Epidemiological study on Reproductive Pathogens with particular focus on Bovine Viral Diarrhoea Virus in dairy cattle in Tajikistan

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Epidemiologisk studie över abortpatogener med fokus på Bovint virusdiarrévirus hos mjölkkor i Tajikistan

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ABBREVIATIONS

BVDV	Bovine Viral Diarrhoea Virus
ELISA	Enzyme-Linked Immunosorbent Assay
FAO	Food and Agriculture Organization
OIE	The World Organisation for Animal Health
PCR	Polymerase Chain Reaction
PI	Persistently Infected
TAU	Tajik Agrarian University

ABSTRACT

Bovine Viral Diarrhoea Virus (BVDV) is considered to be one of the most important infectious diseases of cattle. It causes substantial economic losses worldwide mainly due to reproductive disorders. *Neospora caninum* and *Brucella abortus* are other important diseases causing major economic losses due to reproductive failure. *B. abortus* is also an important zoonotic disease and many countries, like Tajikistan, have a vaccination program in order to control the disease. Tajikistan, located in Central Asia, is a country where approximately 80% of the households own livestock. Many people are dependent on their cattle for survival. Reproductive disorders among cattle can therefore be greatly disadvantageous to the work on reducing poverty in the country.

The aims of this study were primarily to investigate the occurrence of BVDV in the western Tajikistan among dairy cattle and to study risk factors relevant for controlling the disease. The secondary aim was to investigate the occurrence of the differential diagnoses *N. caninum* and *B. abortus* among dairy cattle in the western Tajikistan.

In total, 305 serum samples were collected from six governmental farms and four private farms. The samples were analysed for presence of antibodies to BVDV, *N. caninum* and *B. abortus*. Bulk milk was also collected from five governmental farms and one private farm and analysed for presence of antibodies to BVDV. The overall seroprevalence of BVDV was 77%. Governmental farms had higher seroprevalence than private farms. Young animals in private farms had lower seroprevalence than young animals in governmental farms. This might indicate that within the private farms there was ongoing self-clearance of BVDV. The overall seroprevalence of *N. caninum* was 21%. The seroprevalence was higher in private farms than in governmental farms. This might be due to more frequent horizontal transmission within the private farms. This is the first report, to my knowledge, of infection with BVDV and *N. caninum* in Tajikistan. The overall seroprevalence of *B. abortus* was 11%. Two thirds of the animals in this study were vaccinated against *B. abortus*. Although the seroprevalence was mainly due to vaccination, the results indicate that there was infection with *B. abortus* in the study area.

SAMMANFATTNING

Bovint virusdiarrévirus (BVDV) anses vara en av de viktigaste infektionssjukdomarna hos nötkreatur. Sjukdomen orsakar stora ekonomiska förluster världen över framför allt på grund av reproduktionsstörningar. *Neospora caninum* och *Brucella abortus* är två andra viktiga sjukdomar som orsakar reproduktionsstörningar och därmed stora ekonomiska bortfall hos nötkreatur. *B. abortus* är även en viktig zoonos och många länder, däribland Tajikistan, har vaccinationsprogram för att försöka kontrollera sjukdomen. Tajikistan, som ligger i Centralasien, är ett land där uppskattningsvis 80% av hushållen äger boskap, och många är beroende av dem för sin överlevnad. Reproduktionsstörningar hos mjölkkor kan därför ha en stor negativ inverkan på fattigdomsbekämpningen i landet.

Målen med denna studie var primärt att undersöka förekomst av BVDV hos mjölkkor i västra Tajikistan samt att studera riskfaktorer relevanta för sjukdomskontroll. Sekundärt var målet att undersöka förekomst av *N. caninum* och *B. abortus* hos mjölkkor i samma område.

Totalt samlades 305 serumprover in från sex statliga och fyra privata gårdar. Proverna analyserades för förekomst av antikroppar mot BVDV, *N. caninum* och *B. abortus*. Tankmjölk samlades också in från fem statliga gårdar och en privat gård och analyserades för förekomst av antikroppar mot BVDV. Den totala seroprevalensen BVDV var 77 %. Seroprevalensen var högre inom statliga gårdar jämfört med inom privata gårdar. Unga djur i privata gårdar hade lägre seroprevalens än motsvarande djur i statliga gårdar. Detta kan tyda på högre sannolikhet för självsanering (self-clearance) av BVDV inom privata gårdar jämfört med statliga. Seroprevalensen av *N. caninum* var 21 %. Den var högre inom privata gårdar jämfört med statliga gårdar. Detta kan bero på mer frekvent horisontell transmission inom de privata gårdarna. Detta är, mig veterligen, den första rapporten om infektion av BVDV och *N. caninum* hos mjölkkor i Tajikistan. Den totala seroprevalensen *B. abortus* var 11 %. Två tredjedelar av mjölkkorna i denna studie var vaccinerade mot *B. abortus*. Även om merparten av seroprevalensen speglar antikroppar från vaccination tyder resultaten på att infektion med *B. abortus* fanns inom studieområdet.

INTRODUCTION

Tajikistan

Tajikistan is situated in Central Asia with Afghanistan bordering to the south, Uzbekistan to the west, Kyrgyzstan to the north and People's Republic of China to the east (figure 1). It is populated by 7.2 million people (CIA 2008), most of them Sunni Muslims. In total, 93% of the country area is mountainous. Following independence, after the collapse of the Soviet Union in 1991, a civil war ravaged the country during the years 1992-1997. It was estimated, in 2004, that 60% of the population lived below poverty line (Magnusson et al., 2005).

Figure 1. Map over Tajikistan (www.maps.com Magellan Geographix (1997) Tajikistan political map, 1:5000 000, (805)685-3100).

