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Fetopathic effects of experimental Schmallenberg virus infection in pregnant goats

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ABSTRACT

Schmallenberg virus (SBV) is an emerging virus responsible for congenital malformations in the offspring of domestic ruminants. It is speculated that infection of pregnant dams may also lead to a significant number of unrecognized fetal losses during the early period of gestation. To assess the pathogenic effects of SBV infection of goats in early pregnancy, we inoculated dams at day 28 or 42 of gestation and followed the animals until day 55 of gestation. Viremia in the absence of clinical signs was detected in all virus-inoculated goats. Fetal deaths were observed in several goats infected at day 28 or 42 of gestation and were invariably associated with the presence of viral genomic RNA in the affected fetuses. Among the viable fetuses, two displayed lesions in the central nervous system (porencephaly) in the presence of viral genome and antigen. All fetuses from goats infected at day 42 and the majority of fetuses from goats infected at day 28 of gestation contained viral genomic RNA. Viral genome was widely distributed in these fetuses and their respective placentas, and infectious virus could be isolated from several organs and placentomes of the viable fetuses. Our results show that fetuses of pregnant goats are susceptible to vertical SBV infection during early pregnancy spanning at least the period between day 28 and 42 of gestation. The outcomes of experimental SBV infection assessed at day 55 of gestation include fetal mortalities, viable fetuses displaying lesions of the central nervous system, as well as viable fetuses without any detectable lesion.

1. Introduction

Schmallenberg virus (SBV) first emerged in late summer/autumn 2011 in Western Europe as the causative agent of an outbreak of diarrhea, drop of milk production, and fever in adult cattle. Metagenomic analyses on blood samples from affected animals housed in the municipal area of Schmallenberg (North Rhine-Westphalia, Germany) led to the identification of this new orthobunyavirus (Hoffmann et al., 2012). SBV was later found to induce congenital malformations in the offspring of cattle, sheep, and goats. The most common malformations in affected newborns include arthrogryposis (with muscular hypoplasia), malformations of the central nervous system (CNS), and vertebral malformations (van den Brom et al., 2012; Herder et al., 2012). However, in non-pregnant adult ruminants, clinical signs are generally mild or absent (Wernike et al., 2014).

As determined by phylogenetic analyses, SBV belongs to the Simbu serogroup within the genus *Orthobunyavirus* of the *Peribunyaviridae* family. Several viruses in this serogroup, namely Akabane virus (AKAV), Aino virus, and Shamonda virus, can cause diseases in domestic ruminants (Hoffmann et al., 2012). AKAV has been extensively studied for more than 25 years. It is responsible for abortions, stillbirths, premature births and congenital malformations in newborns while it elicits only

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subclinical or asymptomatic disease in non-pregnant adult animals. Pregnant ewes and goats are most susceptible between 28 and 56 days of gestation, and infection at this stage leads to arthrogryposis and/or CNS malformations in the offspring (Spickler, 2009).

The data from a study on SBV seroprevalence in the region of France with the highest goat population (Poitou-Charentes), suggested that goats are generally less susceptible to SBV infection than sheep and cattle (Valas et al., 2014). Another study on the impact of SBV in France in 2012–2013 revealed that 8% of lambs, 3% of the calves, and 2% of goat-kids born in SBV-affected herds showed congenital malformations, with farmers reporting early embryonal losses in dams from all 3 ruminant species (Dominguez et al., 2014). Until today, the window of susceptibility to vertical SBV infection in pregnant goats still remains unknown.

In this study, we performed experimental SBV infections of pregnant goats at early stages of gestation (day 28/42 after artificial insemination). Our specific objectives were: (i) to assess whether embryos/fetuses are susceptible to SBV infection when pregnant goats are inoculated at day 28 or 42 of gestation; (ii) to describe the pregnancy outcomes and the lesions in the developing fetuses; (iii) to determine the tissues and cells targeted by SBV in the fetus, in the case of successful transplacental SBV transmission.

2. Materials and methods

All experiments were conducted in accordance with the guidelines of the European Council Directive (2010/63/UE) and with French laws and regulations (Articles R214-87 to R214-137 of the Rural Code and decree n°2013-118 dated February 1, 2013 published on February 7, 2013). All experimental procedures were evaluated and approved by the Ministry of Higher Education and Research and the ethics committee of the Val de Loire (CEEA VdL, committee number n°19, number 00770.02)

2.1. Animals and experimental design

2.1.1. Animals

13 adult Alpine goats and 16 adult Saanen goats were obtained from the INRA animal facility in Nouzilly (France) or purchased from local French breeders. During the trial, all animals were housed in the insect-proof, Biosafety Level 2, animal facilities at the INRA Experimental Infection Platform PFIE (INRA Centre de recherche Val de Loire, France). All purchased animals were tested seronegative for SBV (ELISA, before and after arrival at the PFIE), Q fever (complement fixation test, before arrival at the PFIE) and brucellosis (complement fixation test and seroagglutination test, before arrival at the PFIE). They also scored negative for the presence of Border disease virus (before arrival at the PFIE) and SBV (before and after arrival at the PFIE), as determined by RT-qPCR.

