *Chlamydia* psittaci (60.3%), IBR (35.4%), and Neospora (14%) (Figure 40).

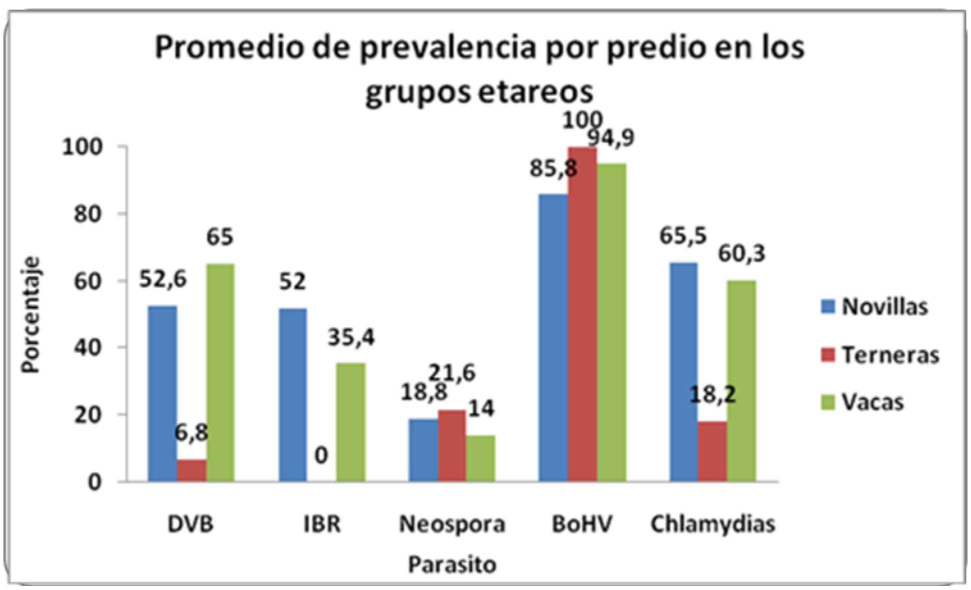

Figure 40 Farm Average prevalence in the age groups

7.5 Relation between Prevalence - Abortion Report

In the area of study, the processing of reproductive records is limited and the few farms that make this are incomplete. Therefore, this research could not made a correlation or a statistical analysis between the seroprevalence of each disease on each farm or the positivity of every animal, with the open days, the interval between births and the number of deliveries per conception. We were just able to collect in each case, general information about the occurrence of abortions each year in the herd or the absence of it, and from there we could just give a descriptive relation between studied diseases and the presence of abortion.

7.5.1 Bovine Viral Diarrhea

According to the results of this study we conclude that of the five farms whose prevalence was 100%, four reported annually abortions superior than 10%. On farms with seropositivity between 80 and 99%, half of the animals had problems of abortions. These results are consistent with that reported by the literature where the presence of this virus is associated with reproductive problems such as increasing the interval between births, because it leads to increased production of cortisol which inhibits the production of luteinizing hormone, which have a role in ovulation. In addition, the virus affects the pellucid area and can avoid the implantation of the embryo (Rondo, 2006).

The literature indicates that one of the triggers of alterations in reproductive parameters is the presence of this virus, either by direct alteration on the estrous cycle or pregnancy (Quispe, *et al.,* 2008) or because inducing these states of immunosuppression constitutes a getaway for other abortive pathogens (Evermann, and Ridpath, 2002; Mainar *et al.,* 2000). The aforementioned could explain but not confirm the presence of abortions in the seropositive farms, where there may be other triggering factors.

7.5.2. Neosporosis

While most of sampled farms in this study reported reproductive alterations, specifically high number of open days and abortions at different stages of gestation, in all cases the parasite seroprevalence was less than 40%. Those farms where we found higher positivity (20-34%) correspond to farms where the presence of abortions was reported. In Colombia there has been reported a seropositivity to *N. caninum* of 54.1% in animals that had abortions (Zambrano *et al*., 2001), by which what was found in this work is considered low. With regard to the relation between the parasite and the presentation of reproductive problems, several reports indicate that *N. caninum* is one of the major infectious agents that causes abortion in cattle (Koiwai *et al*., 2005), due to the passage of the tachysoites to fetuses (Pabón 2007; Zambrano *et al*., 2001). In a study conducted in Montería we found a prevalence of 10.2% and some of the positive animals, reported recurrence of estrus, abortion, and fetal mummification. Therefore, coinciding with the conclusions presented by the authors of the previous work, in this study we can suppose that the report of abortions on farms could be due also to the presence of *N. caninum*.