Livestock

Since independence, the number and productivity of livestock in Tajikistan have been much reduced. However, small-scale farming is common and about 80% of the households own livestock. Good production and healthy animals are therefore very important in order to reduce poverty in the country. Since 1991, the support from the government to animal health service has diminished. This has impaired the veterinary and laboratory work as well as diagnostics of livestock diseases (Magnusson et al., 2005).

There are governmental owned farms and private farms in the country. Most privately owned farms in the villages (kishlaks) are small, with approximately ten sheep or goats kept for meat production. Sometimes the private farms own cattle for milk production. The urban kishlaks more often own cattle than the rural kishlaks. The private households in a kishlak use the same pasture for their livestock. A kishlak can therefore be considered as one unit for transmission of infectious diseases due to communal grazing and trading of animals. The animals in kishlaks are housed during the night and also during the winter. The urban kishlaks often have smaller groups for communal grazing which makes it easier to control infectious diseases. The productivity of the livestock in kishlaks is often low due to low reproduction rates, uncontrolled breeding, problem with utilization of pastures and with control of infectious diseases (Jackson et al., 2007).

The governmental owned farms in Tajikistan own cattle for milk production but also sheep and goats for meet production. These farms were more common during the Soviet era but some still remain. Many diseases are better controlled in the governmental farms because they are well attended by veterinarians working for the government (Jackson et al., 2007).

Reproductive pathogens

Introduction

In a country like Tajikistan, where many people are dependent on milk and meat production from cattle, reproductive disorders can be of significant economic importance. This is due to negative effects on the individual animal and on the net return per cow. Bovine Viral Diarrhoea Virus (BVDV) is considered to be one of the most important infectious diseases of cattle. It causes substantial economic losses worldwide mainly due to reproductive disorders (Goyal & Ridpath., 2005). Other diseases, which cause abortions in cattle, are *Neospora caninum* and *Brucella abortus*. *N. caninum* is a major cause of economic loss in many countries due to reproductive failure (Radostits et al., 2007). *B. abortus* is a zoonotic disease with worldwide distribution and also a cause of substantial economic losses (Jackson et al., 2003).

Bovine Viral Diarrhoea Virus

The virus

In general, 60-80% of the cattle more than one year of age are estimated to be seropositive to BVDV worldwide (Radostits et al., 2007). The infection is caused by a Pestivirus within the family Flavirviridae. The virus is closely related to Classical swine fever virus in pigs and Border disease virus affecting sheep. BVDV, which is a single-stranded RNA enveloped virus, primarily infects ruminants (Quinn et al., 2002).

Pathogenes and clinical signs

The name BVDV is somewhat misleading since the virus can cause several symptoms other than diarrhoea. The symptoms depend on many interactive factors such as if the animal has been exposed to the virus before, if the animal is pregnant or not and on the virulence of the individual BVDV strain (Goyal & Ridpath., 2005).

Seronegative non pregnant cattle

Infection in non pregnant susceptible cattle (i.e. cattle that have not been exposed to the virus before) is often subclinical. The animal can however suffer from mild symptoms such as fever, transient leukopenia, inappetence and depression. Especially young animals can suffer from severe diarrhoea and respiratory signs. The virus has an immunosuppressive action and can therefore predispose for secondary infections. After infection with BVDV, the animal will develop lifelong immunity (Radostits et al., 2007).

Seronegative pregnant cattle

The outcome of infection in pregnant cattle that have not been exposed to BVDV before depends on when, during gestation, the animal becomes infected. Infection during pregnancy can cause abortion at any time. The virus can cross the placental barrier and cause foetal infection. If this happens during embryonic period it often results in embryonic death. During the first four month of pregnancy, before the foetus becomes immunocompetent, infection may result in birth of a PI (persistently infected) calf. These calves shed BVDV throughout life and are the most important transmission sources of infection. PI calves are seronegative to BVDV. They are often small and week at birth but can also appear normal. They can suffer from other infections due to their impaired immune system. Infection after three month of pregnancy can result in many congenital defects such as cerebellar hypoplasi and ocular abnormalities. At approximately 150 days of gestation, the calves can develop a normal immune system and therefore be able to eliminate the virus (Radostits et al., 2007).

Transmission

The most important source of infection within a herd are PI animals. They shed the virus in all body-fluids and faeces. The virus is transmitted by direct contact between a PI animal and a susceptible animal or transplacentally from dam to foetus (Radostits et al., 2007). Between farms, the virus can be spread through trading with PI animals or through a dam carrying a PI calf. Transmission can also occur through semen from a PI bull. There is also a risk of indirect transmission when veterinarians use the same needle between animals or the same glove at rectal palpation between different animals (Goyal & Ridpath., 2005).

Diagnosis

BVDV can be diagnosed using indirect or direct methods. Indirect methods are based on serological tests for antibody detection, and direct methods detect virus or virus antigen.

Indirect methods

Indirect methods are used to distinguish infected herds from non-infected herds. They are also used for monitoring non-infected herds within a control program using herd level tests such as bulk milk tests or spot tests (serum samples from young animals), and to control serological status of individual animals within a possibly infected herd to identify possible PI animals (Lindberg & Alenius., 1999). It is important to remember that seropositivity in an individual animal does not mean ongoing BVDV infection. Rather, it indicates that the animal has been in contact with BVDV sometime during life. A disadvantage with serological methods is the difficulty to differ antibodies produced due to infection with those from vaccination or maternal antibodies from dam to offspring. One serological method commonly used is antibody ELISA (Enzym-Linked Immunosorbent Assay) which is based on BVDV antigen that captures virus specific antibodies if existing in a sample (Goyal & Ridpath., 2005).

