VIRUS-HOST INTERACTION AS THE HANDLE TO ERADICATION OF BOVINE VIRAL DIARRHEA

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Bovine viral diarrhea may be one of the most widespread infections of bovines, yet measures to control this infection are of relatively recent date. Here, we shall briefly describe how the interaction of this virus with its hosts impacts the control programs and describe approaches chosen to eradicate BVD. Emphasis will be on the Swiss BVD eradication program.

As discussed in more detail by Vilcek (this meeting), BVD virus belongs to the pestivirus genus of the flaviviridae family. BVD viruses cause either transient or persistent infections. The latter are unique among persistent infections because they are characterized by immunotolerance to the infecting viral strain. The two types of infection caused by BVD viruses are linked with two biotypes of the virus and with the time point at which infection is initiated. BVD viruses are either cytopathic (cp) or non-cytopathic (ncp), as differentiated by their effect in cultured cells. Cp BVD viruses may be viewed as "accidents in viral replication" that are generated by diverse mutations when multiplying in animals persistently infected with an ncp biotype of BVD virus (for a review, see Peterhans et al., 2010). Persistent infection is initiated by a transient infection of heifers or cows with ncp BVD virus between days 30 to 120 of gestation. The virus reaches the fetus as a result of viremia of the pregnant animal. When infected during this early stage of development, the fetus may develop and be born normal but remains persistently infected for life. However, BVD virus is immunologically tolerated by such hosts, i.e. persistently infected animals do not form antibodies or a T cell response to the infecting viral strain. In addition, due to the lack of immunoglobulin transfer through the epitheliochorial placenta, viral multiplication is not affected by the adaptive maternal immune response that terminates infection in the pregnant animal. Notably, also calves born to persistently infected cows are persistently infected.

Work in the last few years has shown that immunological tolerance may not only comprise the adaptive, but also the innate, immune response of the host. Thus, ncp BVD virus fails to trigger the generation of type-I interferon (IFN-I) in cultured cells, including macrophages. Ncp BVD virus does not only fail to induce interferon in cultured cells, but also in fetuses infected during the early stage of development (Charleston et al., 2001). The absence of IFN-I generation in response to BVD virus may be important also for the induction and maintenance of adaptive immune tolerance to this virus because the innate and adaptive arms of the immune system are interrelated. Specifically, IFN-I is known to be a key activator of the adaptive immune response. In this context, it is remarkable that the lack of interferon induction by ncp BVD virus applies only to persistent infection, whereas acutely infected animals readily produce interferon and mount an adaptive immune response to ncp BVD virus (Charleston et al., 2002). In contrast to ncp, the cp biotype BVD viruses readily stimulate interferon in cultured cells, fetuses and also in animals infected post-natally. Cp biotype BVD viruses never cause persistent infections.This unique way of interacting with the immune system is not only crucial for the successful persistence of ncp BVD virus in individual hosts, but also for persistence in the host population. Transient infections are much more frequent than persistent ones and induce immunity to reinfection, which eliminates these animals from the pool of potential hosts for the virus (for a review, see Peterhans and Schweizer, 2010).

How does the strategy of BVD virus for persistence in the population compare to that of other viruses? - In general terms, viruses are able to persist in a population either by a strategy of "hit and run" or "infect and persist". In the first one, viruses infect hosts transiently, from which they are transmitted to new hosts. In the second, viruses are transmitted to new hosts from persistently infected animals. The two strategies differ in their interactions with individual hosts. A "hit and run" strategy may lead to immunity or death of their hosts – the final result is the same, as neither dead, nor immune, hosts can serve as replicators for BVD viruses again. In contrast, the "infect and persist" strategy is characterized by persistent infection in individual hosts. Obviously, the requirements for these strategies to be successful differ between "hit and run" and "infect and persist". The first strategy requires dense populations with high turnover, be that due to short generation time, or high mobility. In addition, viruses must be highly contagious, or be transmitted efficiently by other mechanisms. Examples of the highly contagious viruses include measles in humans, whereas rabies virus is successfully transmitted by inducing behavioral changes that ultimately lead to biting and injection of virus-containing saliva. In contrast, an "infect and persist" strategy is successful also in host populations that lack the dynamics associated with density and mobility. In the absence of susceptible new hosts, viral persistence in the population is achieved by viral persistence in individual animals. Well-known examples are infections caused by lentiviruses. For example, in Switzerland, up to 80% of the goats were persistently infected with caprine arthritis encephalitis virus before the initiation of a control program in the early 80's of the last century. Only one third of the infected animals, however, showed any signs of infection, and development of symptoms took months to years in those animals (Krieg and Peterhans, 1990).

