Bulgarian Journal of Veterinary Medicine, 2021, **24**, No 4, 487–496
ISSN 1311-1477; DOI: 10.15547/bjvm.2299

Original article

CHARACTERISATION AND PATHOGENICITY OF *PASTEURELLA MULTOCIDA* CAPSULAR SEROGROUP A ISOLATES FROM AWASSI SHEEP IN SYRIA

H. AL-HAJ ALI & B. AL BALAA

Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria, Damascus, Syria

Summary

Al-haj Ali, H. & B. Al Balaa, 2021. Characterisation and pathogenicity of *Pasteurella multocida* capsular serogroup A isolates from Awassi sheep in Syria. *Bulg. J. Vet. Med.,* **24**, No 4, 487–496.

The aim of the present study was to evaluate the prevalence of serogroup A of *Pasteurella multocida* in Syrian Awassi sheep. Of 1630 samples collected from nasal swabs of healthy and pneumonic sheep (125 herds) and pneumonic sheep lungs, a total of 228 (13.9%) strains were isolated and identified as *P. multocida* subsp. *multocida* by phenotypic and biochemical characterisation. However, of them only 117 (51.3%) were identified as serogroup A of *P. multocida* when PCR assay with specific primers for serogroup A strains was applied. The highest rate of serogroup A isolation was from apparently healthy sheep (49.6%) with consideration that all lung isolates (23 isolates) belonged to serogroup A. Geographical and seasonal distribution showed that about 60% of positively isolated bacteria originated from Syrian desert (29 isolates) and central parts of semi-arid step zone (41 isolates). A significant increase ($P \leq 0.05$) in the rate of positive isolates was observed in winter as compared to spring. Pathogenicity tests of 10 isolates with 50 or 10 LD_{50} values showed that 5 isolates were able to induce symptoms of fowl cholera in challenge-exposed chickens indicating that migratory Awassi sheep might serve as a carrier for serogroup A of *P. multocida* and that ovine isolates may be virulent for local breed of chickens.

Key words: Awassi sheep, climatic factors, *Pasteurella multocida*, pathogenicity, PCR, serogroup A

INTRODUCTION

P. multocida serogroup A and at a lesser extent, serogroup D are the causative agents of bronchopneumonia and septicaemia in domestic and bighorn sheep (Gilmour & Gilmour, 1989; Biberstein & Hirsh, 1999; Watson & Davis, 2002). As primary and secondary animal pathogens, these organisms have adapted to parasitic life on the oral and upper respiratory epithelia of apparently healthy animals, in certain conditions activating and invading the host tissues to the lungs causing infec-

tion (Harper *et al.*, 2006). In Syria, pasteurellosis is an officially recognised disease in Awassi sheep, the only breed of sheep with population of 19.651 million heads as reported by FAO (FAOSTAT, 2007). However, as in most developing countries, there is lack of reliable animal disease statistics on sheep pasteurellosis that limits the information on prevalence and the impact of the outbreaks on Awassi sheep industry (Younan *et al.*, 1988; Al-haj Ali & Al Balaa, 2018). Diagnosis of sheep pasteurellosis is based on clinical symptoms, standard bacteriological assays, serotyping and DNA based techniques. The methods that rely on bacterial isolation and identification are time-consuming and labour-intensive, what is more, investigations on the specificity of serotyping as diagnostic tool demonstrated that some isolates from ruminants are untypable (Younan *et al.*, 1988; Dziva *et al.*, 2007). In recent years, due to advancements in molecular biology involving DNA hybridisation studies and *16S rRNA* sequencing, taxonomical position of *P. multocida* has been changed within the genus *Pasteurella* (Mutters *et al.*, 2005; Christensen *et al.*, 2005) therefore, prevalence and epidemiological studies related to this organism should be subjected to constant update. Moreover, genotyping methods especially PCR assay allow the detection of microorganisms by dramatically improving the sensitivity and decreasing the time required for bacterial identification. To date, no PCR assay has been used for detection of *P. multocida* in Syria.