Using ELISA on bulk milk, as a herd level test for BVDV, is easy and cheap. The results are categorized in 4 different classes. Class 0-1 have no or low level of antibodies and indicates that infection with BVDV is absent within the herd. Class 2-3 have moderate to high level of antibodies and indicates that the herd may have current infection. Class 3 also indicates that PI animal(s) is or has been present within the herd. Bulk milk testing is also a good way to monitor herds free from infection (Alenius & Larsson, 1996).

Spot tests are individual serological tests on young animals (6-12 month old). If antibodies are detected among young animals, and they are not maternal antibodies, it is an indication of recent or ongoing infection within the herd. If a PI animal is present within a group of animals, the other animals will have high levels of antibodies due to the effective transmission of virus between a PI animal and susceptible animals. PI animals are BVDV seronegative. If no antibodies are detected in a spot test, this indicates that the herd has not been infected during the time since the sampled calves were born (Lindberg & Alenius, 1999).

Direct methods

Direct methods are aimed to detect virus or virus antigen. It can be done in many different ways. Commonly used is antigen-ELISA. With this method antigen can be detected in whole blood, serum or plasma. The method is based on specific BVDV antibodies that capture antigen if existing in the sample. If antigens exist, a complex is formed which can be visualized in a reaction using enzyme-conjugated antibodies. The test is used on the seronegative animals within a herd in order to detect PI animals which are BVDV antigen positive (Goyal & Ridpath., 2005).

Prevention and control

A control program against BVDV can be based on bulk milk testing with antibody ELISA and spot tests in all herds included in the program. The herds are, according to results from bulk milk screening and spot tests, divided into infected or non-infected.

Non-infected herds

Herds that are declared free from an ongoing infection, using bulk milk and spot tests, must be protected from infection using adequate biosecurity. It is crucial that no animals are introduced into the herd without being confirmed free from infection. It is also important to avoid that free herds share pasture with infected herds, and therefore good information regarding biosecurity must be given to the farmers (Lindberg & Alenius., 1999).

The control program, which started in Sweden and Scandinavia in 1993, has been very successful and today all of the Scandinavian countries are free or almost free from BVDV (Ståhl., 2006). The animal health and production have much been improved since the herds were declared free from infection with BVDV in Sweden and Scandinavia (Alenius & Larsson., 1996).

Infected herds

Intervention

In infected herds, the most important intervention is to recognize and remove PI animals by individual screening of seronegative animals in order to prevent further transmission within the herd. After removing the PI animals, further monitoring should continue, e.g. using spot tests. To prevent reintroduction, direct contacts with possibly infected herds must be avoided. It is very important that adequate information is given to the farmers with infected herds (Lindberg & Alenius, 1999).

Self-clearance

Self-clearance is when a BVDV infected herd is cleared from infection without intervention. This is a phenomenon seen in infected herds due to the nature of BVDV with high mortality among PI calves and effective transmission of infection within a herd. This is provided that infection is not re-introduced into the herd (Alenius & Larsson., 1996).

Vaccines

Different vaccines have been used in order to control BVDV infection. The use of vaccines have in some studies been questioned and the vaccines have failed to prove substantial effect. Vaccines were not included in the eradication program in Scandinavia (Ståhl., 2006).

Neospora caninum

The parasite

Neospora caninum is a reproductive pathogen that causes substantial economic losses worldwide. It is the most common diagnosed cause of abortion in cattle in most countries (Radostits et al., 2007). *N. caninum* is a protozoan parasite within the family Sarcocystidae. It was first recognized in dogs in 1988 and has structural similarities with Toxoplasma gondii. *N. caninum* infects a wide range of animals but primarily cattle and dogs (Urquhart et al., 1996).

Transmission

Dogs are known to be definitive hosts of *N. caninum* and cattle the main intermediate host. There are two routes of infection for cattle (Radostits et al., 2007). The main route is vertical transmission, which is estimated to occur in 85% of all calvings by seropositive dams (Frössling., 2004). The other route of transmission is horizontal through ingestion of food or water contaminated with dog-faeces containing oocysts, or infectious products such as placenta and foetus from abortion. The infection with *N. caninum* is persistent and lifelong (Radostits et al., 2007).

Clinical signs

The main clinical sign following infection with *N. caninum* is abortion. The foetus can be reabsorbed, mummified, stillborn, born weak or born chronically infected though appearing normal. Congenitally infected calves often appear to be normal but can have neurological deficits (Radostits et al., 2007).

Diagnosis

Serological test, like ELISA is often used to diagnose *N. caninum* and a positive result indicates that the animal is infected. Diagnosis can also be made by serological analyses of foetal sera or pleural fluid. PCR (polymerase chain reaction) can be used to detect *N. caninum* antigen (Radostits et al., 2007).

Prevention

To prevent *N. caninum*, it is important to control risk factors such as faecal contamination from dogs in food and water as well as on pastures. Consequently, it is also important to remove placentas, aborted foetuses and dead calves so that neither dogs nor cattle come into contact with this material (Radostits et al., 2007).

Brucella abortus

The bacteria

Bovine brucellosis is caused by the bacteria *Brucella abortus* which is a small, non-motile Gram-negative bacteria (Quinn et al., 2002). The disease plays a major role in developing countries without national programmes aimed to control or eradicate the disease. It is one of the most important and widespread zoonotic diseases in the world (Radostits et al., 2007). It is OIE listed and must be reported, according to OIE, if diagnosed (OIE, 2006).