7.5.4. Infectious Bovine Rhinotracheitis.

In this study IBR prevalence was relatively low, only in one farm it was found over 80% and it does not report problem of abortions, so it can be inferred that the presence of the virus does not trigger the problem in the farm tested. However, in two farms, whose prevalence was between 50 and 80% annual abortions rate was over 10%. Literature indicates that 74.4% of repeat cows and 70% of cows with reported abortion, are positive to IBR, however it is determined that there is no direct correlation between the disease and the previously mentioned alterations (Betancur, *et al.*, 2006), which coincides with the findings of this work. It has been shown that the BoHV-1 in its respiratory form brings as a consequence abortion, usually between the fourth and eighth month of pregnancy, but the pathophysiology of the process is not known (Muylkens, et al., 2007). However, in a similar way to what was indicated for the BVDV, this virus also can pass to the implanted embryo and can trigger embryonic death.

7.5.5. Infection with Bovine Herpes Virus Type 4

In the studied farms, 13 out of 19 that had prevalence above 50%, report presence of abortions and a link between the recorded seropositivity to BoHV-4 and abortions cannot be excluded, as also reported by many authors who directly associate presentation of abortion with the presence of infection (Gür y Dogan, 2010; Frazier et al., 2001; Kalman et al, 2004).

7.5.6. Chlamydiosis

Eleven out of the 17 farm that showed prevalence above 50% reported at least an abortion. In literature, many authors indicate that this bacteria produces abortions between 3 and 5 month of pregnancy and is a triggering factor of it. (Borel et al., 2006; DeGraves et al.; 2003; Cavirani et al., 2001).

8. CONCLUSIONS AND RECOMMENDATIONS

In general terms, on the basis of the results obtained by this work, we can say that the most prevalent disease in the sampled municipalities was BoHV-4, which is an important report, taking into account that the presence of the disease had never been reported in any region of Colombia, including the area of study. Finding 95.4% of animals positive to the virus, it is suggested to do further studies on this subject in order to learn more about the serological dynamic, risk factors and potential impacts on health and animal production.

The second most infectious agent was *Chlamydia abortus* (63.2%), followed by BVDV (39.3%), IBR (15.4%) and *Neospora caninum* (8.7%), which was the less prevalent.

Differences in the prevalences of all sites surveyed, for each of the diseases were found. In the case of BoHV-4, the seropositivity range was between 11.1 and 100%; for the BVD was between 12 and 100%, for IBR from 0 to 88% and *Neospora caninum* between 0 and 34.6%, this indicates that the data cannot be extrapolated from a zone to another and that is why it is necessary to advance studies of this type, in each municipality with livestock farming, to know the epidemiological situation of these diseases throughout the country.

In a manner similar to that reported by other authors, (Betancour *et al*, 2006. Otte *et al*, 1996) this work found no clear relationship between the seroprevalences and the age of the animal, which suggests that it may prove more important, to take serum samples at different stages of the life of the animal, by adhering to such factors as delivery, time of year or reproductive stage, since stressful situations can change the serological status of a herd.

On the other hand, and because of the difficulty to get complete and up-to-date reproductive records on the farms study areas, it was impossible to determine the influence of these diseases on infertility. With regard to the report of abortions we could determine that there is a relative relation between positivity to each of the studied infectious agents and the reporting of abortions. However, because there are other triggering factors of this events, it is not possible to define a clear association between the presence of these microorganisms and abortions; is the only etiology. Because of this we suggest that properties with greater prevalence of the diseases or those with important reproductive problems should conduct a study with females showing abortion, including seriated sampling of aborted fetuses.

In most of the evaluated farms, we found reduced sanitary measures and biosafety, which may explain the presentation of many of the studied diseases. Having several animal species in the same farm was common and therefore exposure of birds and canines with cattle can frequently happen. This can lead to the transmission of *Clamydia psittaci*, *Leptospira spp*. and *Neospora caninum*. This situation can be overcome with the correct technical guidance of the owners and the animal handlers.