Due to the above-mentioned circumstances, the objective of the present study was to evaluate the prevalence of capsular serogroup A of *P. multocida* in Syrian Awassi sheep by identification and characterisation of isolated bacteria from Awassi sheep and their lungs using biochemical analysis and PCR assay. Also, it aimed to verify the potential role of ovine isolates in the outbreak of fowl cholera in local breeds of chickens since most of sheep herds, following a migratory style of life, pass nearby numerous poultry farms that suffer from fowl cholera in Syria.

MATERIALS AND METHODS

Sampling and transportation

The present study was conducted between 2008 and 2012. A total of 1520 nasal swab samples (634 from pneumonic cases and 886 from apparently healthy sheep) were collected from Awassi sheep aged from 3 months to 5 years, representing 125 herds reared in Euphrates Basin (North east), Syrian Desert (East) and central zones (semi-arid steppe zones) of Syria (Fig. 1). In addition, 110 samples were collected from pneumonic lung tissued from slaughterhouses in Hama and Damascus prefectures (Al-haj Ali & Al Balaa, 2018). The sites were selected on the basis of the large sheep population, frequent reports by the Syrian Directorate of Animal Health on respiratory diseases and farmers cooperation. Nasal swabs were placed in sterile test tubes containing 2 mL of transport medium (Amies medium), kept in ice and were then cultured in Erlenmeyer flasks containing brain heart infusion broth (BHI) (Difco) at 37 ºC overnight for further analysis.

Cultural and biochemical identification

The cultural, morphological and biochemical tests were carried out according to standard procedures of Cappuccino & Sherman (2001) and identification of *P. multocida* subsp. *multocida* was perfor-

med according to the characteristics described by Alton & Carter (1990) and Mutters *et al.* (2005). Samples were cultured by direct inoculation on BHI agar containing 5% sheep blood (BHI/blood) and incubation at 37 °C for 18 h. A single colony from each plate was subcultured and examined for *P. multocida*-like colony

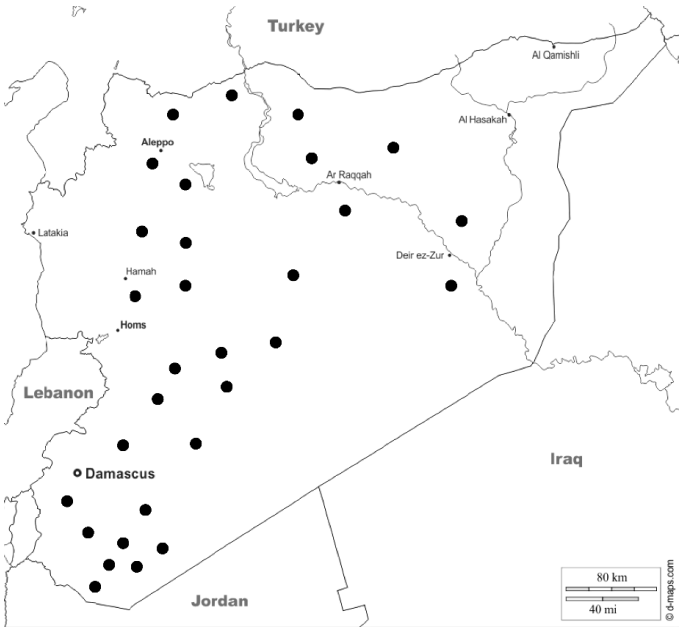

Fig. 1. General map of Syria showing selected sampling sites in the present study.

Table 1. Phenotypic characteristics of identified ovine isolates of *P. multocida* subsp. *multocida* in the present study

Test	*P. multocida* subsp. *multocida*[*]	No of isolates (%)	
		Positive	Negative
Gram staining	–	0	228
Motility	–	0	228
β-haemolysis	–	0	228
Growth on MacConkey agar	–	0	228
Indole	+	228	0
Oxidase	+	228	0
Nitrate reduction	+	228	0
Catalase	+	228	0
Urease	–	0	228
ODC[1]	+	189	39
Lactose	–	19	209
Trehalose	d	47	181
L-arabinose	–	21	207

(+): ≥ 90 % of the strains are positive; (–): ≥ 90 % of the strains are negative; d: different results observed; ODC: Ornithine decarboxylase activity; [*]Alton & Carter (1990) and Mutters *et al.* (2005).

morphology, negative results of blood haemolysis and Gram staining, motility and growth on MacConkey agar. Biochemical analysis and carbohydrate fermentation are listed in Table 1.

