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Review

Diagnosis of caseous lymphadenitis in sheep and goat

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ABSTRACT

Caseous lymphadenitis (CLA) is a transmissible, insidious, serious disease of sheep and goat. It was firstly reported in the last decades of the 19th century. To date control of such disease is mostly unsuccessful because of its nature which leads to relapse of the problem even if a single case escapes diagnosis. Consequently, diagnosis of CLA is still a matter of intense research for more than a century. There is no single diagnostic test that could identify all cases or even different stages of the disease. This review discusses the most common diagnostic approaches for CLA in sheep and goat and to illustrate the opportunities and limitations of each approach and its validity to build up a diagnostic plan for CLA.

1. Introduction

Caseous lymphadenitis is a chronic, pyogenic, contagious disease of sheep and goat (Ivanovic et al., 2009). The disease is caused by *Corynebacterium pseudotuberculosis* (*C. pseudotuberculosis*): a facultative intracellular, non-capsulated, non-motile, fimbriated, gram positive pleomorphic bacterium (Connor et al., 2000; Ivanovic et al., 2009). *C. pseudotuberculosis* possesses two major virulence factors: a mycolic acid rich cell wall and potent phospholipase-D (PLD) exotoxin (Bernheimer et al., 1985). Clinically, CLA appears either as palpable superficial abscesses or shows signs of viscera involvement, which may occur independently or coexist with each other (Dorella et al., 2006; Al-Gaabary et al., 2009). Detection of infected animals is the key factor for success of the control measures (Prescott et al., 2002; Menzies et al., 2004). Clinical diagnosis is only suggestive as there are many bacterial organisms besides *C. pseudotuberculosis* that are able to induce superficial abscesses in small ruminants (Arsenault et al., 2003; Mohan et al., 2008). However, abscesses which are caused by other pyogenic bacteria mostly occur sporadically in contrast to CLA lesions which usually involve the flock (Baird and Fontaine, 2007). Isolation and identification of *C. pseudotuberculosis* is the most powerful confirmatory diagnostic method for CLA, but isolation failure and inaccessibility of visceral lesions to be sampled are limitations for such diagnostic method (Laven et al., 1997; Komala et al., 2008; Al-Gaabary et al., 2010). Serological detection of *C. pseudotuberculosis* enables the detection of clinical and subclinical cases. The enzyme linked immunosorbent assay (ELISA) is the most common serological test used to detect immune responses against *C. pseudotuberculosis* (Meyer et al., 2005; Chirino-Zárraga et al., 2009; Hoelzle et al., 2013). However, a scientific debate about specificity and sensitivity of different serological tests to diagnose *C. pseudotuberculosis* infection exists (Williamson, 2001; Prescott et al., 2002; Sting et al., 2012). Genomic diagnosis of CLA by PCR is considered a promising method that is used for either colonial identification or direct detection of *C. pseudotuberculosis* in pus samples (Cetinkaya et al., 2002; Pacheco et al., 2007). Unfortunately,

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inaccessibility of visceral lesions for sampling and the questionable results of PCR when performed on blood samples are obstacles facing such technique to detect CLA cases (Pacheco et al., 2007). Recently, ultrasonography and radiography are being used to detect the site and the size of CLA visceral lesions (Williamson, 2001; Ferrer et al., 2009). In summary, there is no reliable single test or single testing policy to diagnose all CLA cases. There is a need to construct a diagnostic plan that fits the geographical and temporal needs of each flock, depending on intended outcomes, apparent prevalence, available facilities and testing interval that would be determined by veterinary supervision.

2. Clinical signs

Caseous lymphadenitis expresses itself in external and visceral forms, either separately or together (Williamson, 2001; Al-Gaabary et al., 2009). External CLA lesions appear initially as abscesses that convert later on to pyogranulomas ranging in size from millimeters to centimeters. These external lesions are mostly located within superficial lymph nodes, but infrequently in subcutaneous tissues (Kuria and Nagattia, 1990; Pepin et al., 1999; Hassan et al., 2011). Wool or hair over CLA lesions may be lost due to the weak dermonecrotic action of *C. pseudotuberculosis* exotoxins and the pressure atrophy of overlying skin by the lesions (Quinn et al., 1994; Zaitoun and Ali, 1999). Incision of CLA lesions reveals milky, creamy or caseated white to greenish-white pus surrounded by a thick fibrous capsule (Baird and Fontaine, 2007). Visceral lesions are not detectable clinically but express themselves according to their number, site and effect on the involved organ. Progressive weight loss, respiratory disorders and chronic recurrent ruminal tympany are the most prominent signs that may accompany visceral CLA lesions (Al-Gaabary and El-Sheikh, 2002; Kolychev and Zabolotnykh, 2002). Occasionally, CLA cases may be accompanied by orchitis, cellulites, mastitis or rarely death (Ladds, 1993; Scott et al., 1997; Sunil, 2006; Baird and Fontaine, 2007). Although signs of CLA are considered characteristic and highly suggestive (Baird and Fontaine, 2007; Al-Gaabary et al., 2009) particularly if several animals within the same flock are affected, clinical signs are not always confirmatory. Reliability of clinical diagnosis of CLA is negatively impacted by the occurrence of subclinical cases, too small undetectable lesions, and the accidental evacuation of some superficial lesions that reappear after a period during which the animal may appear clinically normal (Williamson, 2001; Ivanovic et al., 2009).