Transmission

Within a herd, the major source of transmission is waste from the aborting cow like foetus, placenta and vaginal exudates. Other cows can be infected directly when they come in contact with this material or indirectly from a contaminated environment. The calf can also be infected in utero although it is probably not very common. The bacteria enter the body mainly through ingestion but can also penetrate through the skin or conjunctiva, or may enter through inhalation (Biberstein & Chung Zee., 1990). Between herds, infection occurs by movement of an infected animal to a susceptible, noninfected herd (Radostits et al., 2007). *B. abortus* can also be transmitted by artificial insemination with infected sperm (Biberstein & Chung Zee., 1990).

Clinical signs

As the name implies, the most important symptom of *B. abortus* is abortion. Outbreaks of abortions are most commonly seen in unvaccinated heifers after the fifth month of pregnancy. Bulls can also develop orchitis and epididymitis if infected (Radostits et al., 2007).

Diagnosis

B. abortus can be diagnosed using serological methods such as ELISA and Rose Bengal test. There are also methods aimed to detect virus or virus antigen like PCR test and culture (Radostits et al., 2007).

Control and prevention

Vaccination is the main method to prevent infection by *B. abortus* in many countries. Many of these countries, like Tajikistan, have therefore vaccination programmes. There are different kinds of vaccines available in the market. Infection can be transmitted through artificial insemination and it is therefore important to use bulls free from infection. It is also important to remove risk factors like wastes from abortions to minimize horizontal transmission. Some countries eradicate the disease by test and slaughter (Biberstein & Chung Zee., 1990).

INTRODUCTION TO STUDY

This study was conducted in cooperation with the Tajik Agrarian University (TAU) and the Food and Agricultural Organization (FAO) in Tajikistan. I travelled and did all the practical work together with my friend and veterinary student Birgitta Gralén.

Aims of the study

1. The primary aim was to investigate the occurrence of BVDV in dairy cattle in the western Tajikistan and to study risk factors relevant for controlling the disease by using methods from the Swedish BVD control program.

2. The secondary aim was to investigate the occurrence of the differential diagnoses *Neospora caninum* and *Brucella abortus* among dairy cattle in the western Tajikistan.

BVDV

To my knowledge, there are no seroprevalence studies on BVDV in dairy cattle in Tajikistan. Studies made in Europe show that in countries, without systematic control, 60-80% of the cattle population are seropositive for BVDV and 1-2% of the animals are PI (EU Thematic network, 2001). A prevalence study made in Mashhad-Iran, including 141 dairy cows, showed that 69% of the cows were ELISA seropositive. All the herds in the study were seropositive for BVDV and the seroprevalence increased with age (Talebkhan Garoussi, 2008). Another study from Spain also showed that seroprevalence increased with age and that farms, which had bought many animals from markets, had higher seroprevalence within the herd compared to those who had not bought animals from markets (Mainar-Jaime et al., 2000). A study from Jordan showed that the seroprevalence was significantly higher in large herds than in small herds (Talafha et al., 2008). One of the most important risk factor for transmission of BVDV is livestock trading without controlling BVDV status (Lindberg & Alenius, 1999).

Neospora caninum

To my knowledge, there are no seroprevalence studies on N. *caninum* in Tajikistan. Results from a study made in Thailand showed that the seroprevalence in dairy herds varied between 0 and 46% (Chanlun et al., 2002). Another study from India, including 427 cows, showed that the overall seroprevalence was 8% (Meenakshi et al., 2004). Abortions due to *N. caninum* can be epizootic or sporadic. Up to 45% of the cows can abort during an outbreak of epizootic abortions, but the abortion rate in infected herds is usually between 5 and 10%. The seroprevalence within herd varies widely between 7 and 70%. Seropositive dams have 3-7 times higher risk of abortion than seronegative dams (Radostits et al., 2007).

Brucella abortus

Brucella abortus is considered to be one of the most important zoonoses in Tajikistan and the country has a vaccination program in order to control the disease in cattle (Magnusson et al., 2005). Brucellosis causes undulant fever in humans (Radostits et al., 2007) and the number of reported cases of human brucellosis has increased in the country (Magnusson et al., 2005). The vaccine used in Tajikistan is a strain 82 vaccine. The aim is to include all cattle in governmental farms in the vaccination program. The calves are vaccinated at four and ten months of age and then once a year. Antibodies can be detected in blood for approximately five months after vaccination although this varies between individuals. If antibodies are detected longer time after vaccination this can indicate an ongoing infection (Sattorov, TAU, personal message).

A study made on *B. abortus* in cattle from Tajikistan showed that the overall seroprevalence ranged between 3 and 7% and the mean prevalence was 2,1%. The seroprevalence ranged between 0 and 23% among cattle in different urban kishlaks. The animals in this study came from two different regions, Khatlon and the Region of Republican Subordination (RRS). Sera were also collected from six governmental farms from the same regions. Some of the farms included in this study had vaccinated the cattle against *B. abortus*, and the ELISA used could not distinguish between seropositivity due to natural infection and those due to vaccination (Jackson et al., 2003).

MATERIAL AND METHODS

Study area

The farms included in the study were located in the western part of Tajikistan in four different districts: Sharinau, Rudaki, Gisar and Tursunzade (figure 2).

Figure 2. Map with location of the farms (www.theodora.com/maps).

The governmental farms were situated close to the capital Dushanbe. The private farms in the kishlaks were situated further away from the city. Some of them were located close to the mountains and had little access to nearest village. In September, when we collected the samples, the area was very dry and the pastures had little grass on them (figure 3 and 4). There was a lack of water in many kishlaks due to the dry season. Approximately 150 000 cattle were kept within the study area which provided the region, including Dushanbe, with milk (Sattorov, TAU, personal message).

Figure 3. Pasture belonging to a rural kishlak (private photo).

Figure 4. Pasture belonging to a rural kishlak in a mountainous area (private photo).