PCR assay

The PCR amplification was carried out with specific primers from within genes *hyaC-hyaD* of region 2 cap biosynthetic locus of *P. multocida* (Townsend *et al.*, 2001). A small amount of a single colony grown on BHI/blood agar was picked with a micropipette tip and used as the DNA template in the PCR amplification mixture (25 µL) containing 10 pmol of each forward (CAPA-FWD: 5'- TGC CAA AAT CGC AGT CAG -3') and reverse (CAPA-REV: 5'- TTG CCA TCA TTG TCA GTG -3') primers (Townsend *et al.*, 2001), dNTPMix (0.2 mM of each), 1.5 mM $MgCl_2$, 1× *Taq* buffer and 1.25 U *Taq* DNA polymerase (Fermentas). The cycling conditions were: one cycle of 95 °C for 5 min; 35 cycles (at 95°C for 60 s; at 55 °C for 60 s; at 72 °C for 60 s), then one cycle at 72 °C for 10 min. The reference strain *E. coli* NIHJ was used as negative control and the results of PCR were observed under UV light after electrophoresis in agarose gel pre-stained with ethidium bromide.

Pathogenicity in mouse

Colony forming units (CFU) of each isolate were determined by a serial dilution method. Pathogenicity and 50% lethal dose (LD_{50}) for 10 positive isolates (3 from infected lungs and 7 from pneumonic sheep) were examined in 4–6 week old ddy mice (Al-haj Ali *et al.*, 2004). Briefly, a single colony grown on BHI/blood agar was transferred to BHI broth and incubated at 37 °C for 6 h. The number of bacteria was adjusted to 1×10^6 bacteria/mL and serial 10-fold dilutions of each bacterial suspension were made with BHI broth. A dose of 0.1 mL from each dilution was injected into ddy mice (5/group), intraperitoneally. As negative control group, 5 mice were inoculated with BHI broth. Before injection, 0.1 mL of each dilution was spread on to BHI agar to enumerate the CFU. For determination of LD_{50} mortality rates were recorded daily for 10 days after exposure and the LD_{50} values were determined by the method of Kärber (1931). Liver and spleen of dead mice were collected and cultured on BHI/blood agar for recovery of *P. multocida*. The identity was confirmed by PCR assay described above. Recovered bacteria were used in chickens infection test.

Pathogenicity in chicken

Groups of 25–30-day-old White Leghorn chickens (5/group) (Saidnaya Chicken Establishment, Syria) free from clinical signs of fowl cholera were challenged with approx. 10 or 50 LD_{50} of ovine *P. multocida* serogroup A in BHI broth, orally (Dahi *et al.*, 2002). As negative control, a group of chickens were inoculated with BHI broth. Chickens were kept isolated in plastic cages and observed for clinical signs for 10 days. Survived and died chickens were euthanised and their spleens and livers were subjected for bacteriological examinations and PCR assay. The experimental procedures on mice and chickens and the facilities used to hold the experimental animals were in accordance to the National law (Real Decreto 233/1988, in BOE number 67).

Statistical analysis

Seasonal effect on the rate of bacterial incidence was statistically analysed. The differences between percentages were

evaluated by means of test of proportions using Fisher's exact test.

RESULTS

Based on phenotypic and biochemical characterisation, 228 (14%) isolates were identified as *P. multocida* subsp. *multocida* (205 from nasal swabs and 23 from lungs samples) (Table 1). Colonies of identified isolates appeared small, mucoid with no zone of *β*-haemolysis. The bacteria were Gram negative rods, nonmotile, did not grow on MacConkey's agar, were urease negative, produced indole, oxidase and catalase, and reduced nitrate. The isolates differed in their ability to ferment lactose, trehalose, L-arabinose, and the presence of ODC activity.

