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Research Article

Serotyping of *Mannheimia haemolytica* Strains Isolated from Pneumonic Lungs of Sheep in the Aydın Region of Turkey*

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Abstract: This study was conducted to serotype isolates of *Mannheimia haemolytica* in the Aydın region of Turkey. A total of 200 lung samples showing pneumonia signs were obtained from slaughterhouses in the Aydın region. Twenty-four strains were isolated as *Mannheimia haemolytica* complex. Twenty-two strains were identified as *Mannheimia haemolytica* and 2 were as *Pasteurella trehalosi* for the first time in the region. The serotyping studies of strains isolated from pneumonic lung samples showed that *Mannheimia haemolytica* serotypes A1, A2, A6, A7, A8 and *Pasteurella trehalosi* serotype T4 were present in Aydın.

Key Words: *Mannheimia haemolytica*, *Pasteurella trehalosi*, isolation, identification, serotyping

Türkiye'nin Aydın Yöresindeki Pnömonili Koyun Akciğerlerinden İzole Edilen *Mannheimia haemolytica* Suşlarının Serotiplendirilmesi

Özet: Bu çalışma *Mannheimia haemolytica*'nın Türkiye'nin Aydın yöresinde serotiplendirilmesini sağlamak amacıyla yapıldı. Aydın bölgesindeki mezbahanelerden pnömoni semptomu gösteren 200 adet akciğer örneği toplandı. Örneklerden 24 adet *Mannheimia haemolytica* complex izole edildi. Aydın bölgesinde ilk defa 22 suş *Mannheimia haemolytica* ve 2 suş *Pasteurella trehalosi* olarak identifiye edildi. *Mannheimia haemolytica* suşları A1, A2, A6, A7, A8 olarak serotiplendirildi ve *Pasteurella trehalosi* serotip T4 varlığı ortaya konuldu.

Anahtar Sözcükler: *Mannheimia haemolytica*, *Pasteurella trehalosi*, izolasyon, identifikasyon, serotiplendirme

Introduction

Mannheimia haemolytica (formerly *Pasteurella haemolytica*) is the most common aetiological agent of pneumonic pasteurellosis, septicaemia and mastitis and is considered one of the most important pathogens of lambs, calves and goats (1).

Mannheimia haemolytica strains were formerly evaluated into two biotypes as biotype A and T. *Mannheimia haemolytica*, based on differences in phenotypic capsular polysaccharide, has been identified as 17 serotypes (2-5). Previous reclassification was based on the DNA relationships (6). Current taxonomical studies based on genotypic and phenotypic analysis (7-9) propose a reclassification of 17 serotypes of former *Mannheimia haemolytica* complex to three genetically distinct species: serotypes 1, 2, 5, 6, 7, 8, 9, 12, 13, 14, 16 and 17 were described as *Mannheimia haemolytica* (10); serotypes 3, 4, 10 and 15 were described as *Pasteurella trehalosi* (6); and serotype 11 was described as *Mannheimia glucosida* (10). In addition, approximately 10% of isolates from ruminants are untypable (4).

Mannheimia haemolytica can cause pneumonia or pleuropneumonia in ruminants of all ages, septicaemia in suckling lambs, mastitis in ewes, and arthritis, meningitis and middle-ear infections in sheep, while *Pasteurella trehalosi* strains are responsible for acute systemic pasteurellosis in feedlot lambs (11,12).

Serotyping for *Mannheimia haemolytica* in sheep has been carried out in many countries including the USA (13), Great Britain (1), New Zealand (14), and Hungary (15).

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Mannheimia haemolytica strains have been isolated and serotyped regularly in central and eastern Anatolia in Turkey in recent years (16-18). Kaya and Kırkan (19) isolated *Mannheimia haemolytica* strains from sheep nasal discharge of pneumonic animals in the Aydın region of Turkey. However, no studies have been conducted on serotyping *Mannheimia haemolytica* strains in the Aegean region of Turkey.

The aim of this study was to demonstrate the presence of *Mannheimia haemolytica* in pneumonic lungs of sheep and to determine serotypes isolated in the Aydın region of Turkey.

Materials and Methods

Pneumonic lung samples

Thirty-two scientific visits between 1999 and 2002 were carried out to 4 slaughterhouses (Aydın, Incirliova, Ortaklar and Soke slaughterhouses) in the Aydın region of Turkey.

A total of 200 pneumonic lung samples were brought to the Department of Microbiology laboratories, Faculty of Veterinary Medicine, Adnan Menderes University. The pneumonic lung samples were transferred to laboratories in an ice-pack container.

Isolation of *Mannheimia haemolytica*

Samples of sheep lungs with lesions of pneumonia were taken into sterile containers. Isolation of *Mannheimia haemolytica* was obtained from pneumonic lung tissue by scorching the lung surface with a hot spatula and directly smearing of the cut surface onto blood agar plates and incubating at 37 °C for 24 h. After incubation, the suspected colonies with *Mannheimia*-like morphology, colour, feature and haemolysis were stained by using Gram staining technique and Gram negative bipolar bacilli were examined at the microscopic examination (20,21).

Identification of *Mannheimia haemolytica*

Identification was made on the basis of colony morphology, haemolysis, Gram staining and biochemical tests. Biochemical characteristics of the isolates were determined by using catalase, oxidase, nitrate reduction, ONPG, H_2S, ornithine decarboxylase, indol, urease, growth on MacConkey Agar, voges-proskauer and fermentation of glucose, lactose, mannitol, raffinose, salicine, trehalose, xylose and arabinose (20,21).