How does BVD compare to these examples? – BVD is unusual because its strategy combines both, "hit and run" and "infect and persist". Persistently infected animals shed large amounts of virus lifelong. The spread to non-immune animals is the "hit and run" part of BVDV's strategy. However, since transiently infected animals are themselves inefficient in transmitting the virus (Moen et al., 2005; Nickell et al., 2011) only those "hit and run" transmissions that result in the generation of persistently infected fetuses and the birth of persistently infected calves significantly contribute to viral persistence in the cattle population. Within a closed herd, the high infectious pressure originating from a persistently infected animal increases herd immunity. This, in turn, decreases the chances that further persistently infected animals are generated. In the absence of control measures, approximately 0.6-1% of the animals are persistently infected, 60% are seropositive (immune), and the remaining ones are seronegative and susceptible to infection with BVD virus (Rьfenacht et al., 2000). Moreover, genetically and antigenically diverse BVD viruses of low virulence co-exist in endemically infected cattle populations (Stalder et al., 2005; Bachofen et al., 2008; 2010). These figures show that a very low percentage of persistently infected animals is sufficient for maintaining persistence of BVD virus in a population.

Transient infection is mostly associated with mild clinical signs but, rarely, may lead to severe thrombocytopenia and bleeding. Most of the severe outcomes of infection are associated with BVD virus-2 strains (Ridpath et al., 2000) but occasionally also BVD virus-1 strains may be virulent (Ridpath et al., 2007). Persistent infection may also be clinically mild, but recurrent infections with other agents, ill thrift and growth retardation are frequently observed. Calves born persistently infected may show cerebellar ataxia. Together with the fatal mucosal disease, these two forms of persistent infection most often draw the attention of veterinarians and farmers to the presence of BVD virus in a cattle herd. However, especially at epidemiological equilibrium, both transient and acute infections may be overlooked. This may explain why BVD control programs were initiated later than for other cattle disease such as IBR. The pioneers of BVD control programs are clearly the Scandinavian countries. A country-wide eradication program was initiated also in Austria in 2004 and in some regions of other countries (e.g. France, Italy) (for reviews on control programs, see Moennig et al., 2005; Lindberg et al., 2006).

Key points of any BVD control programs are information and sensitization of the stakeholders. In the European context, an important contribution came from an EU-sponsored thematic network program. Specialists in virology, immunology, epidemiology, veterinary public health and economy held several meetings to discuss the various aspects of BVD. The consensus reached was summarized in a position paper that contains both the theoretical and practical aspects of BVD control (http://www.bvdv-control.org/). Meetings dedicated to BVD control were also held in the USA (http://www.bvdinfo.org/). In Switzerland, we established a website (http:// www.bvd-info.ch) that contains information for farmers (in German and French) and for veterinarians (in German and English).