Regard to the grade of modernization only one out of the 21 sampled farms can be considered as of high modernization and another one with the worst handling conditions; the rest has low to regular handling conditions. The prevalence of the studied diseases did not show a specific pattern in any of the farms. However, we should suggest improvement of every health and biosecurity aspect even in the farm with greater modernization since in this farm all diseases were present.

With regard to Leptospira, despite partial results, the presence of infection has been demonstrated in sampled cattle in the area of study. Also collecting data regarding dogs of each estate could be interesting to define the epidemiology of infections by the serovar tested in the studied farms. The zoonotic characteristic of leptospirosis make particularly important these informations.

REFERENCE

Ackermann, M; Engels M. (2006). Pro and contra IBR-eradication. Vet Microbiol; 113:293-302.

Agudelo-Flórez, P; Restrepo Jaramillo, B, N. (2007). Situación de la Leptospirosis en el Urabá Antioqueño Colombiano: estudio seroepidemiológico y factores de riesgo en población general Urbana. Cad. Saúde Pública, Rio de Janeiro. 23(9), 2094-2102.

Anderson, M, L. (2007). Infectious causes of bovine abortion during mid- to late-gestation. Theriogenology 68 474 – 486.

Bartha, A; Juhasz, M; Liebermann, H. (1966). lsolation of a bovine herpesvirus from calves with respiratory disease and keratoconjuntivitis. Acta Veterinaria Academiae Scientiarum Hungaricae 16: 357-358.

Barr, B; Anderson, M; Dubey, J; Conrad, P; (1991). Neospora-like protozoal infections associated with bovine abortions. Vet. Pathol. 28: 110-116.

Bernal, E, J; (1988). Pastos y Forrajes Tropicales. Producción y Manejo. Ediciones Banco Ganadero. Bogotá, Colombia. Cap.1, 14 a 18.

Betancour, H.C.A., Gogorza, L.M., Martinez, F, G. (2007). Seroepidemiología de la diarrea viral bovina en Montería (Córdoba, Colombia). Analecta Veterinaria; 27(2) 11- 16.

Betancour, H.C.A.; González, M.T.; Reza, L.G.. (2006). Seroepidemiología de la rinotraqueitis infecciosa bovina en el municipio de Montería, Colombia. Revista MVZ, Córdoba. 11(2): 830-836.

Bjerkas, I; Dubey, J. (1991). Evidence that Neospora caninum is identical to the toxoplasma-like parasite of Norwegran dogs. Acta. Vet. Scand. 32: 407-410.

Bolmann, S.; Schettino, D.; Disanto, M.; Arroyo, G. (1997). Seroepidemiología del herpes virus bovino tipo 1, en rodeos lecheros de Tandil, Argentina (1994-1995). Avances en Ciencias Veterinaria. 12 (1): 25-30

Booker, C W.; Abutarbush, S M.; Morley, P S.; Guichon P; Wildman B K.; Kee Jim G; Schunicht, O C.; Pittman T J; Tye Perrett J A.; Greg E. (2008). The effect of bovine viral diarrhea virus infections on health and performance of feedlot cattle. Canadian Veterinary Journal 49:253–260.

Borel, N.; Thoma, R.; Spaeni, P.; Weilenmann, R.; Teankum, K.; Brugnera, E.; Zimmermann, D.R.; Vaughan, L.; Pospischil, A. (2006). Chlamydia related abortions in cattle from Graubunden, Switzerland. Vet. Pathology. 43: 702 - 708

Cárdenas, R.E.A. (2006). Alternativas forrajeras para clima frio en Colombia. Disponible en http://mvz.unipaz.edu.co/textos/lecturas/generalidades/file_eventosenti10332.pdf. Consultado el 5 de agosto de 2011. 20 p.

Cavirani, S. (2002).La diarrea virale del bovino - Il Virus. Utet Divisioni Periodici; 3-6.

Cavirani, S; Cabassi C.S; Donofrio G; De Laco B; Taddei S; Flammini C.F (2001). Association between Chlamydia Psitacci sepositivity and abortion in Italia dairy cows. Preventive Veterinary Medicine 50, 145 – 151.

