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Dissemination of intestinal pathogens between lambs and puppies in sheep farms

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ABSTRACT

Objectives of the present work were (i) to confirm pathogens implicated in cases of diarrhoea in newborn and young lambs in sheep farms in Greece and (ii) to investigate a possible relation in dissemination of pathogens between lambs and dogs present in the farm. Work was carried out in 22 sheep farms, with (i) flock size over 150 animals, (ii) presence of clinical signs of diarrhoea in lambs in the flock and (iii) close and continuous contact and movement of shepherd dogs within the animal shed of each farm. Faecal sample collection from lambs was performed within 48 h of onset of clinical signs and prior to administration of any antimicrobial or antiparasitic medication to lambs. Faecal samples were also collected from puppies in the farm. In total, samples were collected from 126 lambs and 58 puppies. Samples were processed by using established techniques for isolation of bacteria, detection of viruses and observation of protozoan oocysts. *Escherichia coli* isolates obtained during the study, were tested for antimicrobial resistance against a variety of antimicrobial agents. In total, 236 bacterial isolates were recovered from faecal samples of lambs and 165 isolates from faecal samples of puppies. *E. coli* was the most frequently isolated microorganism: 104 isolates from lambs and 109 isolates from puppies were recovered. Other bacteria isolated were *Enterobacter* spp., *Proteus* spp., *Klebsiella* spp., (lambs and puppies), *Clostridium perfringens*, *Citrobacter freundi*, *Salmonella enterica* subsp. *diarizonae* (only lambs) and *Streptococcus* spp. (only puppies). Group A *Rotavirus* was detected in samples from lambs (2.5%) and *Parvovirus* in samples from puppies (5%). *Cryptosporidium* spp. oocysts were observed in samples from lambs and puppies. This is the first report of isolation of *S. enterica* subsp. *diarizonae* and of detection of *Rotavirus* from lambs in Greece. Rates of *E. coli* isolates from puppies resistant to antimicrobial agents were, in general, smaller than respective rates in isolates from lambs. Two pairs of isolates from the same farm (one from a lamb and one from a puppy) with identical patterns of resistance to antimicrobial agents were detected, which provides some evidence in support of a hypothesis that members of each pair might possibly have been spread from one animal species to the other.

1. Introduction

In newborn and young lambs, enteric infections are frequent problems, occurring in enzootic form or as acute outbreaks (Rook et al., 1990; Sargison, 2008). Enteric infections are caused by a variety of pathogens and are the predominant cause of neonatal mortality of lambs. They can cause clinical disease and/or may lead to suboptimal growth rate of affected animals (Sargison, 2008).

The role of bacteria and parasites in causation of the problem has been well documented. Among bacteria, *Escherichia coli*, *Salmonella* spp. and *Clostridium* spp. are the predominant pathogens causing clinical disease, although other organisms (e.g., *Campylobacter*)

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may be involved less frequently (Harp et al., 1981; Munoz et al., 1996; Scholes et al., 2007; Yang et al., 2014). Among parasites, *Cryptosporidium* spp. and *Giardia* spp. have now been recognised to be of significant involvement in the aetiology of the problem (Causapé et al., 2002; O'Handley and Olson, 2006; Geurden et al., 2008; Yang et al., 2009; Minetti et al., 2014). However, there is significantly less knowledge regarding a potential role of viruses, especially their implication as causative agents of the disease (Martella et al., 2015); rotaviruses, coronaviruses and adenoviruses could be of greater significance, although other viruses (e.g., picornaviruses, bunyaviruses) could also play a role in the problem (Lehmkuhl and Hobbs, 2008; Alkan et al., 2012; Chatzopoulos et al., 2013; Hubalek et al., 2014).

In establishing control measures for the syndrome, limiting the spread of potential causal agents is significant for effective management of disease. Transmission of intestinal microbial pathogens between sheep and dogs cannot be ruled out. There is extensive documentation regarding transmission of infective forms of parasites (helminthes or protozoa) between sheep and dogs (Vasileiou et al., 2015). However, there is no information regarding the potential role of dogs in the dissemination of enteric microbial pathogens in sheep flocks, although these animals are present in sheep farms around the world (bar in intensively managed flocks). Dogs also are in close proximity with humans, hence pose an increased risk to transmit zoonotic pathogens.

Objectives of the present work were (i) to identify and confirm pathogens implicated in cases of diarrhoea in newborn and young lambs in sheep farms in Greece and (ii) to investigate a possible relation in dissemination of intestinal microorganisms between lambs and dogs present in the farm.