Study population

The cows were milked twice a day. In the governmental farms (figure 5), the animals were kept outside in open pens. The animals in private farms were housed during night and winter (figure 6). All the cattle from private farms, in the same village, shared pasture every day. There were many dogs kept together with the cattle on private farms. There were different breeds in the study population: Holstein, Russian black and white, Local breed, Mixed breed, Latvian red and Carpat. Holstein and Russian black and white were strictly milking breeds. The Local cows and Mixed cows were smaller and did not produce as much milk as Holstein and Russian black and white. The Mixed breed was often Local breed mixed with some other breed. Latvian red, was bigger than the Local breed and not very common in the study population. Carpat cows were primarily used for meat production although some farms kept them for milk production as well. The governmental farms in our study owned approximately 100-900 cattle. The private farms in Kishlaks owned approximately 3-4 cattle and there were approximately 400 cattle totally in each Kishlak.

Figure 5. Governmental farm with Holstein breed (private photo).

Figure 6. Animals belonging to private farms on its way to pasture in a rural kishlak. Local breed (private photo).

Sampling and data collection

Questionnaire

A questionnaire, translated into Russian, was given to the veterinarians working at the governmental farms and to the owners of the private farms. It consisted of questions regarding farm type, herd size, age of the animal, breed, animal health, vaccination programmes, abortions and where the animals originated from.

Collection and treatment of samples

Samples were collected during September 2008 from the coccygeal vein or the jugular vein into sterile tubes. The blood samples were kept in a cool-box and transported to the laboratory where serum were removed and kept in freezer until analyzed.

The sampled animals were selected by the farmers. We wanted to collect samples from big farms (governmental) and small farms (private). The TAU decided which farms to visit and arranged the visits. The aim was to take spot-tests of young animals, but due to lack of young animals in some farms this was not always possible. Blood-samples were collected from 305 dairy cattle (50% estimated prevalence, 90% confidential interval, 5% precision) from six governmental farms (n = 180) and four private farms (n = 125) (figure 7). Totally, 16 private farms were included in the study. They belonged to four different kishlaks and due to communal grazing, trading and mixing of animals, every kishlak were considered as one epidemiological unit. Sixteen private farms were therefore considered as four private farms. The goal was to collect approximately 30 samples from each farm although this was not always possible (table 1). Tank milk was also collected from five governmental farms and one private farm and kept frozen until being analyzed. In figure 8, the distribution of collected blood samples among different breeds is summarized. Figure 9 shows the distribution of different breeds between farm types.

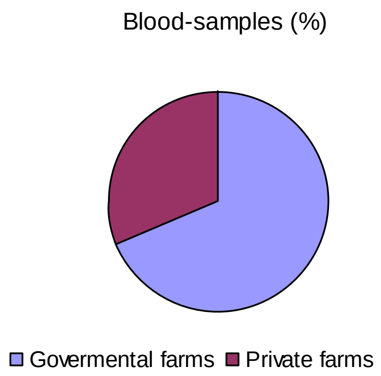

Figure 7. Number of blood-samples in % collected from governmental and private farms.

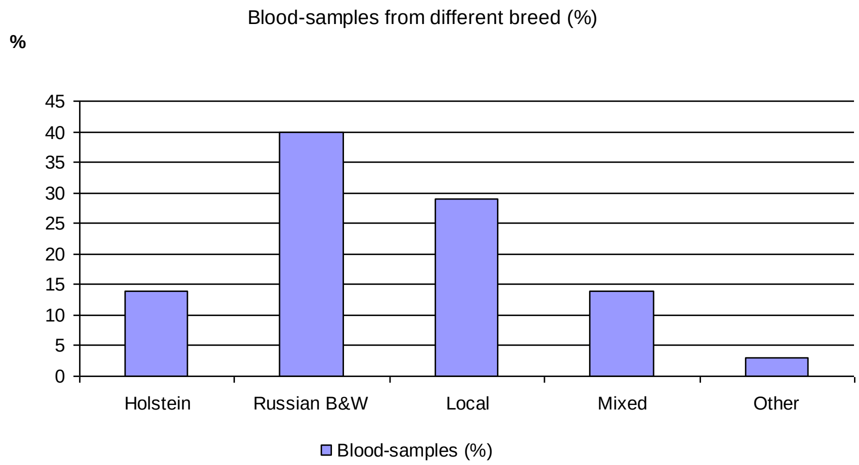

Figure 8. Distribution of collected blood-samples among different breeds. (Other: Carpat and Latvian red).

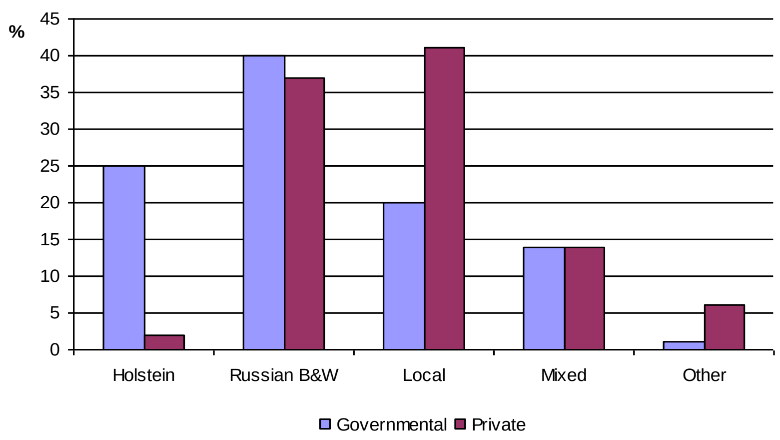

Figure 9. Distribution of different breeds between farm types. (Other: Carpat and Latvian red).