Castrucci, G.; Frigeri, F.; Ferrari, M.; Di Luca, D.; Traldi, V. (1991). A study of some biologic properties of Bovid herpesvirus-4. Comp lmmunol Microbiol lnfect Dis. 14(2):197-201

Corsaro, D.; Greub, G. (2006). Pathogenic potential of novel Chlamydiae and diagnostic approaches to infections due to theses obligate intracellular bacteria. Clinical Microbiology Review. 19 (2): 283-297

DeGraves, F.J.; Gao, D.; Hehnen, H.R.; Schlapp, T.; Kaltenboeck, B. (2003). Quantitative detention of Chlamydia psitacci and C. pecorum by high sensitivity real time PCR reveals high prevalence of vaginal infection in cattle. Journal of Clinical Microbioloy. 42 (4): 1726 - 1729

Donofrio, G.;Herath , S.; Sartori, C.; Cavirani, S.;Flammini C.F.; Sheldon M. (2007). Bovine herpesvirus 4 (BoHV-4) is tropic for bovine endometrial cells and modulates endocrine function. Reproduction; 134(1): 183–197.

Donofrio, G.; Franceschi, V.; Capocefalo, A.; Cavirani, S.; Sheldon, M. (2009). Isolation and characterization of bovine herpesvirus 4 (BoHV-4) from a cow affected by post partum metritis and cloning of the genome as a bacterial artificial chromosome. Reproductive Biology And Endocrinology 7 (83): 1 -12.

Dubey, J.; Carpenter, J.; Speer. C.; Topper, M.; Uggla, A. (1988). Newly recognized fatal protozoan disease of dogs. J. Am. Vet. Med. Assoc. 192: 1269-1285.

Dubey, J.; Lindsay, D. (1989). Transplacental Neospora Caninum infection in dogs. Am.J.Vet.Res. 50, 1578-1579.

Dubey, J.; Barr, B.; Barta, J. (2002). Redescription of Neóspora Caninum and its differentiation from related Coccidia. International Journal of Parasitology 32: 929-946.

Dubey, J. (2003). Neosporosis in cattle. J. Parasitol. 42-56.

Ellis, J.; Luton, K.; Baverstock, P. R.; Brindley, P. J.; Nimmo, K. A.; Johnson, A. M. (1994). The phylogeny of Neospora Caninum. Mol. Biochem. Parasitol. 64: 303-311.

Egyed, L. (1998). Replication of bovine herpesvirus type 4 in human cells in vitro, J. Clin. Microbiol. 36, pp. 2109-2111.

Engels, M.; Ackermann, M. (1996). Pathogenesis of ruminant herpes virus infections. Vet Microbiol; 53: 3-15.

European Commission, Health and Consumer Protection Directorate General . Avian clamydiosis as a zoonotic disease and risk reduction strategies. Report or the Sicentific Committee on Animal Health and animal Welfare. Sanco AH-R26. 26 p

Evermann, J.F.; Ridpath, J.F. (2002). Clinical and epidemiology observation of bovine viral diarrhea virus in the northwestern United States. Veterinary Microbiology. 89: 129 - 139

FAO. (1985). Organización de las Naciones Unidas Para la Agricultura y la Alimentación. Manual de Técnicas de Diagnóstico Virológico.

Frazier, K.; Pence, M.; Mauel, M.J.; Ligget, A.; Hines, M.E.; Sangster, L.; Lehmkuhl, H.D.; Miller, D.; Styler, E.; West, J. (2001). Endometritis in postparturient cattle associated with bovine herpes 4 infections: 15 cases. Journal of Veterinary Diagnostic Investigation 13:502-508.

Frazier, K.S.; Baldwin, C.A.; Pelce, M.; West, J.; Bernard, J.; Liggett, A.; Miller, D.; Hines, M.E. (2002). Seroprevalence and comparison of isolates of endometriotropic Bovine Herpesvirus-4. J. Vet. Diagn. 14: 457-462

Fulton, W R.; Whitley, M E.; Johnson J B.; Ridpath J F.; Lurinda K S.; Burge, B.; Cook A W. (2009).Prevalence of bovine viral diarrhea virus (BVDV) in persistently infected cattle and BVDV subtypes in affected cattle in beef herds in South Central United States. The Canadian Journal of Veterinary Research. 7:283–29.

Gillian D.; Berke, O.; Smith, R R .; Ojkic, D.; Prescott, J F. (2009). Increase in seroprevalence of canine Leptospirosis and its risk factors, Ontario 1998–2006. The Canadian Journal of Veterinary Research 73,116-175.

