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Short Communication

Survey on pneumonic pasteurellosis in slaughtered sheep and goats at the Ziaran abattoir

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ABSTRACT

This study was carried out at the Razi Institute during 2005-2006 with inspecting 12168 lung tissue of slaughtered sheep and goats at the Ziaran Abattoir. Pneumonia were diagnosed in 282 cases and the affected lung tissue was collected and transferred to the Bacteriology and Pathology Departments for isolating of *Pasteurella spp.* and interpretation of histopathologic lesions. *Pasteurella multocida* was isolated from 120 cases. Histopathology sections indicated purulent bronchopneumonia 0.51%, purulent interstitial bronchopneumonia 0.17%, purulent bronchitis / bronchiolitis 0.12%, purulent pleuritis / pleuropneumonia 0.07%, purulent fibrinous bronchopneumonia 0.04%, purulent pneumonia 0.09% and progressive pneumonia 0.01%. Statistical analysis of data showed that the frequency of outbreak in the various seasons was significantly different in sheep ($P<0.001$).

Keywords: pneumonia, Pasteurella multocida, bronchopneumonia, pleuritis, pleuropneumonia

INTRODUCTION

Since 1969 lung tissue condemnation data from all abattoir have been reported (Stewart 1970, Rahman & Iyer 1979, Simmons and Cuthbertson 1985). *Pasteurella spp.* is as a primary pathogen for inducing pneumonia after virus and mycoplasma infections (Timoney *et al* 1988). The disease is as high as 80% mortalities and is associated with decreased performance and product *Pasteurella multocida* is part of the commensal flora in the upper respiratory tract and induces disease if it is toxigenic (Eisenstein 1990) . Assays based on polymerase chain reaction (PCR) are contributing to diagnostic microbiology (Eisenstein 1990, Hunt *et al* 2000). PCR is specific and sensitive for identification of toxigenic isolates (Nagai *et al* 1994, Lichtensteiger *et al* 1996).

In this study pneumonia and pleuropneumonia due to *Pasteurella spp.* infections have been recorded and its histopathological aspects were studied and the results for detecting mycoplasma will be published in another paper.

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MATERIALS AND METHODS

10129 sheep and 2039 goats were inspected at the Ziaran abattoir weekly within the year (fall 2005 - fall 2006). Totally 282 lung tissue condemnation showed consolidation which were collected in situ and then transferred to the Pathology Department in the Razi Institute.

Microbiology. The lung tissue was cultured on the blood agar and McCankey's agar plates incubating at 37 °C and confirming it by using oxidase tests and other biochemical reactions. Polymerase Chain Reaction (PCR) studies were also carried on DNA from samples of *Pasteurella multocida* (Townsend *et al* 2001, Jabbari *et al* 2006).

Genomic DNA preparations. All 120 isolates of *P. multocida* that were used to analyse by method of PCR. Briefly the cells were lysed by 10% SDS and proteinase K. The DNA was extracted with phenol / chloroform / isoamylalchol (25:24:1), precipitated with cold ethanol, washed and dried at room temperature. The DNA pellets were resuspended in Tris EDTA buffer (pH 8) measured spectrophotometrically at 260 nm and stored at -20 °C until required (Wilson *et al* 1995).

Polymerase Chain Reaction (PCR). The PCR amplification mixture (25µl) was contained each primer at a concentration of 3.2 µm,each deoxinucleoside triphoshate at a concentration of 200 µm, 1xPCR buffer, 2mM MgCl2 and 0.5 U of Taq DNA polymerase. Twenty nano -grams of template *P.mutocida* genomic DNA was added to the mixture. All amplifications were performed with the eppendorf PCR system. Amplification was performed for 30 cycles. The cycling temperature were initial denaturation at 95 ̊C for 30 sec., annealing at 55 ̊C for 30 sec. and extension at 72̊C for 30 sec. The final cycle was followed by an extension at 72°C for 5min. The amplified products were separated by electrophoresis in 2% agarose gels and visualized by ethidium bromide staining.

Primers. Sequences of the oligonucleotides used in the *P. multocida* were as follows: These primers were described by Townsend *et al* 2001. KMT1T7: ATCCGCGATTTACCCAGTGG. KMTSP6: GCTGTA AACGAACTCGCCAC.

Pathology. 282 pneumonic lung tissue for istological examination was fixed in 10% formalin. Sections were cut 5 µm and staining by Haematoxylin and Eosin.

