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Prevalence of border disease virus in Spanish lambs

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Abstract

The prevalence of border disease virus (BDV) viraemia in Spanish lambs was determined from 2089 sera randomly collected at two slaughterhouses in 2001 and 2003, as well as in 126 sera obtained in 2004 from a fattening unit with an acute disease problem. BDV was detected with an indirect peroxidase monolayer assay (IPMA), and for the fattening unit sera also by an antigen ELISA. A subset of sera was additionally tested for BDV antibodies. The BDV prevalence in the slaughterhouse sera was 0.24%, whereas 7.1% of randomly selected and 38.6% of sera from clinically affected lambs in the fattening unit were virus positive. Pestivirus antibodies were found in 17.6% of the slaughterhouse sera and 28.6% of those from randomly selected lambs in the fattening unit. In total, 33 virus isolates and 3 antigen positive samples were identified. Genetic typing of 5′-UTR sequences classified all 36 pestiviruses as of BDV type 4. This shows that from a low BDV prevalence in apparently healthy lambs in the entire sheep population, clinical problems associated with BDV can develop when viraemic sheep are brought into intense rearing units.

Keywords: Border disease virus; Lamb; Prevalence; IPMA; Genetic typing

1. Introduction

Border disease (BD) is the clinical outcome of pestivirus infection in sheep. The signs are very variable and can include: barren ewes, abortions, the birth of lambs with tremor and other neurological signs, abnormal fleece or body conformation, or small offspring with poor growth rate and viability (Nettleton, 2000). Besides the important economical losses caused as primary pathogens, pestiviruses may compromise the normal immune response to other pathogens and increase the severity of other infections in sheep (Hussin and Woldehiwet, 1994). The key for pestivirus control is the prompt identification and removal of persistently infected (PI) individuals, combined with improved biosecurity measures to avoid reinfection. PI animals have survived transplacental infection with a non-cytopathogenic (ncp) biotype of pestivirus before onset of immunological competence, and usually remain virus positive and

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antibody negative throughout their lives. Their survival rate is lower than normal animals, but some can survive for years (Nettleton et al., 1992).

Three virus species in genus *Pestivirus* are known to infect naturally and produce BD in sheep: BD virus (BDV), bovine viral diarrhoea virus (BVDV) type 1 and 2 (Vilcek et al., 1997; Sullivan et al., 1997; Pratelli et al., 2001). Thus for identification of a virus causing BD, antigenic or genetic typing of virus isolates, or comparative virus neutralisation studies are required (Paton et al., 1995). For genetic typing, analysis of the conserved 5′-untranslated region (5′-UTR) of the genome or the N^{pro} gene are most commonly used (Vilcek et al., 1994, 1997; Valdazo-González et al., 2007). Molecular characterisation of pestiviruses isolated from Spanish sheep has so far only identified BDV (Hurtado et al., 2003; Valdazo-González et al., 2006, 2007; Berriatua et al., 2006).

Pestivirus infections in sheep have been reported worldwide. Serological studies have shown seroprevalences ranging between 5 and 50% for various countries and regions within countries (Nettleton, 2000). In Northern Spain, serological surveys have shown 4–21% of the adult sheep and 10–93% of the sheep flocks to be pestivirus seropositive (Álvarez et al., 1989; Mainar-Jaime and Vázquez-Boland, 1999; Berriatua et al., 2006). Several studies worldwide have reported prevalences of PI or viraemic sheep ranging from 0.3 to 20% in flocks with clinical BD (Buonavoglia et al., 1994; Braun et al., 2002; Berriatua et al., 2004; Valdazo-González et al., 2006). In contrast, few investigations for BDV have been carried out in sera from randomly sampled sheep flocks; however two such found no evidence of pestiviruses in adult sheep (Hyera et al., 1991; Heckert et al., 1994).

Spain is the second largest sheep meat producing country within the European Union, responsible for 24–25% of the meat production from 32% of the sheep slaughtered (Anonymous, 2001–2003). The Spanish sheep meat demand is for lightweight carcasses. Hence, approximately 96% of slaughtered sheep are 25–45 days old lambs (suckling lamb), or lambs weaned and fed ad libitum in the originating flock or fattening units up to 70–100 days of age (young light lambs). This complicates studies on the prevalence of pestivirus viraemia in Spanish sheep, in that a large proportion of the population, that in fact is most likely to be BDV positive, is only available for sampling a relatively short time after birth, with difficulties in obtaining convalescent samples to clarify differential diagnostic issues of persistently versus acutely infected animals.

The aim of this study was to determine the prevalence of pestiviruses in Spanish lambs. In samples obtained at slaughter, such data would provide background prevalence information in a subpopulation of healthy lambs previously not investigated for pestiviruses. Furthermore, samples from a fattening unit that was suffering an outbreak of disease were also included, to assess the potential impact of BDV in lambs managed in an intense production unit.

2. Material and methods

2.1. Sample collection

Plain blood samples were obtained from two different slaughterhouses in the Province of Leon, and a fattening unit in the Province of Teruel. The slaughterhouses were visited mostly at the yearly peak time for slaughter of sheep, in December 2001 (A) and 2003 (B). Blood was collected during the bleeding, from 10% of randomly selected lambs in each batch from every supplier of animals (farmer, fattening unit, dealer or other trading agents). Altogether around 7–8% of all lambs slaughtered during these months were sampled. The sampled lambs came from dairy and meat flocks in western Castile and Leon (central northern Spain) and from Extremadura (western Spain). Of a total of 2089 sera, 1198 samples were collected at slaughterhouse A whereas 891 came from slaughterhouse B. Of these, 1285 were from suckling and 804 from young light lambs, respectively (Table 1).

From the fattening unit, clotted blood samples from 126 lambs were received in March 2004. This 50,000 capacity unit, which contained only 15,000 animals at the moment of sampling, was divided in three buildings (entrance, classification and fattening), each with pens for 250–300 lambs. Animal supplies came from dairy and meat flocks from the whole of Spain, southern Portugal, and occasionally from