Góngora, A.; Parra, J L.; Aponte, L H.; Gomez, L A. (2008). Seroprevalencia de Leptospira Spp. en grupos de población de Villavicencio, Colombia. Revista De Salud Pública. 10 (2).

Góngora, A,; Villamil, LC.; Vera, V.; Ramírez, G.; Parra J. (1995). Diagnóstico de las principales enfermedades reproductivas en toros de la Sabana de Bogotá. Rev Med Vet Zoot; 43: 37-41.

Goyal, S.M.; Naeem, K. (1992). Bovine herpesvirus-4: a review, Veterinary Bulletín 62, pp.181-201.

Gür, S.; Dogan, N. (2010).The possible role of bovine herpesvirus type-4 infection in cow infertility, Animal Science Journal. 81: 304–308.

Griffiths, IB.; Gallego, MI.; Villamil, LC. (1982). Factores de infertilidad y pérdidas económicas en ganado de leche En Colombia. Publicaciones ICA. P.168.

Instituto Geográfico Agustín Codazzi (IGAC). (2008). Disponible en www.igac.gov.co. Consultado 1 de diciembre de 2011

Instituto Geográfico Agustín Codazzi (IGAC). Regiones Naturales de Colombia. Disponible en www.igac.gov.co:8080/igac_web/contenidos/-home.jsp. Consultada 3 de agosto de 2011.

Jara D V. (2008). Estudio de Seroprevalencia de diarrea vírica bovina (DVB) y rinotraqueítis infecciosa bovina (IBR) en la provincia de Loja (Ecuador) por medio de Enzyme Linked Immunosorbent Assay (ELISA) y su distribución epidemiológica geoespacial. Trabajo de grado para optar el título de ingeniero agropecuario. Universidad Catolica de Loja. Escuela De Ingenieria Agropecuaria. Loja. 80 P

Jiménez, R.L.C. (2006). Región espacio y territorio en Colombia. Editorial Universidad Nacional de Colombia. Bogotá, Colombia

Kadohira, M; Tajima, M. (2010). A Case Control Study of bovine viral diarrhea virus (BVDV) persistent infection (PI) in Betsukai, Hokkaido, Japan Journal Veterinary Medicine. Sciense. 72 (5), 635-638.

Kalman, D.; Janosi, S.; Egyed, L. (2004). Role of herpes virus 4 in bacteria bovine mastitis. Microbial Pathogenesis. 37:125 -129

Kauffold, J.; Henning, K.; Bachmann. R.; Hotzel, H.; Melzer, F..(2007). The prevalence of clamydiae or bulls from six bull studs Germany. Animal Reproduction Science 102:111-121

Koiwai, M.; Hamaoka, T.; Haritani, M.; Shimizu, S.; Kimura, K.; Yamane, I. (2005). Proportion of abortion due to Neosporosis among dairy cattle in Japan. Journal Veterinary Medical Science. 67 (11): 1173- 1175

Lemaire M. (2000). Production of bovine herpesvirus type 1 seronegative Latent Carriers by administration of a live attenuated vaccine in passively immunized calves. J Clin Microbiol; 38: 11.

Lindsay, D. S.; Dubey, J.P.; Macallister, M.M. (1999). Neospora Caninum and The Potential For Parasite Transmission. Compen. Cont. Edu. Pract. Vet. 21: 317-321.

Longbottom, D.; Coulter, L.J. (2003). Animal Chlamydiosis and zoonótico implications. J. Comp. Path. 128:217-244.

López, G.; Restrepo, B.; Restrepo, M.; Lotero, M.; Murillo, E.; Cano, J.; Giraldo, J. (2007). Estudio Para Evidenciar La Presencia De Neospora Caninum En Bovinos De La Hacienda San Pedro, En El Municipio De Fredonia. Revista Ces / Medicina Veterinaria y Zootecnia. 2 (1): 7-19.

López, V.G.; Restrepo, J.B.N.; Restrepo, I.M.; Lotero, C.M.A.; Murillo, E. V.E.; Chica, A.; Cano, j.; Giraldo, J.M. (2007). Estudio para evidenciar la presencia de Neospora caninum, en bovinos de la hacienda San Pedro en el Municipio de Fredonia. Revista CES, Medicina Veterinaria y Zootecnia. 2(1): 7-19.

