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Araştırma Makalesi – Research Article

Studies on Caseous lymphadenitis in Chinkara Deer (*Gazella bennettii*), Pakistan

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Abstract

Corynebacterium pseudotuberculosis is an important cause of caseous lymphadenitis, a complex, chronic devastating and destructive disease of small ruminants. In present study the postmortem examination of Chinkara deer (n=25) was conducted from April 2012 to April 2013. Pus samples suggestive of caseous lymphadenitis were collected according to standard microbiological procedures from the superficial lymph nodes, liver, spleen and lungs during necropsy for isolation of bacterial pathogens and their molecular analyses. Out of 25 pus samples collected from the carcasses presenting clinical lesions 19 carcasses were infected with *Corynebacterium pseudotuberculosis* on the basis of culture characteristics. The frequency of *Corynebacterium pseudotuberculosis* bacterium was increased in old animals. Grossly, multiple tubercles of variable size having caseous material were observed in liver, lungs, spleen and lymph nodes. Histopathologically, tissue sections from all the visceral organs were extensively plugged with abscess. For confirmation of isolates polymerase chain reaction technique was develop to amplify the specific proline iminopeptidase (PIP) gene present in *Corynebacterium pseudotuberculosis* bacterium. The PCR technique developed in present study confirmed 17 isolates.

Keywords: Chinkara deer, *Corynebacterium pseudotuberculosis,* caseous lymphadenitis, PCR

Introduction

Corynebacterium pseudotuberculosis is pleomorphic rod, gram-positive, facultative anaerobic, non motile and non spore-forming bacterium causes significant loss in small ruminants (Kumar et al., 2012). *C. pseudotuberculosis* is an intracellular bacterium, proliferate inside macrophages and is causative agent of caseous lymphadenitis (Pavan et al., 2011). Clinically the disease is characterized by enlargement of superficial, submandibular, prescapular, supramammary and prefemoral lymph nodes and in visceral organs such as lungs (Zavoshti et al., 2012). *C. pseudotuberculosis* biovar ovis is highly resistance to low temperature and rapidly enter into the hosts through skin injuries in humid environment. Moreover, the visceral form of caseous lymphadenitis is normally detected in abattoirs (Yeruham et al., 2004). *C. pseudotuberculosis* bacterium has phospholipase D, a major virulence factor which favors the pathogen to disseminate into the vascular system by inducing increased vascular permeability and disruption of vacuolar membrane (Selvy et al., 2011). The caseous lymphadenitis disease due to *C. pseudotuberculosis* bacterium has worldwide distribution and indicate high prevalence in different meat producing countries like New Zealand Australia, United States, South Africa, Brazil, Canada and India (Arsenault et al., 2003; Dorella et al., 2009; Kumar et al., 2012). C. *pseudotuberculosis* disease has financial impact in different agro-ecological zones particularly the arid, tropical and subtropical areas across Africa, Asia, Central and South America where more than 80% world's goat population is present (FAOSTAT, 2011). The *C. pseudotuberculosis* infection has been found in different domestic and wild

species such as horse, buffalo, lama, camels, alpaca, and deer (Fontaine and Baird, 2008). The *C. pseudotuberculosis* is usually disseminated in sheep and goats and results in significant economic losses to farmers due to reduced milk yield, less meat production, loss of fertility, increased culling and condemnation of affected animals (Williamson, 2001; Guimaraes et al., 2011). Exact diagnosis of *C. pseudotuberculosis* primarily based upon the clinical observations of various lymph nodes having pus, culturing and determining the phenotype of *C. pseudotuberculosis* and biochemical tests (Williamson, 2001). However; gene-based PCR assay is useful and reliable for the estimation of prevalence of *C. pseudotuberculosis* in animals (Cetinkaya et al., 2002). This is the first ever study depicting absolute characterisation of *C. pseudotuberculosis* originating from lungs, liver, spleen and lymph nodes of deer and their gross and histopathological lesions in semi-arid tropical region of Punjab in Pakistan.