3. Bacteriological diagnosis

For bacteriological identification of *C. pseudotuberculosis*, lesion contents should be collected aseptically after sterilization by ethanol (Chirino-Zárraga et al., 2006). Disinfection before sampling is tremendously important. Otherwise, saprophytic bacteria will overshadow *C. pseudotuberculosis* on culture medium (Brown et al., 1987; Mubarak et al., 1999). Direct microscopy of stained pus smears is rarely helpful unless they are prepared from early lesions, as old lesions usually contain few viable *C. pseudotuberculosis* cells (Baird and Fontaine, 2007) which consequently lowers isolation chance from old calcified lesions (Mubarak et al., 1999; Baird and Fontaine, 2007). However, it is possible to isolate *C. pseudotuberculosis* from old lesions several years after the infection if such lesions are still containing viable *C. pseudotuberculosis* cells (Baird and Fontaine, 2007). Isolation is mostly performed under aerobic conditions, occasionally under anaerobic conditions The organism is grown on a highly enriched medium at 37 °C for 48–72 h in a pH of 7.0–7.2 with the addition of blood or serum for enhancing the growth (Selim, 2001; Baird and Fontaine, 2007). Some CLA lesions may be non culturable rather than negative (Binns et al., 2007) due to either the presence of other bacteria such as Staphylococci and Streptococci that overshadow sensitive *C. pseudotuberculosis* on culture media (Mubarak et al., 1999) or sampling from a sterile part of the abscess (Brown et al., 1987). Colonies appear creamy and opaque on most agar surfaces (Dorella et al., 2006), they are yellowish white and β-hemolytic on blood agar while they acquire blackish color on Hoyle's tellurite lysed blood agar (Mohan et al., 2008; Ivanovic et al., 2009). In liquid medium, *C. pseudotuberculosis* usually forms granular deposit and a surface pellicle (Chirino-Zárraga et al., 2006; Mohan et al., 2008). Stained smear of *C. pseudotuberculosis* colony yields a gram positive palisade or Chinese letter arranged bacilli, coccobacilli or occasionally cocci (Baird and Fontaine, 2007; Ivanovic et al., 2009). The coccoid form of *C. pseudotuberculosis* was numerically reported in a previous study (Chirino-Zárraga et al., 2006) who had 44 isolates from goat, 8 of which showed cocci morphology and 36 shaped as cocco-bacilli. Consequently, it is important not to exclude *C. pseudotuberculosis* if the bacterial smear occasionally showed cocci shaped bacteria. Staining by Loffler, Albert or Neisser stains, *C. pseudotuberculosis* exhibits spiral metachromatic volutin granules which exist in bacillary form, but absent in coccoid form (Dorella et al., 2006; Ivanovic et al., 2009).

C. pseudotuberculosis biovar ovis behaves biochemically as catalase, gelatin liquefaction, arginine hydrolysis, urease, methyl red, citrate, maltose, starch, arabinose, fructose, galactose, dextrose and mannose fermentations positive (Skalka et al., 1998; Literák et al., 1999; Mohan et al., 2008; Ivanovic et al., 2009). In contrast, it behaves biochemically negative for nitrate reduction, acetone production, esculin and bile-esculin hydrolysis, oxidase, mannitol, salicin, trehalose, xylose, rhamnose, lactose, trehalose, raffinose, dextrin and glycerol fermentations (Skalka et al., 1998; Literák et al., 1999; Mohan et al., 2008; Ivanovic et al., 2009). Fermentation of sugars by *C. pseudotuberculosis* yields acid, but not gas. However, a problematic extensive variability in biochemical performance of *C. pseudotuberculosis*, particularly in sugar fermentation, is reported (Sutherland et al., 1993; Mubarak et al., 1999).

Similarity in biochemical performance of *C. pseudotuberculosis* serovar ovis and serovar equi, which infects equines and bovines, is reported and the differentiation is based on nitrate reduction test as the former serovar does not reduce nitrate while the latter does (Sutherland et al., 1996). In fact, it is not important to consider biochemical differences between the two biovars of