Mainar, J.R.C.; Berzal, H.B.; Arias, P.; Álvarez, M.; Rojo, V.F.A.. (2000). Epidemiological situation of bovine viral diarrhea virus (BVDV) infections in dairy cattle population from the Asturias region of Spain. Proceedings of the 9th International Symposium on Veterinary Epidemiology and Economics. Disponible en www.sciquest.org.nz. Consultado el 18 de octubre de 2011.

Mcallister, M.; Dubey,J.; Lindsay, D.; Jolleyw, R.: Wills, R.; Mcguire, A. (1998). Dogs Are Definitive Hosts of N. Caninum. Int. J. Parasitol. 28: 1475-1478.

Ministerio de Agricultura y Desarrollo Rural. (2010). Boletin De Analisis Por Producto (Leche) : (6), 1 – 20.

Mockeliunienė, V.; Salomska, A.; Mockeliunas, R.; Petkevicius, S.. (2004). Prevelences and epidemiological features of bovine viral diarrhoea virus infection in Lithuania. Veterinary Microbiology. 99: 55-57.

Monno, R.; Fumarola, L.; Trerotoli, P.; Cavone, D.; Giannelli, G.; Rizzo, C.; Ciceroni, L.; Musti, M.; (2009). Seroprevalence of Q Fever, Brucellosis and Leptospirosis in farmers and agricultural workers in Bari, Southern Italy, Ann Agriculture Environment Med.16, 205–209.

Morgan, S.; Sorensen, O.; Wu, J T Y.; Chow, E.; Manninen, K.; VanLeeuwen, J. (2006). Seroprevalence of Mycobacterium avium subspecies paratuberculosis, Neospora caninum, Bovine leukemia virus, and Bovine viral diarrhea virus infection among dairy cattle and herds in Alberta and agroecological risk factors associated with seropositivity. Can vet J, 47:981-991.

Muylkens, B.; Thiry, J.; Kirten, P.; Schynts, F.; Thiry, E.(2007).Bovine Herpesvirus 1 infection and infectious bovine rhinotracheitis. Vet. Res. 38 181–209.

Naeem, K.; Caywood, D.D.; Werdin, R.E.; Goyal, S.M. (1990). Evaluation of pregnant rabbits as a laboratory model for bovid herpesvirus-4 infection. Am i Vet Res. Apr 51(4):640-4

Nikolin, V.M.; Donofrio, G.; Milosevic, B.; Taddei, S.; Radosavljevic, V.; Miicevic, V. (2007). First Serbian isolates of bovine herpesvirus 4 (BoHV 4) from a herd with a history of postpartum metritis. New Microbiological. 30: 53-57.

Obando, C.; Bracamonte, M.; Montoya, A.; Cardenas, V. (2010). Neospora Caninum en un rebaño lechero y su asociación con el aborto. Rev. Científica Venezuela FCV-Luz 20 (3) 235-239.

Ochoa, J E.; Sánchez, A.; Ruiz, I. (2000).Epidemiología de la Leptospirosis en una zona andina de producción pecuaria. Revista Panamericana de Salud Publica. 7(5), 325 – 330.

Otte, M J.; Ravenborg, T.; Hiittnerb, K. (1995) A Pilot study of elevated abortion and stillbirth ratios in cattle in the foothills of the easter plains of Colombia. Preventive Veterinary Medicine 22, 103-113.

Otte, E.; Navarrete, M.; Orjuela, J. (1985). Resultados de una encuesta realizada sobre producción y salud animal en Montería - Córdoba, Colombia.1982-1984: Proyecto Colombo Alemán Ica-Gtz. Informe Técnico.; P.1-125.

Otranto, D.; Llazari, A.; Testini, G.; Traversa, D.; Frangipani D.G.A.; Badan, M.; Capelli, G.. (2003). Seroprevalence and asocciated risk factors of neosporosis in beef and dairy cattle in Italy. Veterinary Parasitology. 118:7-18.

Oviedo, T; Betancourt, C.; Alberto, M.; Marco, G. Reza, L.; Calonge, K. (2007). Estudio Serológico Sobre La Neosporosis en bovinos con problemas reproductivos en Montería, Córdoba, Colombia. Revista MVZ Córdoba. 12 (1): 929-933.