Material and Method

Necropsy and Histopathology

The present study was conducted over a period of 12 months (April 2012 to April 2013). During this period necropsies of Chinkara deer (n=25) were carried out within 1 h after death at university college of veterinary and animal sciences, The Islamia University of Bahawalpur. The pus samples were collected from external lesions suggestive of caseous lymphadenitis. The presences of various external and internal gross lesions were carefully recorded in all the visceral organs. For histopathological investigations tissue samples were collected from liver, lungs, lymph nodes and spleen and were fixed in 10% neutral buffered formaldehyde solution for further use. All the fixed tissues were washed in running water, dehydrated in ascending grades of alcohol and cleared in xylene. After embedding through paraffin technique, 4-5 µm sections were cut and stained with hematoxylin and eosin following standard histopathological techniques at Department of Pathology University of Agriculture, Faisalabad and histopathological lesions observed with help of light microscope.

Isolation of pathogens

For bacterial isolation, pus samples were collected from all infected organs stored at 4 °C and were sent in sterile disposable containers to the quality control laboratory, Veterinary Research Institute Zarrar Shaeed Road Lahore, Pakistan. All these samples were inoculated on blood agar based medium supplemented with 5% sheep blood. Bacterial pathogens have convex, dry, whitish, opaque and hemolytic colonies were obtained after 72 h of incubation at 37 °C. These isolates were further purified on cystine tellurite blood agar base supplemented with 5% sheep blood. Finally the suspected growth was subjected to Gram-stained staining for morphological characteristics (Quinn et al., 2011). The purified black colonies of the isolates were used for various biochemical tests including catalase, nitrate reduction and urease. All the nitrate negative and catalase and urease positive cultured growths were considered as *C. pseudotuberculosis*.

Polymerase chain reaction technique

C. pseudotuberculosis isolates were finally identified by using polymerase chain reaction technique by amplification of most important oligonucleotide gene (PIP) of *C. pseudotuberculosis*. Previously used primers for amplification of PIP gene forward, 5-ACTGCGGCTTTCTTTATTC-3′ and reverse 5′- GACAAGTGGGAACGGTATCT-3 were used (Kumar et al., 2012). The bacterial DNA was extracted using commercially

available DNA extraction kit (Vivantis, USA). A total of 30 µl PCR reaction mixture containing DNA template, specific primers and 1X master mix was used for gene amplification. The PCR conditions used for amplification were: an initial cycle for denaturation at 94 °C for 5 min, 30 cycles of each at 94 °C for 35 s, annealing at 55 °C for 45 s and primers elongation for 45 s at 72 °C and finally the extention cycle was carried out at 72 °C for 5 min. The amplified PCR products were observed using 1% agarose gel stained with ethidium bromide. The data regarding age and sex was subjected to chi-square analysis, odd ratios and 95% confidence intervals were also determined.