Table 1. Total number of animals and collected samples from each farm.

Farm	Number of cattle	Number of samples
1	900	10
2	270	15
3	400	35
4	140	35
5	18	8
6	400	31
7	88	30
8	680	35
9	420	56
10	384	50

Laboratory analyses

The analyses were made at the laboratory of TAU and at the laboratory of the Tajik Veterinary Research Institute in Dushanbe, Tajikistan. Serum samples were analysed for antibodies using indirect antibody-ELISA (Svanova Biotech AB, Uppsala, Sweden). We used BVDV-Ab ELISA (specificity (sp) 98,2%, sensitivity (se) 100%), *Brucella*-Ab I-ELISA (sp 100%, se 89,6%) and *N. caninum* iscom ELISA (sp 96%, se 99%). All seronegative BVDV samples were also tested for BVDV antigen using Herdcheck BVDV Ag/Serum plus (sp 95%, se 95%) (IDEXX Laboratories B.V, Netherlands). Milk samples were tested for BVDV using BVDV-Ab ELISA. All analyses were made according to instructions from the manufacturers. Both negative and positive controls were included in every assay. Percent positive (PP) values were calculated as:

$PP = COD \times 100 / COD_{\text{positive control}}$ (COD = correlated optical density).

PP values >14 were considered positive for BVDV Ab-ELISA, >20 for *N. caninum* Ab-ELISA and > 40 for *B. abortus* Ab-ELISA respectively.

For BVDV antigen the results were considered positive if $OD_{\text{sample}} - OD_{\text{negative control}} > 0{,}300$ and negative if the result were $\leq 0{,}300$.

Statistical analysis

Multivariat logistic regression was used to analyze associations between serological responses (BVDV and *N. caninum* respectively) and relevant herd and animal factors such as farm type, breed and age. Factors with $p < 0,05$ were considered as statistically significant. Nine animals (eight Carpat and one Latvian red) were excluded from the study when comparing prevalence between different breeds because these groups were too small for the statistical analysing program.

RESULTS

Questionnaire

The results from the questionnaire regarding farm type, breed, age and herd size are summarized in table 2. The variables, regarding animal health and abortions, were difficult to interpret and therefore excluded from the study. The governmental farms had vaccination programmes against *B. abortus*, Foot-and-mouth disease, Tuberculosis, Anthrax and *Clostridium chauvoei*. The cattle from private farms were vaccinated against Foot-and-mouth disease, Anthrax and *Cl. chauvoei*. None of the animals were vaccinated against BVDV. In total, 195 animals in the study were vaccinated against *B. abortus* and 110 animals had never been vaccinated against the disease.

Laboratory analyses

BVDV

The overall seroprevalence of BVDV was 77% (table 2), and all farms included in this study had seropositive animals. The governmental farms had significantly higher seroprevalence than the private farms. There was also a significant difference in seroprevalence among different age groups. Young animals (0-1 year old) had lower seroprevalence than older animals in the study (table 2). The difference was even clearer when comparing governmental and private farms where the seroprevalence were lower among young animals in private farms than in governmental farms (figure 10). Seventy-one animals tested negative for BVDV antibodies in the study. One of these tested positive for BVDV antigen.

Table 2. Results showing seroprevalences of BVDV and N. caninum for the variables farmtype, breed and age. Seroprevalence measured with indirect ELISA (Svanova, Biotech, Uppsala, Sweden).

		BVDV		*Neospora caninum*		
Variable	Category	No.pos (%)	No. neg	No.pos (%)	No. neg	Total
Farm type	Governmental	153 (85) a[1]	27	26 (14) a	154	180
	Private	82 (66) b[1]	43	38 (30) b	87	125
	Total	235 (77)	70	64 (21)	241	305
Breed	Russian B&W	92 (76)	29	39 (32) a	82	121
	Local	72 (81)	17	17 (19) b	72	89
	Holstein	36 (84)	7	7 (16) b	36	43
	Mixed	33 (77)	10	1 (2) b	42	43
Age	0-1	32 (46) a	37	10 (14) a	60	70
	>1-3	67 (81) b	16	26 (31) b	57	83
	>3	135 (88) b	18	28 (18) a	124	152

[1] a significant difference compared to b.

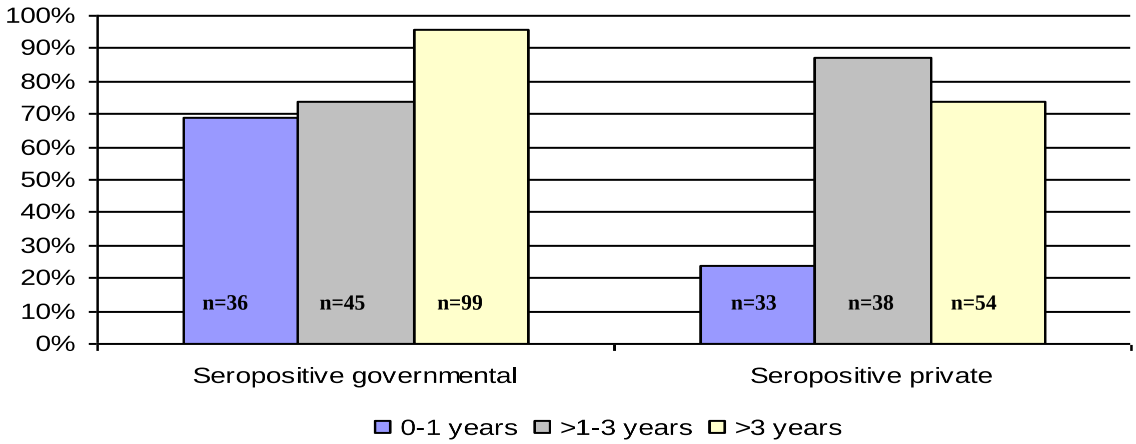

Figure 10. Comparing seroprevalence (%) of BVDV measured with indirect ELISA (Svanova, Biotech, Uppsala, Sweden) between governmental farms (n=180) and private farms (n=125) according to age.