Oviedo, S.T.J.; Betancurt, C.; Mestra, P. A.; Reza, G.L.. (2006). Estudio serológico e histopatológico sobre neosporosis en bovinos con problemas reproductivos en Montería, Córdoba. Universidad de Córdoba, Facultad de Medicina Veterinaria Zootecnia. Montería, Córdoba.

Pabón, V. M..(2007). Estudio de la neosporosis en vacuno lechero en Cataluña. Departamento de Sanidad y Anatomía Animales. Trabajo de tesis Doctoral. Universidad Autónoma de Barcelona. España.

Pérez, M.J.; Storz, J.. (1987).Género Chlamydia: biología básica, propiedades antigénicas y potencial patogénico. Ciencia Veterinaria- 4: 37-60.

Piedrahita, L.E.; Montoya, L.M.; Pedraza, F.J.. (2010). Herpes virus bovino tipo-1(BoHV-1) como posible causa de encefalitis en bovinos de la región del Magdalena Medio Colombiano. Estudio serológico y análisis epidemiológico. 23:191-198.

POT, Plan de Ordenamiento Territorial del Municipio de Oicatá. (2000). Disponible en www.**oicata**-boyaca.gov.co/apc-aa-files/. Consultado el 15 de noviembre de 2011.

POT, Plan de Ordenamiento Territorial del Municipio de Tunja. (2001). Disponible en http://www.tunja.gov.co/?idcategoria=4878/. Consultado el 1 de octubre de 2011

Prescott, J.F.; Miller, R.B.et al. (1988). Seroprevalence and association with abortion of Leptospirosis in cattle in Ontario. Canadian Journal of Veterinary Research, 52, 210-215.

Quevedo, J.V.; Chávez, A.; Rivera, H.; Casas, E; Serrano, E. (2003). Neosporosis en bovinos lecheros en dos distritos de la Provincia De Chachapoyas. Rev. Inv. Vet. Perú. 14 (1): 36-37.

Quispe, R.; Ccama, A.; Rivera, G.H.; Arainga, M.L. (2008). El virus de la diarrea viral en bovinos criollos de la Provincia de Melgar, Puno. Revista de Investigación Veterinaria de Perú. 19 (2): 176-182

Radostits, Om.; Gay, Cc.; Blood, Dc.; Hinchcliff, KW. (2000). Veterinary Medicine: A Textbook of the diseases of cattle, sheep, pigs, goats and horses, 9th.W.B. Saunders Company Ltda: P.1173-1184.

Reinhardt, G.; Carrasco, L.; Tadich, N.; Riedemann, S.(2001). Comparación entre dos técnicas de diagnóstico para diarrea viral Bovina (DVB) en 50 predios de la X región, Chile: Seroneutralización y Enzimo inmunoensayo Indirecto (Elisa-I)*. Archivos. Medicina. Veterinaria 33 (2), 173 - 183.

Rodriguez G. (2000). Estado actual de la Leptospirosis. Revista MVZ Córdoba 5(1), 61 – 64.

Rondón L. (2006). Diarrea Viral Bovina: Patogénesis E Inmunopatología. Revista. MVZ Córdoba 11(1), 694-704.

Solís, J.J.; Segura, V.M.; Segura, J.C.; Alvarado, A..(2003). Seroprevalence of and risk factors for infectious bovine rinotracheitis in beeh cattle herds of Yucatán, México. Preventy Veterinary Medicine. 57:199-208.

Soracá. 2010. Información general sobre el municipio. Disponible en www.soraca-boyacá.gov.co. Consultado el 3 de octubre de 2011.

Sotaquirá. 2010. Información general del Municipio de Sotaquirá. Disponible en http://sotaquira-boyaca.gov.co/nuestromunicipio.shtml?apc=mfxx1-&m=f#geografia, con acceso el 10 de diciembre de 2011

Scott ,H M.; Ole, Sorensen.; Wu, J Ty.; Chow E Yw.; Manninen, K.; Vanleeuwen, J A. (2006). Seroprevalence of mycobacterium avium subspecies paratuberculosis, Neospora Caninum, Bovine Leukemia Virus, And Bovine Viral Diarrhea Virus Infection Among Dairy Cattle and herds in Alberta and agroecological risk factors associated with seropositivity. Canadian Veterinary Journal; 47: 981–89.