Results and Discussion

In present study the frequency of *C. pseudotuberculosis* infection was significantly ($P < 0.003$) increased in older carcasses (Table 1) which represent chronic nature of disease. Earlier studies have reported that caseous lymphadenitis (CLA) is a chronic infection and increases with increase in age of animals (Arsenault et al., 2003; Zavoshti et al., 2012). From 25 carcasses inspected clinically, 9(36%) adult deer revealed variable enlargement and pus formation in submandibular lymph nodes, 2(8%) carcasses showed pus formation in cervical lymph nodes and 1(4%) deer showed abscess formation in lymph nodes of brisket region. All the affected lymph nodes were harder in consistency and on cutting showed creamy white, odorless and pasty abscess. Previously, similar characteristics pathognomonic abnormities induced by *C. pseudotuberculosis* infection have been reported in slaughtered sheep (Zavoshti et al., 2012). In 13(52%) carcasses no gross abnormalities including injuries, swelling of lymph nodes and pus formation were recorded in any of superficial lymph nodes. However, the visceral lesions were present in liver, spleen, lungs and lymph nodes. Previously, Fontaine and Baird (2008) and Zavoshti et al., (2012) reported that the enlargement of lungs and lymph nodes with pus are the characteristic features of visceral form of *C. pseudotuberculosis*. 19(76%) of these 25 cases were positive for *C. pseudotuberculosis* bacterium on the basis of cultural characteristics, colony morphology, catalase and Gram-positive reaction. Bacterial colonies of *C. pseudotuberculosis* isolates were obtained after 72 h of inoculation and appeared as small, convex, whitish and hemolytic on 5% sheep blood ager. Similar bacterial growth characteristics have been reported for *C. pseudotuberculosis* identification (Quinn et al., 2011; Kumar et al., 2012). However, the necropsy of these carcasses indicated caseous material in liver, lungs, spleen and internal lymph nodes. From 25 carcasses examined internally, variable size of tubercles having creamy white abscess were observed in 16(64%) of liver tissues (Figure 1), 16(64%) of lungs (Figure 2), 19(76%) of spleen (Figure 3) and 17 (68%) of internal lymph nodes (Figure 4). All the carcass having external lymph nodes abnormalities indicated abscess formation in spleen, liver and internal lymph nodes. Similar postmortem changes have been reported due to *C. pseudotuberculosis* infection (Fontaine and Baird 2008; Zavoshti et al., 2012). Histopathological examination of all the tissue sections obtained from visceral organs revealed extensive abscess formation. These findings are also similar to previous study (Zavoshti et al., 2012). The polymerase chain reaction procedure confirmed *C. pseudotuberculosis* infection in 17 carcasses (Table 1) by amplification of specific and conserved fragment PIP gene 551 bp. Previously PCR amplification of PIP gene has been used to confirm *C. pseudotuberculosis* infection (Kumar et al., 2012). Previously in small ruminants including sheep and goats no reports are available about *C. pseudotuberculosis* infection in Pakistan. Therefore, this is the first ever study in Chinkara deer in Pakistan and the results of present study can be applied to sheep and goat-producing areas for control and diagnosis of *C. pseudotuberculosis*.

Table 1. Frequency of isolation of *Corynebacterium pseudotuberculosis* in different age groups in both sexes (n=25)

Sex/age	No. of Animal	PCR positive		95% CI	Odd Ratio / P value
		N	%		
Sex					
Female	11	8	72.7	42.19 to 92.55	OR = 1.48 [reciprocal = 0.68]
Male	14	9	64.3	44.55 to 90.19	
Age groups					
1-2 Year	6	1	16.7	0.83 to 59.09	Mantel-Haenszel chi-sq P < 0.003
3-5 Years	9	7	77.7	43.79 to 96.09	
6-8 Years	10	9	90.0	59.65 to 99.50	

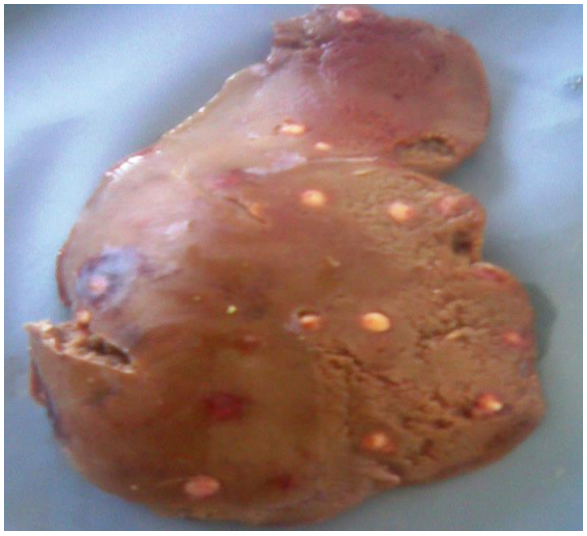

Figure 1. Multiple tubercles of variable size showing caseous material in liver tissue due to *Corynebacterium pseudotuberculosis* infection