The results of PCR assay are shown on Fig. 2. Of 228 identified isolates only 117 (51.3%) were found to give an amplified product of ~1044 bp size using CAP-A primers (94 from nasal swabs and 23 from lungs samples) and thus were considered as serogroup A of *P. multocida*. No bands were observed when the same PCR conditions were applied on *E. coli* NIHJ strain.

The effect of clinical condition of sampled sheep or lungs on the rate of isolation is shown in Table 2. All lungs isolates of *P. multocida* subsp. *multocida* were found to belong to serogroup A of *P. multocida* (100%), followed by apparently healthy animals (49.6%) and the lowest incidence was that from samples of sheep suffering from pneumonia (35.7%). Geo-

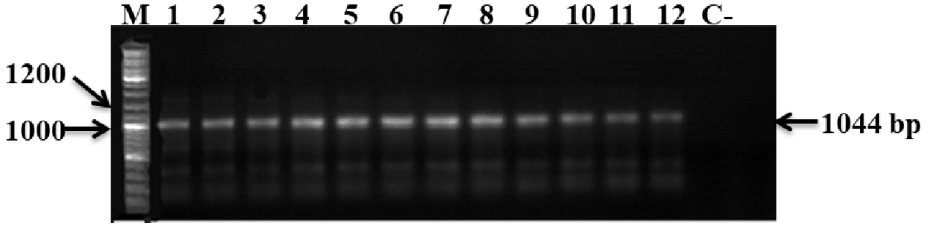

Fig. 2. PCR product of 12 ovine isolates of serogroup A of *P. multocida*using CAP-Aprimers in the present study. M: DNA marker; lanes from 1 to 4: Bacteria isolated from pneumonic lungs; from 5 to 12: Bacteria isolated from nasal swabs of pneumonic cases; C-: reference strain *E. coli* NIHJ with CAP-Aprimers (negative control).

Table 2. Incidence of *P. multocida* subsp. *multocida* and serogroup A of *P. multocida* in respiratory tract of Syrian Awassi sheep

Source	Symptoms	No of samples	Identified bacteria	
			P. multocida subsp. *multocida* (%)[1]	*P. multocida* serogroup A (%)[2]
Nasal swabs	Apparently healthy	886	149 (16.8)	74 (49.6)
	Pneumonic	634	56 (8.8)	20 (35.7)
Lungs	Pneumonic	110	23 (20.9)	23 (100)
Total (%)		1630	228 (13.9)	117 (51.3)

[1]Identification was based on phenotypical and biochemical characterisation; [2]Identification was based on PCR assay with CAP-A primers (Townsend *et al.*, 2001).

Table 3. Seasonal variation in the incidence of ovine serogroup A of *P. multocida* isolation in Syria

Season	Number of samples[1]	No. of positive isolates / year				Total (%)
		2008	2009	2010	2011	
Spring	339	5	4	2	2	13 (3.8)*
Summer	223	4	3	3	5	15 (6.7)
Autumn	482	10	6	9	8	33 (6.8)
Winter	476	13	18	4	6	41 (8.6)*
Total	1520					102 (6.7)

[1]Nasal swabs only; *P<0.05 compared to spring.

Table 4. Pathogenicity of ovine serogroup A of *P. multocida* (10 isolates) for ddy mice and white leghorn chickens

Strain	Source	CFU	LD_{50} in mouse	Infection in chicken[1]	
				10 LD_{50}	50 LD_{50}
Q13	Nasal swab	1.3×10^8	≤ 37	0/5	0/5
Ho10	Lung	3.8×10^9	≤ 88	1/5	2/5
Ho9	Nasal swab	3.5×10^9	≤ 64	1/5	2/5
Ha25	Lung	6.4×10^8	≤ 110	1/5	2/5
Ha13	Lung	1.2×10^9	≤ 26	0/5	0/5
Ha7	Nasal swab	1.7×10^8	≤ 85	0/5	0/5
Rq5	Nasal swab	6.8×10^8	≤ 94	0/5	0/5
Rq6	Nasal swab	4.6×10^8	≤ 122	0/5	0/5
Dz12	Nasal swab	9.8×10^9	≤ 320	1/5	2/5
Dz15	Nasal swab	5.4×10^9	≤ 114	0/5	1/5
Control		–	–	0/5	–