Due to difficulties in receiving bulk milk from all private farms, bulk milk samples were only collected from five governmental farms and one private farm. Three farms belonged to BVD class 3, two farms to BVD class 2 and one farm to BVD class 1. No farms belonged to BVD class 0.

Neospora caninum

The overall seroprevalence of *N. caninum* was 21%. There was a significant difference in seroprevalence between governmental farms and private farms. In governmental farms, the seroprevalence was lower than in private farms. There was also a significant difference in seroprevalence between breeds where Russian black and white had higher seroprevalence than the other breeds in the study. A significant difference was also seen in seroprevalence between different age groups where group >1-3 years old had higher seroprevalence than the other groups (table 2). The difference was even clearer when comparing farmtypes; the seroprevalence were higher in private farms than in governmental farms among animals >1-3 years old (figure 11).

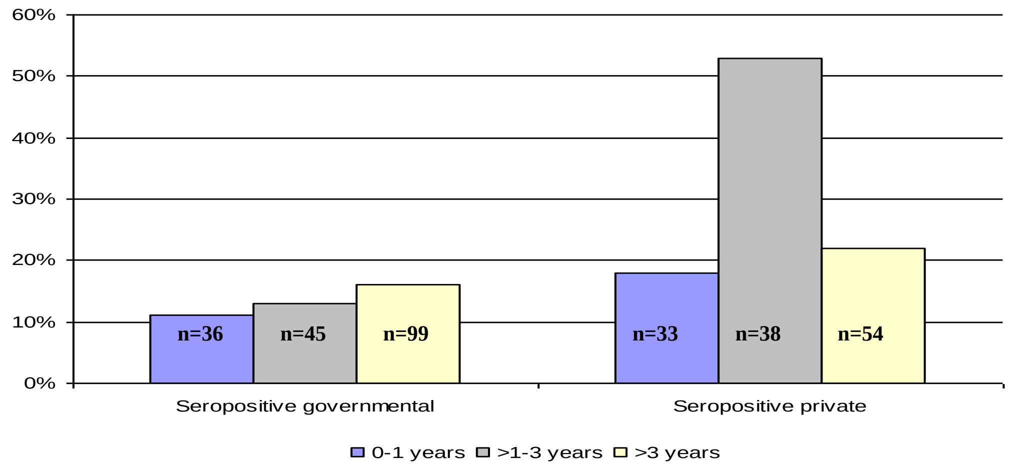

Figure 11. Comparing seroprevalence (%) of Neospora caninum measured with indirect ELISA (Svanova Biotech, Uppsala, Sweden) between governmental farms (n=180) and private farms (n=125) according to age.

Brucella abortus

The overall seroprevalence *B. abortus* was 11% (32/305). There were three private farms and one governmental farm that had never vaccinated against *B. abortus* (n=110) and five governmental farms and one private farm that had vaccinated against the disease (n=195).The seroprevalence was 1% (1/110) among non vaccinated animals. One farm had vaccinated their calves 1,5 months before we collected samples and the older animals 7 months before. Following, there were single farms that had vaccinated 5, 18 and 60 months before sampling. Two farms had vaccinated 11 months before. Results from *B. abortus* seroprevalence study are summarized in figure 12.

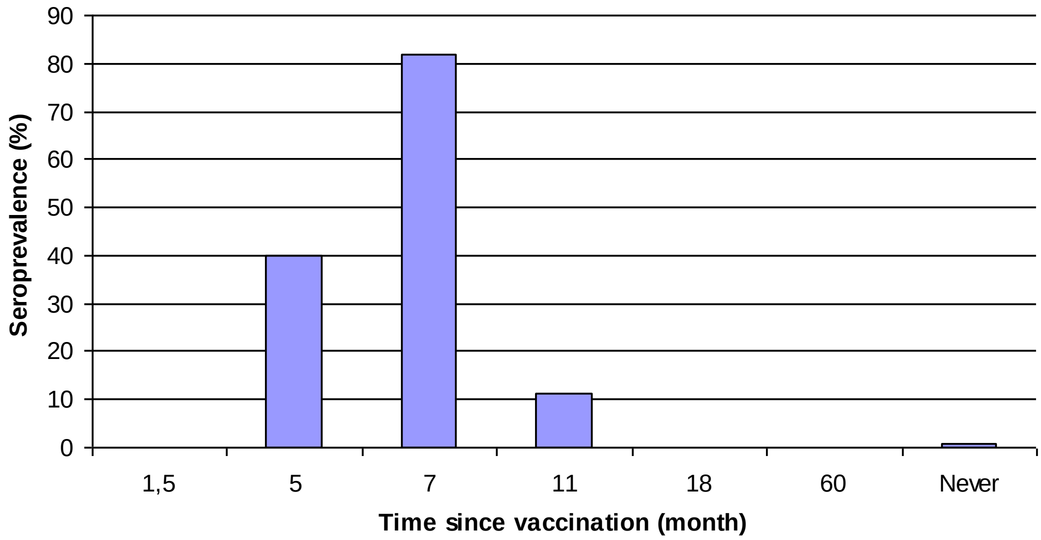

Figure 12. Seroprevalence of Brucella abortus measured with indirect ELISA (Svanova, Biotech, Uppsala, Sweden) in vaccinated (n=195) and non-vaccinated (n=110) animals. Seroprevalence measured in month ago from vaccination.