Schefers, M J.; Collins, E J.; Goyal, M S.; Ames, R T. (2009). Detection, characterization, and control of bovine viral diarrhea virus infection in a large commercial dairy herd. Canadian Veterinary Journal 50,1075–1079.

Thiry, E.; Bublot, M.; Dubuisson, I.; & Pastoret, P.P. (1989). Bovine herpesvirus-4 (BHV-4) infections of cattle. In Herpesvirus Diseases of Cattle, Horses, and Pigs, pp. 96±115. Edited by G. Wittmann. Boston: Kluwer.

Vargas D, S. Jaime, J. Vera, V. (2009). Perspectivas Para El Control Del Virus De La Diarrea Viral Bovina (Bvdv). Revista Colombia Ciencias Pecuarias 22,677-688.

Valla G. (2008). Virus della diarrea viral del bovino (BVDV): strategie di controllo. Mr Maus Comunicazione – Segrate (Mi). 85 P.

Valla G. (2009). Virus della rinotracheite infettiva del bovino (BoHV-1): strategie di controllo. Mr Maus Comunicazione – Segrate (Mi). 90 P.

Van Oirschot J. (1995). Bovine Herpesvirus 1 in semen of bulls and the risk of transmission: a brief review. Vet Q; 17: 29-33.

Van Balen, J.; Hoet, A.; D’pool G.; Gil M.; Escalona, F.; Díaz, D. (2009). Análisis retrospectivo de las pruebas diagnósticas de leptospirosis bovina procesadas en la unidad de investigación y diagnóstico de leptospirosis de la universidad del zulia, 1998-2001. Revista Científica, FCV-UZ, 19 (6), 598 – 606.

Vázquez, M.H. (2007). Presencia de *Neospora caninum* y otros parásitos gastrointestinales en perros procedentes de poblaciones de riesgo en España. Revista Complutense de Ciencias Veterinarias. 1(2): 394 - 402

Wentink G, Van Oirschot J, Verhoeff J. (1993). Risk of Infection With Bovine Herpes Virus 1 (BHV-1): A Review. Vet Q: 15: 30-33.

Wiedmann, M. (1993).Detection of Bovine Herpesvirus-1 in bovine semen by a nested PCR assay. J Virol Methods; 44.

World Organization For Animal Health (OIE). (2009). Manual of diagnostic tests and vaccines for terrestrial animals. Avian Chlamydiosis.

Whetstone, Ca.; Miller, Jm.; Bortner, Dm.; Vander, Maaten Mj. (1989). Changes in the bovine herpesvirus 1 genome during acute infection, after reactivation from latency and after super infection in the host animal. Arch Virol; 106: 261-279.

Wheelhouse, N; Katzer, F; Wright,F; Longbottom, D. Novel Chlamydia like organinms as cause of bovine abortions, UK. (2010). Emerging Infectious Diseases. 16 (8) P 1323 – 1324.

Woodbine, K.A.; Medley, G.F.; Moores, S.J.; Ramírez, V.A.M.; Mason, S.; Green, L.E.. (2009). A four year longitudinal sero-epidemiological study of bovine herpervirus type-1 (BHV-1) in adult cattle in 107 unvaccinated herds in south west England. Veterinary Research. 5(5):1-12

Zambrano, P.F. (1998). Colombia País de Regiones Edición Cinep-Colciencias. Tomo I,II,III Bogotá, Colombia.

Zambrano, J.; Cotrino, V.; Jiménez, C.; Romero, M.; Guerrero, B.. (2001). Evaluación serológica de *Neospora caninum*, en bovinos de Colombia. Revista Acovez. 26: 5 - 10

Zimmermann, W., BroIl, H.; Ehlers, B.; Buhk, HJ.; Rosenthal, A.; Goltz, M. (2001).Genome sequence of bovine herpesvirus 4 a bovine rhadinovirus, and identification of an origin of DNA replication, J. Virol. 75, pp. 1186—1194.

Zúñiga, A.I.; Ossa, L.J.E.; Hincapié, N.J.O.. (1978). Prevalencia de rinotraqueitis infecciosa bovina en reproductores del Urabá antioqueño para 1977. Revista Colombiana de Ciencias Pecuarias (Colombia). 1 (2): 135-148