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SCHMALLENBERG VIRUS (SBV) INFECTIONS IN RUMINANTS IN POLAND

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Schmallenberg virus (SBV) was first identified in Germany and the Netherlands at the outbreaks of unknown disease of cattle in 2011. The animals showed non-specific clinical signs such as drop of milk yield, watery diarrhoea, and increased body temperature. The causative agent was identified as a new virus, which belonged to Orthobunyaviridae genus, not yet reported in Europe.

Paper includes data for diagnostic methods and first SBV cases in Poland. The SBV seroprevalence in domestic ruminants has been analyzed. Results of detection of SBV in insect vectors were presented. Role of wild ruminants in SBV spread was described.

Keywords: Schmallenberg virus, diagnostics, surveillance, seroprevalence, vectors.

In August 2011, Schmallenberg virus (SBV) was first identified in Germany and the Netherlands at the outbreaks of unknown disease of cattle. The animals showed non-specific clinical signs such as drop of milk yield, watery diarrhoea, and increased body temperature. The causative agent was identified as a new virus, which belonged to *Orthobunyaviridae* genus, not yet reported in Europe. SBV is an arbovirus transmitted by *Culicoides* spp. midges also involved in the transmission of bluetongue virus (BTV). The greatest losses due to SBV infection are associated with the malformations, stillbirths, and mortality of the newborns. While in cattle the symptoms often remain unnoticed, losses in sheep and goats may be more severe, affecting up to 50 % of progeny. In spite of many analogies to BTV, SBV has spread faster across Europe.

Diagnostic methods and first SBV cases in Poland. The diagnostic methods which were introduced in Poland in 2012 include virological (real time RT-PCR detecting S segment of SBV and virus isolation in baby hamster kidney BHK-21 cells) and serological (commercial ELISA by IDvet and IDEXX and virus neutralization test) assays. SBV infection in Polish cattle was first reported in August 2012 (Larska et al., 2013a). The outbreak coincided with the introduction of two French bulls into the herds in Goleniów (Zachodniopomorskie province) and Częstochowa districts (Śląskie province), which were SBV positive during the quarantine. The presence of virus RNA was also confirmed in *Culicoides obsoletus*, which were caught in August 2012 in Goleniów district, 5 km away from the examined herd. In 2012, first cases of malformed due to SBV infection foetuses and newborns were observed. More than half of the cases involved calves and lambs which died in the first day after the birth, while the rest were stillbirths. Most common malformations included: scoliosis and kyphosis, arthrogryposis, torticollis and brachygnathia interior. In 10 to 20 % of cases, hypoplasia of the cerebrum, cerebellum and spinal cord was observed at the necropsy. The most suitable material for SBV detection because of the highest concentration of viral RNA proved to be the brain, cerebellum, medulla oblongata and the fetal umbilical cord. The virus was detected in spleens only in 21 % of the cases.

SBV seroprevalence in domestic ruminants. The prevalence of Schmallenberg virus (SBV) specific antibodies in ruminants from 13 Polish provinces was evaluated between January 2010 and August 2013 (Larska et al., 2014). A total number of 1813 serum samples from cattle, sheep, goats, mouflon, wild and farmed cervids, and European bison were tested by ELISA for viral nucleoprotein antibodies. First SBV seropositive animals were identified in August 2012 (1.6 %), and the percentage increased gradually, reaching 57.1 % in December of this year. The proportion of seropositive animals in 2013 at the level of 34.2% increased tenfold in comparison to 2012 (3.4 %), which was particularly significant at the level of individual provinces. In 2013, the highest percentages of SBV seropositive animals were found in Dolnośląskie (92.3 %), Podlaskie (82.3 %), and Zachodniopomorskie (80.9 %) provinces. Significant associations between the seroprevalence and province of origin, month, ruminant species, and insect vector activity were found, while no dependence of animal age on seropositivity was observed. The differences between SBV seropositive large and small ruminants suggested the involvement of some vector exposure factors.