[1] Number of dead/number of injected after 10 days of observation.

graphical distribution of identified *P. multocida* serogroup A over sampled areas revealed that about 60% of positively isolated bacteria originated from Syrian desert (29 isolates) and central parts of semi-arid steppe zone (41 isolates). The role of climatic factors on bacterial incidence is shown in Table 3. Significant increase ($P \leq 0.05$) in the rate of isolated serogroup A of *P. multocida* was observed in winter as compared to spring.

The pathogenicity of serogroup A of ovine *P. multocida* in ddy mice and White Leghorn chickens is demonstrated in Table 4. While infection of mice has led to death and subsequently determination of LD_{50} values, infection of chickens showed variable results; thus only 5 out of 10 isolates (Ho10, Ho9, Ha25, Dz12 and Dz15) induced symptoms of fowl cholera in challenged chickens and 70% of infected chickens died within 5 to 10 days post inoculation. The Gram negative bipolar bacilli were recovered from the livers and spleen of infected chickens only and the identity of recovered bacteria was confirmed by PCR as described above.

DISCUSSION

In the absence of information, the nasal discharge from pneumonic Awassi sheep

was an appropriate choice as sample for isolation of the pathogenic serogroup A of *P. multocida*. It was shown previously that swabs from the naso-pharynx or tonsillar tissue were appropriate specimens for isolating *P. multocida* associated with respiratory infections (De Jong, 1999; Jamaludin *et al.*, 2005). For precise identification and characterisation, cultural and biochemical analysis was applied in combination with genotyping methods with consideration for clinical signs. All the 228 isolates were recognised as *P. multocida* subsp. *multocida* because they matched the properties of *P. multocida* described by Alton & Carter (1990) and Mutters *et al.* (2005) and of them only 117 isolates were confirmed as serogroup A because they gave a positive result by PCR as described by Townsend *et al.* (2001), indicating that other serotypes within isolated *P. multocida* species may be exist. It was shown previously that not only serogroup A, but serogroup D can be involved in infection of sheep with bronchopneumonia (Gilmour & Gilmour, 1989; Biberstein & Hirsh, 1999); moreover, in a previous study 7 isolates of capsular serotype A amongst other *P. multocida* including capsular serotype D, *P. multocida* ssp. *Septica* and *Pasteurella*-like strains were identified from nasal swabs of 15 Awassi flocks as well as samples from 60 pneumonic lungs of dead and slaughtered Awassi sheep collected in Syria by phenotypic and serotyping methods (Younan *et al.*, 1988). The current study both confirms and extends the findings of this earlier study. Identification of *P. multocida* by various techniques and with different rates of isolation from apparently healthy and pneumonic sheep has been reported from various agroclimatic regions such as South Africa (Cameron *et al.*, 1978), Senegal (Doutre & Perreau, 1981), Malaysia (Chandrasekaran *et al.*, 1991), Brazil (Hancock *et al.*, 1991), Mexico (Viera *et al.*, 1993), Turkey (Diker *et al.*, 1994), India (Umesh *et al.*, 1994), and Ethiopia (Deressa *et al.,* 2010). The present PCR method was designed to amplify the *hyaC–hyaD* gene of *P. multocida*. This gene was an ideal amplification target for PCR because hyaluronic acid is a principal component of type A capsule (Chung *et al.*, 1998; Townsend *et al.*, 2001). It is also highly specific for strains genetically capable of producing a serogroup-specific capsule. Moreover, all the isolates used in this PCR system described by Townsend *et al.* (2001) classified as serogroup A by conventional serotyping methods were not affected by their geographical origin (Australia, Vietnam, and the United States) and produced the appropriate amplimer with the serogroup A *cap*-specific primers. The assay described here can be performed with suspected *P. multocida* colonies from primary isolation plates, thus reducing the time required for culture preparation and identification by conventional methods.