2. Materials and methods

2.1. Study design and sampling strategy

Work was carried out from late autumn 2014 to early spring 2015, during the lambing and lactation period in 22 sheep farms in central Greece. A variety of indigenous (e.g., Karagouniko, Chios) or imported (Lacaune, Assaf) breed animals were present in the flocks. As per principal production system in Greece, lambs sucked their dams until 45- to 55-day-old, at which age they would go for slaughter. Sheep management in these farms was of the semi-intensive or semi-extensive type.

Inclusion criteria for flocks were (i) flock size over 150 animals, (ii) presence of clinical signs of diarrhoea in lambs in the flock and (iii) close and continuous contact and movement of shepherd dogs within the animal shed of each farm.

Faecal sample collection from lambs was performed within 48 h of onset of clinical signs and prior to administration of any antimicrobial or antiparasitic medication to lambs. Then, faecal samples were collected from puppies younger than 45 days present in the farm, which had not been vaccinated and had not received any anthelmintic treatment.

In total, faecal samples were collected from 126 newborn lambs younger than two weeks. Within each flock, samples were collected from three to six lambs. Additionally, samples were collected from 58 puppies living in the same farms, two to four puppies in each farm. Initially, a swab was inserted (1.0–1.5 cm) into the rectum of each animal, swirled and then removed. Further, 20 g of faeces were collected into the gloved hand of the investigator. Swabs were placed into transport medium and faeces were maintained in cold storage until transport to the laboratory, which took place within 4 h maximum.

2.2. Laboratory examinations

In all cases, rectal swabs and faecal samples were processed within 24 h of collection. Samples were processed for presence of microbial or parasitic agents by using the techniques described below.

2.2.1. Bacteriological tests

At first, rectal swabs were cultured onto 5% sheep blood agar and McConkey agar and incubated at 37 °C for up to 48 h. Morphology of colonies on each plate was examined; all colonies on the same plate found to be morphologically different between them, were cultured on tryptic soya agar to recover pure colonies. Conventional microbiological techniques, the API rapid identification system (Biomerieux, Marcy-l'-Etoile, France) and the automated identification Vitek 2 system with card GN (Biomerieux) were used for identification of organisms. Swabs were also cultured on CDC Anaerobe 5% Blood Sheep agar (Becton-Dickinson, Franklin Lakes, NJ, USA) for anaerobic incubation up to 72 h for possible isolation of *Clostridium perfringens*. For isolation of *Salmonella* spp., the procedure described in ISO: 6579:2002 protocol was followed, starting with a 20 g of faeces and using Buffered Peptone Water as recovery medium, a Modified Semisolid Rappaport-Vassilliadis Medium and a Xylose-Lysine Desoxycholate agar agar.

For identification of *E. coli*, the following characteristics were primarily taken into account: growth onto McConkey agar (pink coloured colonies with bright pink halo), results of Gram stain (Gram negative rod-shaped), evaluation of bacterial motility (motile organisms), results of lactose fermentation (lactose fermenting), of D-glucose fermentation (glucose fermenting with gas production), of D-mannitol fermentation (mannitol fermenting) and of sorbitol fermentation (sorbitol fermenting) and result of indole production test (indole producing), of Voges/Proskauer test (negative result), of urea hydrolysis test (non-hydrolysing organism), of H_2S production test (non-H2S producing organism), of aesculin hydrolysis (non-hydrolysing organism), of lysine decarboxylase test (positive test), of citrate ulitisation test (negative result) and of ONPG test (positive result) (Barrow and Feltham, 1993; Edens et al., 1997).

2.2.2. Virological tests

For detection of Group A *Rotaviruses*, *Adenovirus*, *Coronavirus* and *Parvovirus* in faecal samples, commercially available rapid test kits (VIKIA Rota-Adeno; Biomerieux—Rota-Corona-Parvo Quicking; Quicking, Shanghai, China), which detected viral antigens, were used, as per manufacturers' specifications. When *Rotavirus* was detected in a sample, the presence was confirmed by using a RT-PCR protocol as previously described (World Health Organization, 2009); faecal homogenates were prepared in phosphate buffer saline and RNA extraction was performed using a commercially available RNA kit (Ambion RNA kit; Thermo Fisher Scientific, Waltham, MA, USA) according to manufacturers' protocol. The extracted double stranded RNA was denaturated at 97 °C for 5 min and placed immediately on ice; a two-step multiplex RT-PCR was performed to characterise G and P types of the *Rotavirus* strains, based on previously published terms and conditions (World Health Organization, 2009); amplicons corresponding to each G and P type were visualised under ultra-violet light on 2% agarose gel.

2.2.3. Detection of Cryptosporidium spp.

For detection of *Cryptosporidium* spp. in faecal samples, a smear from each sample was stained by means of the modified Ziehl-Neelsen technique; additionally, the flotation method using ZnSO4 33.2% solution was performed to detect any *Giardia* spp. oocysts (Ministry of Agriculture, Fisheries and Food, 1986).