The points common to all BVD eradication programs are (i), detection and elimination of all persistently infected animals and (ii), restrictions on animal movements. The initial approach taken for detecting persistently infected animals in the Scandinavian and Austrian programs differs from that taken in Switzerland. Based on the observation that a persistently infected animal in a herd leads to a high percentage of seropositive animals, serology was used in Scandinavia and Austria for the detection of herds likely to contain such animals. Due to the overall high herd immunity in most of the Swiss cattle farms, this indirect approach did not permit reliable identification of herds likely to contain persistently infected animals. It was therefore decided to start the program with testing the entire cattle population for virus. In 2008, the entire cattle population of 1.5 million animals was tested. Ear notches were analyzed for virus using either real-time RT-PCR or antigen capture ELISA. All animals found persistently infected were eliminated. The added benefit of using ear notches is the easy identification of animals by their earmarks. From 2009 through 2011, all newborn calves were tested when applying the ear tags. From 2012 onwards, a combination of testing for virus and serology will be used. In October of 2008 the prevalence of virus-positive ear notches was at 1.1% and has since dropped to reach 0.06% in December of 2011 (Presi et al., 2011; Di Labio, personal communication). As indicated, the seroprevalence before initiation of the eradication program was 60%. The current level is not known, but will have decreased markedly, albeit somewhat slower than the drop in virus prevalence. The reason for the slower decrease is explained by the slow turnover of the population which still contains a significant number of animals that were transiently infected before, or at the early stage of, the eradication campaign. If persistently infected animals have indeed been removed from a herd, all animals born after this time point should be seronegative to BVD virus, once maternal antibodies are no longer present. For serology, there are two possible types of specimen available: serum of calves after weaning of maternal antibody, or, milk of primiparous cows. We propose to collect serum samples rather than milk because serum samples of calves can be tested approximately from 6 months of age onwards, whereas milk of primiparous cows becomes first available around 18 months later, when the animals are 24 months old. The gain in time obtained when testing serum versus milk is important and, in the end, may be more economical than the milk sampling proposed by the Swiss veterinary authorities. Thus, if not detected early, persistently infected animals will continue to transmit virus and initiate new chains of infection. Generation of new persistently infected animals will in fact become more frequent with decreasing herd immunity. A second advantage of serum over milk samples is of technical nature. Experiments done in our laboratory show that one antibody-positive serum can be detected when tested together with four negative ones. In contrast, testing of pooled milk samples is markedly less sensitive.

Like all virus eradication programs, also BVD eradication shows a “tailing phenomenon”, i.e. the initial rapid decrease in the prevalence of infection gives way to a slower decrease, and to reach freedom of infection may take several years. In an unpublished study performed by the Swiss Veterinary Office in 2011, it was noted that approximately 60% of new cases of persistent infection found in farms previously free of BVD originated from unknown sources. This shows that routes of infection may exist that evade analysis by classical epidemiological methods. Innovative work performed in Sweden demonstrated the power of molecular epidemiology as a tool in the analysis of such infections (Stehl et al. 2005). Initial testing for virus of all animals in 2008, and of all newborn calves from 2009 through 2011 provided over 7’000 virus isolates for genetic analysis. Partial sequences of all strains (mainly of the 5’ untranslated region) were obtained and combined with animal data in a large database. New viral strains detected in outbreaks of unknown origin can now be compared to all available sequences. With the help of this tool, we analyzed over 100 outbreaks where conventional epidemiological approaches did not allow to unequivocally identify the source of the outbreak. In addition to being able to trace chains of infection, the in-depth analysis of the sequences also provides new information on the molecular evolution of this virus at a resolution and degree of detail that would be impossible without the unique opportunity offered by a country-wide eradication campaign. Thus, in addition to the farmers and other stakeholders, virology and virologists are among the beneficiaries of this eradication campaign – not so much in money, but in insight into the behavior of a virus in a population of 1.5 million host animals.

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ВЗАЄМОДІЯ ВІРУС-ХАЗЯЇН, ЯК МОЖЛИВІСТЬ ЕРАДИКАЦІЇ ВІРУСНОЇ ДІАРЕЇ ВРХ

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Вірусна діарея великої рогатої худоби є, можливо, однією з найбільш поширених інфекцій великої рогатої худоби, але заходи щодо боротьби з цією інфекцією стали доступними порівняно недавно. У цій статті коротко описується як взаємодія вірусу з його хазяїном впливає на програми ліквідації ВД ВРХ, а також представлені підходи щодо викорінення вірусної діареї ВРХ, що були обрані швейцарською програмою ліквідації ВД ВРХ.

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POSITIVE FINDING FOR SPIRAL BACTERIA SIMILAR TO SPECIES FROM *HELICOBACTER* GENUS FOUND IN PORCINE ULCERATIVE LESIONS