The isolates which were found positive for the catalase, oxidase, nitrate reduction, ONPG, H_2S, growth on MacConkey Agar, mannitol, xylose tests and found negative for the ornithine decarboxylase, indol, urease, voges-proskauer, arabinose, glucose, lactose, raffinose, salicine, trehalose tests were identified as *Mannheimia haemolytica*.

Antisera of *Mannheimia haemolytica*

Serotypes 1-17 of *Mannheimia haemolytica* were kindly provided in the lyophilised culture from Dr. L. Fodor, Department of Epizootiology, University of Veterinary Science, Budapest, Hungary. Antisera to serotypes 1-17 of *Mannheimia haemolytica* were prepared in the New Zealand rabbit in the Department of Microbiology laboratories.

Serological Typing of *Mannheimia haemolytica*

The isolated *Mannheimia haemolytica* strains were serotyped using the indirect haemagglutination (IHA) test introduced by Biberstein for serotyping *Mannheimia haemolytica* (11).

Results

A total of 24 (12%) of 200 pneumonic lung samples were positive for the *Mannheimia haemolytica* complex.

Twenty *Mannheimia haemolytica* strains (83.33%) and two *Pasteurella trehalosi* (8.33%) strains were serotyped by the IHA test, whereas two strains (8.33%) were not able to be serotyped by using the IHA test.

The number of serotypes of *Mannheimia haemolytica* complex isolated were as follows: 6 strains (25%) of serotype A2; 5 strains (20.8%) of serotype A7; 5 strains (20.8%) of serotype A6; 3 strains (12.5%) of serotype A1; 1 strain (4.1%) of serotype A8 and 2 strains (8.3%) of *Pasteurella trehalosi* serotype T4. Two strains (8.3%) were untypable (UT) *Mannheimia haemolytica* strains (Table).

Discussion

Mannheimia haemolytica is an important pathogen of ruminants in Turkey. Its control is an important measure in the prevention of pneumonic pasteurellosis of ruminants in Turkey and the rest of the world. Yearly herd losses costing millions of dollars have led to research focused on vaccine production because the number of

Table. Distribution of serotypes of *Mannheimia haemolytica* (Mh) and *Pasteurella trehalosi* isolated from pneumonic lungs of sheep in different locations in the Aydın region of Turkey

Visited Slaughterhouses	No. of lung Samples	No. of isolates	*Mannheimia haemolytica* serotypes						*Pasteurella trehalosi*
			MhA1	MhA2	MhA6	MhA7	MhA8	MhUT	PtT4
Aydın	70	12	1	4	2	3	-	2	-
İncirliova	50	5		1	1	2	-	-	1
Ortaklar	50	3	2	-	1	-	-	-	-
Soke	30	4	-	1	1	-	1	-	1
Total	200	24	3	6	5	5	1	2	2

strains isolated demonstrates the continuing economic importance of *Mannheimia haemolytica* infection (22).

The primary diseases associated with *Mannheimia haemolytica* were pneumonia in lambs and calves and septicaemia in lambs, as have been extensively recorded elsewhere (23). The isolated serotypes in the present study are also in good conformity with the recognised patterns of disease in sheep (1). Several studies have shown that *Mannheimia haemolytica* and *Pasteurella trehalosi* strains vary from country to country. *Mannheimia haemolytica* A1, A2 and A6 are dominant in United Kingdom (24), *Mannheimia haemolytica* A2 is dominant in New Zealand (25), *Mannheimia haemolytica* A1 is dominant in Denmark (26) and *Mannheimia haemolytica* A1, A2 are dominant in Hungary (27).

In Turkey, Güler et al. (28) serotyped *Mannheimia haemolytica* strains as follows: 23.4% A2, 12.3% A9, 10.4% A7, 9.5% A5, 8.6% A1, 7.8% A12, 4.3% A8, 4.3% A13, 3.4% T15, 3.4% T4, 0.8% T10, 0.8% A11 and 10.4% untypable strains. Beside this, Gündüz and Erganiş (29) were also reported the serotypes of *Mannheimia haemolytica* strains isolated from cattle lungs. The investigators found the *Mannheimia haemolytica* strains A1, A2, A7 and A13.

In this study, *Mannheimia haemolytica* strains are serotyped as *Mannheimia haemolytica* serotypes A1, A2, A6, A7, A8 and as *Pasteurella trehalosi* T4. These results have shown similarities to the results of previous studies conducted in some other regions of Turkey (16,28,29). Thus, this study has the importance of being the first report of the isolation-identification and serotyping of *Mannheimia haemolytica* in the Aydın region of Turkey.

Mannheimia haemolytica serotype A2 is the dominant serotype among the isolates. This finding supports a previous report (1), which demonstrated that the serotype A2 was the most frequent isolate from the cases of ovine pneumonic pasteurellosis although all serotypes and untypable isolates were common in sheep.

Untypable *Mannheimia haemolytica* (UT) isolates were the second most frequently isolated strains encountered. The UT strains have been described as mutants of *Mannheimia haemolytica* which may have lost their ability to produce capsular polysaccharide (30). In this study, two strains were found to be as untypable strains.

In conclusion, pneumonia caused by *Mannheimia haemolytica* has multi-factorial aetiology. Beside this, varieties in the serotypes in various geographical regions could affect the severity of the disease regarding the mortality and economical losses. Therefore, evaluation of serotyping of strains would give the opportunity to find the exact antigenic structure and to prepare the most effective vaccine to prevent disease.

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