DISCUSSION

This is the first report, to my knowledge, of BVDV and *N. caninum* infection among dairy cattle in Tajikistan. From this study it can not be concluded whether the mentioned diseases have a real negative impact on productivity in the study area. This is because the data on abortions were not found to be reliable. However, since both diseases were found to be commonly occurring and are well known to decrease productivity (Radostits et al., 2007), such an assumption would not be without reason. Brucellosis is a very important and prioritized disease in Tajikistan. This study indicates that there was *B. abortus* infection in the study population.

The overall seroprevalence of BVDV in this study was 77%. Similar results have been reported from studies made in other countries without systematic control (EU Thematic network, 2001; Talebkhan Garoussi, 2008). There was a difference in seroprevalence of BVDV between large herds (governmental farms) and small herds (private farms). The governmental farms had higher seroprevalence than the private farms. This finding corresponds with a report from Jordan (Talafha et al., 2008) showing that young animals in private farms had lower seroprevalence than young animals in governmental farms. This might indicate that self-clearance of BVDV is more common in the private farms. This is in line with the theory that self-clearance is more likely to occur in smaller herds due to high mortality among PI calves and effective transmission of infection within the herds (Ståhl., 2006). It is therefore advisable that no animals are introduced into these herds without controlling BVDV status to avoid re-infection. The difference in seroprevalence found in this study between different age groups, where younger animals had lower seroprevalence than older animals, can be expected due to the lifelong immunity developed after BVDV infection. This finding is consistent with other studies (Talebkhan Garoussi, 2008; Mainar-Jaime et al., 2000).

Results from bulk milk analyses proved one farm to belong to BVD class 1. BVD class 1 indicates that this farm was free from infection with BVDV. In order to protect this farm from BVDV infection, it is advisable that new animals are declared free from BVDV before being introduced into the herd. Unfortunately we did not receive spot-tests of young animals from this farm. Two farms belonged to BVD class 2 and had positive spot-tests, which indicates ongoing or recent infection with BVDV within these farms. Three farms were classified as BVD class 3, which indicates that there were, or had been PI animals within these herds (Alenius & Larsson, 1996). The spot-tests from two of these farms were positive indicating ongoing or recent BVDV infection within the farms. The remaining farm belonging to BVD class 3 was negative on spot-test, which indicates that a majority of the lactating animals on this farm have been exposed to the virus, but that it is currently free from infection. Only one of the seronegative animals tested positive for BVDV antigen indicating persistent infection. To definitely classify this animal as PI, the test has to be repeated positive. It is likely that there were more PI animals within the population. In order to find and remove PI animals further investigations are required.

The overall seroprevalence of *N. caninum* in this study was 21%, and seropositive animals are in general considered as being infected with the parasite (Radostits et al., 2007). The overall seroprevalence were higher in private farms than in governmental farms. This might be due to more frequent horizontal transmission within the private farms. This may be explained by more dogs being kept together with the cattle and poorer handling of material from abortions in private farms than in governmental farms. There was also a difference when comparing different age groups where animals >1-3 years old had higher seroprevalence than the other groups. This is in line with results from a study that indicated, in contrary to current knowledge that the infection is lifelong, that some animals can become free from the parasite after infection (Chanlun, et al., 2007). When comparing seroprevalence in animals >1-3 years old between farmtypes it was clear that the seroprevalence was only increased within this group in private farms and not in governmental farms. This difference might indicate that there were more frequent horizontal transmission within this group in private farms and that the older animals had become free from infection. The difference found among breeds, where Russian black and white had higher seroprevalence than the other breeds, can be due to vertical transmission if using seropositive animals for breeding. The same pattern of transmission has been shown by Frössling (2004).

In this study the overall seroprevalence of *B. abortus* was 11% compared to 2% in a previous study from Tajikistan (Jackson et al., 2003). It is difficult to compare these studies because some of the animals in both studies had been vaccinated against *B. abortus*. The ELISA used in these two studies could not distinguish between antibodies due to vaccination or infection. The seroprevalence can therefore reflect the time period from vaccination because antibodies remain in the blood approximately five month after vaccination (Sattorov, TAU, personal message).

One governmental farm in this study had vaccinated against *B. abortus* seven months before our sampling. There was an increased rate of positive animals for *B. abortus* antibodies in this farm compared to the farms which had vaccinated five months before. This contradicts the information that antibodies decreases five months after vaccination (Sattorov, TAU, personal message). The farm which had vaccinated seven months before sampling had one confirmed case of *B. abortus* infection two weeks before we collected the samples (Sattorov, TAU, personal message). *Brucella* Ab I-ELISA can not differ between antibodies from vaccination and infection. Within this farm it would have been interesting to do further analyses on the seropositive animals to distinguish antibodies due to infection and vaccination. The fact that the level of antibodies increases between five and seven months after vaccination may be taken as an indicator of an ongoing infection with *B. abortus* on the farm. It would also have been interesting to collect blood samples from animals vaccinated three and four months before sampling to evaluate the serological response after vaccination further.

CONCLUSIONS

- Infection with BVDV and *N. caninum* are present among dairy cattle in the western Tajikistan.

- The study indicates that there is self-clearance of BVDV in some herds, and that this is more likely to occur within the private farms.

- Results indicate that there is more frequent horizontal transmission of *N. caninum* within private farms compared to governmental farms.

- Infection with *B. abortus* is present within the study population.

- The results demonstrate that BVDV in particular, but also *N. caninum*, are widespread in the study population. Based on this, and on current knowledge of the diseases, it can be assumed that they have a negative impact on bovine reproduction and production in the country. However, to estimate the dimension of this impact, further and more extensive studies are required.

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