Statistics. Frequency of bacteria was analyzed statistically by using chi square (X^2).

RESULTS AND DISCUSSION

282 out of 12168 ovine and caprine lung condemnation collected from the Ziaran abattoir weekly within the year 2005-2006 (2.32%). *Pasteurella spp.* have been isolated from 125 pneumonic cases (44.32%) which consisted of 5 cases of *P. caballi* and the rest of them identified *P. multocida*. Identification of all *P. multocida* isolates were confirmed by species-specific PCR. The PCR amplification of the isolates produced a single band of 460 bp with specific PM primers (Figure 1).

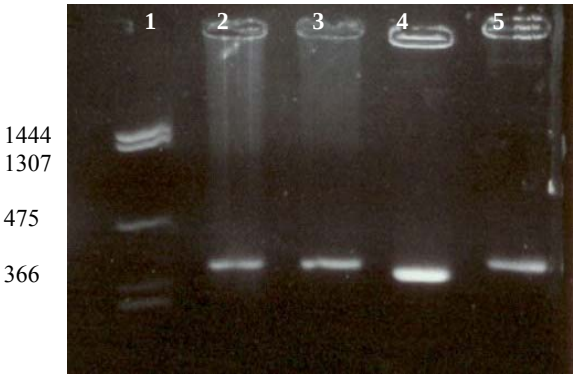

Figure 1. *Pasteurella multocida* specific PCR: *Lane 1* DNA size maker, *Lanes 2-5* contains results of PCR reaction with DNA from representative ovine *P. multocida* isolated.

73 out of 125 isolated Pasteurella showed a little raised lesions and consolidation in tissue at the right cranio-ventral lobes (Figure 3). Relative

frequency of pneumonia in sheep comparable with goats was 2.54% versus 1.22% (Table 1) and Pasteurella cases 11.9 versus 2 in 1000 International Units (I.U.) (Table 2).

Table 1. Relative frequency distribution of affected cases to pneumonia at the Ziaran Abattoir.

Animals	Condition					
	Normal		Infected		Total	
	No.	%	No.	%	No.	%
Sheep	9872	97.46	257	2.54	10129	83.25
Goat	2014	98.78	25	1.22	20.39	16.75
Totall	11886	97.68	282	2.32	12168	100

Table 2. Relative frequency distribution of affected cases to Pasteurella spp. at the Ziaran Abattoir.

Condition	Normal		Infected		Total	
Animals	No.	Per 1000	No.	Per 1000	No.	Per 1000
Sheep	10008	988.1	121	11.9	10129	83.25
Goat	2035	998	4	2	2039	16.75
Total	12043	98.97	125	1.03	12168	100

Statistical data gained out of the Pasteurella case studies were analyzed by using X^2 (chi square) showed the frequency of outbreak in the various seasons within a year was significantly different $P<0.001$ (Table 3). Histopathological observations were comprised of following consequences: The lesions of 125 Pasteurella cases showed Bronchitis/Bronchiolitis and accumulation of exudates obstructed lumen partially. Distribution of neutrophil, lymphocyte and minor plasma cells with thickening of the inter-alveolar septa were present (Interstitial Pneumonia) and scattered poly-nuclear cells were observed within the alveoli in cases of purulent pneumonia. There were whorls of neutrophil cells (Oat Shaped Leukocyte) in 3 cases of Broncho – Pneumonia in sheep. In addition pleural adhesion with mononuclear cells and minor plasma cells were observed. Also proliferation of fibroblast around the bronchials and plasma cells with minor lymphoid cells revealed throughout in cases of progressive pneumonia. The percentage of histological aspects is reported in Table 4.

The mechanisms of the disease due to pasteurella are not fully understood. The portal of entry is usually via the respiratory tract. In fact the virus or mycoplasma may enhance by animal to animal transmission as in pneumonic Pasteurellosis. In chronic cases it is thought that immune complexes may contribute to the lesions (Quinn *et al* 1994). In this study the lesions grossly restricted to the cranioventral portions of the lungs.

The following reasons including: (1) shortness and abrupt branching of airways, (2) greater deposition of infectious organisms, (3) inadequate defense mechanisms, (4) reduced vascular perfusion, (5) gravitational sedimentation of the exudates and (6) regional differences in ventilation (McGavin *et al* 2001) may be suggested. Frequency of the reported Pasteurella cases indicating 11.9 in sheep and 2 in goats in 1000 I .U. The percentage of infection within the four seasons revealed that the relative frequency in infected sheep Pasteurella was the highest in summer time (Figure 2).