2.1.2. Experimental design

All the animals had an acclimatization phase of 15 days before the beginning of the experiments. Vaginal sponges (Syncro-part 45 mg, CEVA) were inserted into the goats' vaginas to synchronize estrus. The protocol for synchronization of the goats was carried out according to the procedures of the CAPGENES company (CAPGENES, Agropole, 2135, route de Chauvigny, 86550 Mignaloux-Beauvoir). Briefly, the vaginal sponges were inserted 15 days before the artificial insemination (AI). Four days before AI, 2 mL of pregnant mare serum gonadotropin (PMSG, 500 IU) and 0.2 mL of Estrumate® (clopostenol) were injected per animal to optimize the estrus synchronization of the goats. The sponges were removed 14 h before the AI. On the day of insemination, each goat was artificially inseminated with two semen straws. The semen straws came from Alpine and Saanen bucks from the CAPGENES company and had been tested negative for SBV. The pregnancy status was assessed at days 35 and 42 after insemination by ultrasonography. Goats that were not pregnant at day 42 post insemination (15/29) were excluded from the study. The number of animals per group given below does not include these animals.

The protocol involved 3 groups: one group inoculated at day 28 post insemination (G28; 5 goats), one group inoculated at day 42 post insemination (G42; 5 goats) and a control group (4 goats). The goats from the G28 group and from the G42 group were subcutaneously inoculated with 1 mL of SBV-containing bovine serum kindly provided by the Friedrich-Loeffler-Institut (FLI), Germany; this serum had been previously shown to contain $10^{2.83}$ infectious doses per mL by in vivo titration in cattle (Wernike et al., 2012). The control group received 1 mL of sterile saline solution by subcutaneous injection at day 28 (2 animals) and day 42 (2 animals) post insemination. In each group, there was as many Saanen goats as Alpine goats. Each group had an equivalent mean of age. The reproductive capacity was harmonized for each group in function of the fitness of each goat in order to have the best rate of fertility.

During the course of the trial, all animals were monitored twice daily, and body temperatures were recorded by telemetric measurement with rumen temperature sensors (Small Bolus®, Médria, Châteaubourg − France). After inoculation, whole blood and serum samples were collected daily during the first week and then once a week until the end of the experiment. The sera were precleared, then centrifugated and stored at + 4 °C before analysis. The whole blood samples were then submitted to hematology analysis (blood cell count, immediately after sample collection) and to RT-qPCR (after blood storage at −80° C). The blood cell count was analyzed with the MS9-5 Hematology Counter® (digital automatic hematology analyzer, Melet Schloesing Laboratories, France). Serum samples were submitted to SBV specific ELISA testing (ID Screen Schmallenberg virus Competition Multispecies®, IDvet) according to the manufacturers' instructions. For feasibility reasons, the animals were euthanized (by intravenous injection of Dolethal®, Vétoquinol) and necropsied over three consecutive days (day 53, 54, and 55 post insemination) at the end of the trial.

2.2. Necropsy, tissue collection and preparation

At necropsy, all the organs from the goats were macroscopically evaluated and a panel of tissue samples was collected for histopathology and RT-qPCR (spleen, prescapular lymph node, ovary, oviduct, uterus, and vagina). In the pregnant goats, the fetuses were examined and measured (crown-rump length) and a panel of tissue samples was collected for histopathology and RT-qPCR (brain, spinal cord within vertebrae, skeletal muscle from the hindlimb, heart, kidney, eye, lung, intestine, liver, thymus, umbilical cord, and mandible from the fetus; placentomes, amniotic and allantoic membranes). Fetal umbilical blood as well as amniotic and allantoic fluid were collected for RT-qPCR only. The collected samples were preserved either by direct freezing in liquid nitrogen and then stored at −80° C (samples for RT-qPCR and viral isolation) or in final 4% formaldehyde at room temperature (histopathology). One aliquot (2 × 2 x 4 mm) of each frozen tissue sample was homogenized with 500 µL of MEM. After centrifugation (2000*g* 5 min at 4 °C), the supernatant was collected and either used as inoculum for viral isolation (after one or two defrosts), or for RNA extraction and consecutive RT-qPCR analyses (after one defrost).

2.3. Assessment of viral presence by RT-qPCR and viral isolation

2.3.1. RT-qPCR

RNA from blood, fetal fluids and homogenized tissue samples was extracted using the LSI MagVet TM Universal Isolation kit (Life Technologies SAS, Saint-Aubin, France) and King Fisher magnetic particle processor (Thermo Scientific™, Illkirch, France) according to the manufacturers' instructions. The samples were then tested for the presence of SBV RNA by RT-qPCR targeting the S segment, as