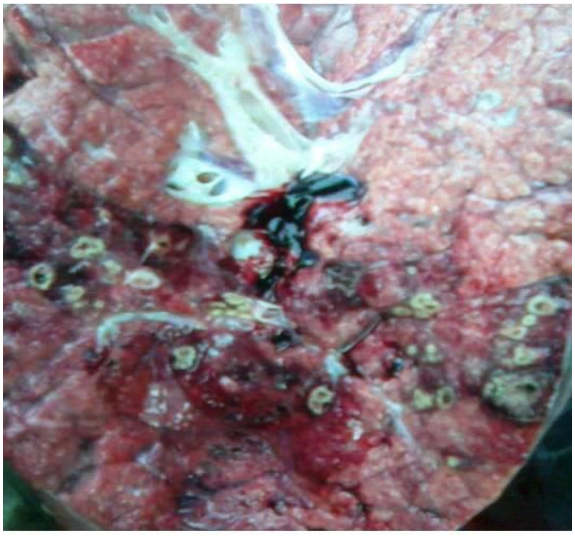

Figure 2. Cut section of lungs infected with *Corynebacterium pseudotuberculosis* showing pus formation

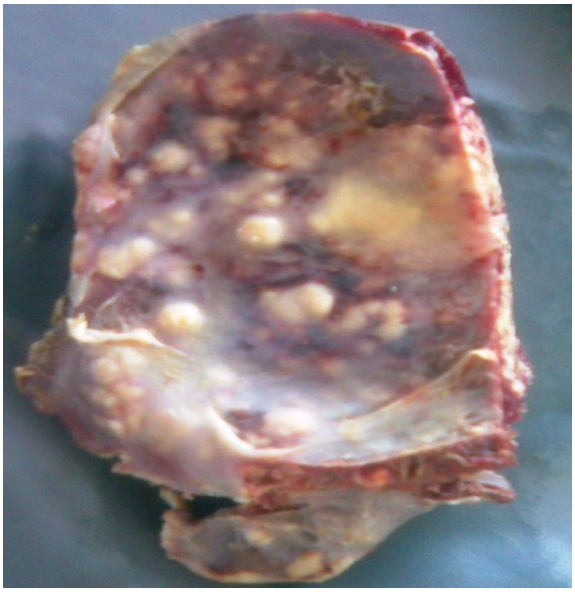

Figure 3. Multiple tubercles of variable size showing caseous material in spleen tissue due to Corynebacterium pseudotuberculosis infection

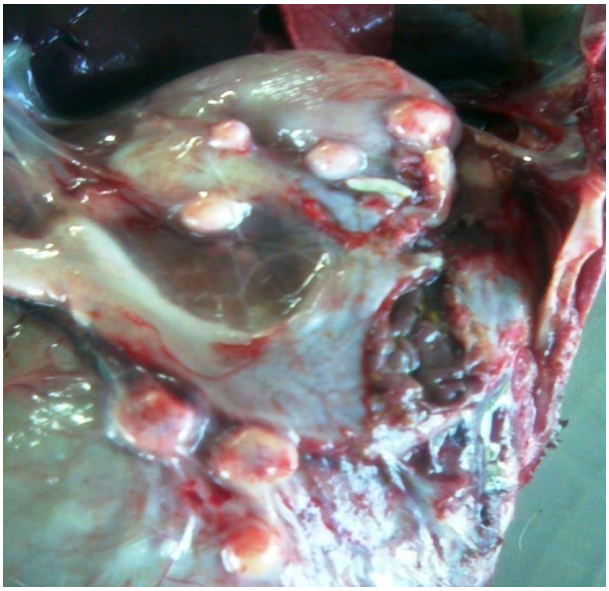

Figure 4. Internal lymph nodes showing pus formation due to *Corynebacterium pseudotuberculosis* infection

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