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Introduction. The presence of hyperkeratosis and ulcers in the region of pars oesophagea of pigs' stomachs has been registered around the world. As a follow-up signs anorexia, chronic anaemia, acute gastric haemorrhage and loss of body mass were reported. Etiological and pathogenetic mechanisms of this syndrome and its economic importance have still not been clearly defined [1]. Although Friendship reports that this infection leads to economic losses [2]. In the past, food and stressogenic factors were noted as etiological factors. It was only recently that infective etiology has been defined [1]. Although the spiral bacteria in gastric mucosa was found with mammals at the end of the nineteenth century, intensive research into this started as late as 1983 year, when Warren and Marshall described the presence of *Helicobacter pylori* in gastric mucosa with people suffering from gastritis and peptic ulcer. Whilst seeking potential reservoirs of *Helicobacter pylori*, Queiroz et al. first identified the presence of spiral, urease-positive bacteria in the samples of gastric mucosa with 10.8% of the examined pigs [3]. These agents were initially marked as *Gastrospirillum suis*, and later on, based on the analysis of 16S rDNK sequence analysis as *Helicobacter heilmannii*. De Groote suggests the name *Candidatus Helicobacter suis* [4]. O'Rourke et al. defined two types of *Helicobacter heilmannii* based on the presence of the 16S rDNK genome sequence and urease gene [5]. *Helicobacter heilmannii* type 1 is morphologically and genetically identical to the bacteria formerly marked as *Helicobacter suis* [6]. This agent is capable of infecting both people and pigs, although its presence has been identified with dogs and cats, too [7]. It is known that with people it can lead to gastritis, peptic ulcer, stomach adenocarcinoma and MALT lymphoma, as well as that through γ-glutamyl transpeptidase it leads to cell necrosis of gastric mucosa with people, just as *Helicobacter pylori* does [8]. Schott proposed factors that enable the infection of people with agents from the group *Helicobacter heilmannii* sensu lato [9]. De Groote reports that *Helicobacter suis* is most frequently present with people in the group of non-*Helicobacter pylori* [10]. Melnichouk et al. report prevalence of the pig infection of 87% [11]. Friendship believes that little data is available about the prevalence of this infection with pigs and reports values of 9.4% and 10.8% [12]. Park et al. have reported values of prevalence of *Helicobacter* sp. infection with pigs of 95.5% confirmed using the PCR method. The same authors report that he values of prevalence range from 8.0% to 77.0%, depending on a geographical region and the applied method of diagnostics. They also say that the values of prevalence are higher when using a PCR test [13]. Different tests are applied for the diagnostics of this porcine infection. Today, a PCR test has been developed to prove the presence of *Helicobacter suis* genome in porcine gastric mucosa samples [1]. Until recently it was considered that this agent cannot be cultivated in conditions *in vitro*, but in 2008. year, Baele reported a successful isolation and characterisation of *Helicobacter suis* sp. nov. from the of pig stomach tissue [6]. To present the main agent, the most frequent staining techniques are haematoxylin/eosin, Warthin-Starry and Giemsa. Urease test is of special significance [12]. The same author believes that no certain etiological connection has been confirmed between the results of spiral bacteria in porcine gastric mucosa and ulcerative lesions [2]. Apart from *Helicobacter suis* and other spiral bacteria like *Arcobacter* strain can be present in pigs' stomachs, although they are morphologically different [14]. Although until today, no clearly established etiology of ulcer disease with pigs has been clearly established, the zoonosical potential of *Helicobacter suis* is known, as well as the fact that pigs are an important source of human infection. The aim of this paper is to point to positive findings of spiral bacteria similar to species from *Helicobacter* genus in ulcerative lesions with pigs.

Material and methods. Stomachs of 60 pigs were collected right after they had been slaughtered in a slaughterhouse in South Bačka Region of Vojvodina, Serbia during 2010 year. After cutting in and opening stomachs along the greater curvature with sterilised instruments, the contents were emptied and mucosa was washed with sterile saline solution. Following this, pathomorphologic examination was done in order to identify aedema, erythema, haemorrhage, exudates, flat erosions, elevated erosions, ulcers, hyperplasia of rugal folds, and rugal folds atrophy, nodularity, and hyperkeratosis. Simultaneously, smears were swabbed from the surface of gastric mucosa in the regions pars fundica ventriculi and pars pyloric ventriculi, as well as the tissue samples. The laboratory examined microbiologically and pathohystologically the biological materials. From the smears from the gastric mucosa surfaces, slides were made, which were stained with Giemsa and Gram stains, and then microscopically scrutinised. With the tissue samples a quick urease test was done (Urease Hp Test), following the directions from the test producer (Cambridge Life Sciences Ltd, UK). For the pathohistological examination, the tissue samples were prepared and stained with haematoxylin and eosin, following a standard protocol.

Results. The pathomorphologic examination of mucosa, ulcer was identified in 20% of the examined stomachs (Figure 1).