Detection of SBV in insect vectors. The distribution of SBV was also studied in *Culicoides* spp. caught in UV traps located in 23 different locations in Poland (Larska et al., 2013 b). The midges were divided into pools according to species and parity status. The study was based on duplex real-time reverse transcription PCR (RT-PCR) for the detection of the SBV S segment and culicoid 18S gene fragments. Forty-four out of 402 midge pools tested (10.9%) were found to be positive for the presence of viral RNA. The SBV positive *Culicoides* came from 10 traps spread randomly across the country and were collected between August and October 2012. The timing of the SBV positive midge collections and the locations of the traps corresponded to the epizootic situation of SBV in ruminants. SBV RNA was most frequently identified in gravid midges (36.4 %), while in nulliparous, blood-fed and parous midges the percentages were 10.8 % 13.0 % and 8.1%, respectively. The majority (82 %) of SBV positive pools belonged to *Culicoides obsoletus/scoticus* complex; however, viral RNA was also found in 8 out of the 149 (5.4%) *Culicoides punctatus* pools tested. The most important findings included identification of *C. punctatus* as a new possible vector of SBV and the recovery of viral RNA from the nulliparous females which may suggest transovarial transmission in the midges.

Role of wild ruminants in SBV spread. The importance of wildlife and sylvatic SBV transmission cycle in the circulation of the pathogen was also investigated. Viral RNA was detected in the serum of an elk (*Alces alces*) calf captured on the outskirts of Białowieża National Park (BNP), close to the border with Belarus in December 2012 (Larska et al., 2013d). The animal died shortly afterwards of acute bronchopneumonia. The study which followed this outbreak included testing 169 serum samples from wild ruminants, including bison, red and fallow deer, originating from eight locations situated in four Polish Provinces, were tested for the presence of SBV-specific antibodies between 2011 and 2013. Although no antibodies were found in samples collected up to July 2012, positive samples subsequently appeared between November 2012 and January 2013 in all of the sampled regions. The introduction of SBV infection to the European bison (*Bison bonasus*) population of BNP between July and November 2012 was also confirmed. The spread of SBV was investigated further in the serosurvey of 580 wild ruminants representing the species of red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), European bison (*Bison bonasus*), fallow deer (*Dama dama),* mouflon (*Ovis orientalis musimon*) which were hunted or immobilized at 34 different locations in the autumn/winter 2013 (Larska and Rola, 2014). Out of 580 sera, 145 (25 %) were considered positive for SBV antibodies. The overall SBV seroprevalence calculated using district population weights was estimated at 27.7 %. The seroprevalence at the district level varied between 0 and 80.0 % with the mean within-district prevalence of 24.0 %. Significantly higher seroprevalence was observed in the animals from Eastern provinces (36.6 %) in respect to Western provinces (22.8 %). SBV seroprevalence varied significantly between different species (higher SBV seroprevalence in larger species such as European bison), population type (free-ranging; captive), age, body weight, percent of the district forest area, part of Poland, and the densities of wild and domestic ruminants at the district and province level. Using statistical multivariable logistic model, population type, age, part of Poland and domestic ruminant density were identified as main risk factors for SBV infection in wild ruminants in Poland. SBV seroprevalence in wild ruminants, similarly to the epizootic situation in domestic ruminants in the country, varied significantly between districts and provinces. Association between SBV seropositivity and animal body weight and age group expressed by a higher prevalence in larger ruminants, and hence their more frequent exposure to midge-vector bites may be explained by the intense excretion (especially when animals are gathered in a group) of CO_2 which is a strong attractant for *Culicoides* spp. The dependence of SBV seroprevalence and population type with higher exposure rates in captive animals suggested also involvement of population density on the SBV transmission. The domestic ruminant densities were found to be an important risk factor which strengthens the hypothesis of wild ruminants as SBV reservoirs promoting the pathogen spillover and spillback events.

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ВІРУС ШМАЛЛЕНБЕРГ (ВШ). ІНФЕКЦІЇ ЖУЙНИХ ТВАРИН У ПОЛЬЩІ

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Вірус Шмалленберг (ВШ) був вперше виявлений в Німеччині та Нідерландах під час спалахів невідомої хвороби великої рогатої худоби в 2011 році. У тварин спостерігали неспецифічні клінічні ознаки, такі як зменшення надоїв, водянисту діареєю і підвищення температури тіла. Збудник був ідентифікований як новий вірус роду Orthobunya viridae, раніше не описаний в Європі.

Стаття містить дані щодо діагностики перших випадків хвороби Шмалленберг (ХШ) в Польщі. Проаналізовано серопрозитивність щодо ХШ серед домашніх жуйних. Були представлені результати виявлення збудника в організмі комах-переносників. Описано роль диких жуйних в поширенні збудника.

Ключові слова: вірус Шмалленберг, діагностика, охорона, серотипи, вектори.