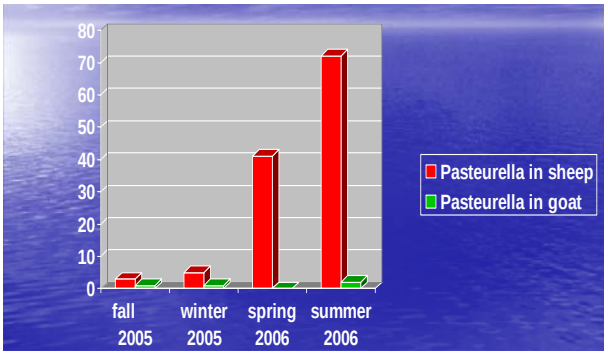

Figure 2. The four seasons revealed that the relative frequency in infected sheep and goat Pasteurella.

Seasons	Spring			Summer			Fall			Winter			Total		
Animals	No.	%	No.s	No.	%	No.s	No.	%	No.s	No.	%	No.s	No.	%	No.s
Sheep	41	1.74	2362	72	2.55	2821	3	0.12	2526	5	0.2	2420	121	1.2	10129
Goat	0	0	178	2	0.22	892	1	0.15	689	1	0.36	280	4	0.2	2039
Total	41	1.61	2540	74	2.05	3713	4	0.12	3219	6	0.22	2700	125	1.02	12168

Table 3. Comparison of relative frequency distribution of affected cases to Pasteurella spp. in the different easons. No.s : number of slaughter.

Animals	No.	PBP	PIBP	B	PFP	PPP_1	PPP_2	PP	PIP
Sheep	10129	0.51	0.17	0.12	0.04	0.07	0.01	0.09	0.088
Goat	2039	0.04	0	0.04	0.04	0.04	0	0	0

Table 4. Distribution of percentage of Pasteurella lesions in lung tissue of sheep and goats. PBP : Purulent BronchoPneumonia, PIBP : Purulent Interstitial BronchoPneumonia, B : Bronchitis , PFP : Purulent Fibrinous Pneumonia , PPP_1 : Purulent Pleuropneumonia , PPP_2 : Purulent Progressive Pneumonia , PP : Purulent Pneumonia, PIP : Purulent Interstitial Pneumoni

In accordance to our studies the combination of isolating *Pasteurella multocida* and *Mycoplasma spp.* from the lung tissue in oneinfected sheep with progressive pneumonia and P. *caballi* and *P. dagmatis* in one infected goat with pleuropneumonia are also recorded. Daniel in 2006 reported that high prevalence of pneumonia in spring born lambs in comparison with the other seasons which was complied with our studies.

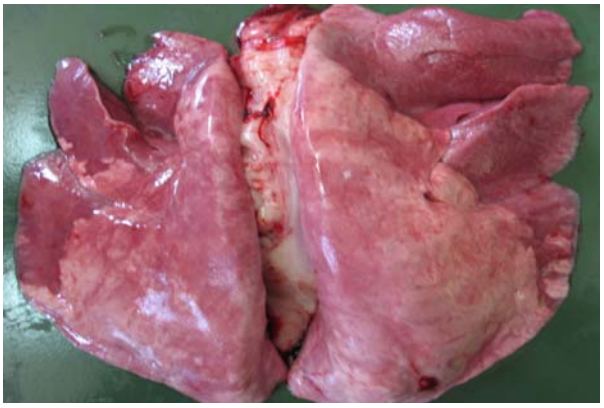

Figure 3. Showing raised lesions and consolidation in lung tissue at the right cranio-ventral lobes.

Assays based on PCR have been carried out although the differentiation between toxigenic and nontoxigenic *P. multocida* need further studies in future. The most important lesions in histopathological slides were Purulent Bronchopneumonia (sheep: 33.9%, goat: 25%) Purulent Interstitial Bronchopneumonia (sheep 19%) Pleuro pneumonia due to Pasteurellosis (sheep: 5.8%, goat: 25%) were determined. The pattern of Oat Shaped Leukocytes in purulent bronchopneumonia was complied with Jubb *et al* 1985. We concluded that isolation and identification of strains of Pasteurella should be used in prospective studies and can be considered in vaccine production.

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