The results of serogroup A of *P. multocida* isolation showed that more than 36% of identified bacteria were isolated from clinical cases and infected lungs with consideration that all isolates of lung origin belonged to serogroup A of *P. multocida* (Table 2), which indicating that not only *M. haemolytica* (Al-haj Ali & Al Balaa, 2018) but serogroup A of *P. multocida* may play an important role in the outbreak of pneumonic pasteurellosis in Syrian Awassi sheep. It was shown previously that sheep pasteurellosis due to infection with *P. multocida* manifests in pneumonic and septicaemic forms (Watson & Davis, 2002) and is often encountered in tropical and subtropical regions

than in the temperate regions where it occurs sporadically (Odugbo *et al.*, 2006).

The rate of bacterial incidence was influenced by the climatic variation of sampled areas where significantly increased rate of isolated bacteria occurred in winter compared to spring (Table 3) and slight increase in the number of positive isolates was observed from the samples of Syrian desert. Previous investigations on the prevalence of *M. haemolytica* and *P. multocida* have shown considerable variation in the rate of bacterial isolation worldwide caused by a number of factors including seasonal variation. In addition it was shown that climatic factors such as cold, wind, dust, shortage of rain and drought have significant impact on the development of pneumonic pasteurellosis in sheep and subsequently, on the rate of isolation (Lacasta *et al.*, 2008; Al- haj Ali & Al Balaa, 2018). The last drought hit Syria was between 2007 and 2009 (Erian *et al.*, 2010). Its impact was clear in the eastern and north-eastern parts of the country where the population of Awassi sheep is high. In the present study, experimental infection with ovine *P. multocida* isolates showed that all isolates were pathogenic for ddy mouse and only 5 were able to induce symptoms of fowl cholera in challenge-exposed chickens (Table 4). Moreover, bacterial examination in combination with PCR assay confirmed the identity of recovered bacteria from infected chickens which may be indicated that migratory Awassi sheep might serve as a carrier for serogroup A of *P. multocida* and that ovine isolates are may be virulent for local breed of chickens. These results are important since the majority of Awassi flocks in Syria are migratory. On their migrations from west to east and northern east in the winter, and from east to west and south in the summer, they cover hundreds of kilometers passing by a huge number of chicken farms where fowl cholera is circulating. Previous investigations suggested that wild birds (Christensen *et al.*, 1999), cats and dogs (Baldrias *et al.*, 1988; Ganiere *et al.*, 1993) are asymptomatic carriers and can transmit infection to domestic birds. More research work is required to clarify this point.

In conclusion, the results of the present investigation demonstrated that serogroup A of *P. multocida* is playing an important role in development of bronchopneumonia in Syrian Awassi sheep. Droughts and shortage of rain may lead to enhancement of proliferation of pathogenic *P. multocida* and subsequently, outbreak of the disease. PCR assay on bacterial isolates from ovine samples can significantly reduce the time required for identification of serogroup A of *P. multocida* from related ovine bacterial species. Migratory Awassi sheep herds may serve as carriers for serogroup A of *P. multocida* that lead to the outbreak of fowl cholera in local breed of chickens.

ACKNOWLEDGEMENTS

The authors wish to express their thanks to the general director of AECS Prof. I. Othman for his support and encouragement.

REFERENCES

Al-haj Ali, H. & B. Al Balaa, 2019. Prevalence of *Mannheimia haemolytica* in Syrian Awassi sheep. *Bulgarian Journal of Veterinary Medicine,* **22**, 439–446.

Al-haj Ali, H., T. Sawada & K. Noda, 2004. Protectivity of an immunoaffinity-purified 39 kDa capsular protein of avian *Pasteurellamultocida* in mice. *Journal of Veterinary Medical Science*, **66**, 1603–1604.

Alton, G. G. & G. R. Carter, 1990. Veterinary Diagnostic Bacteriology: A Manual of

Laboratory Procedures for Selected Diseases of Livestock. Rome, FAO.

Baldrias, L., A. J. Frost & D. O'Boyle, 1988. The Isolation of *Pasteurella*-like organisms from the tonsillar region of dogs and cats. *Journal of Small Animal Practice*, **29**, 63–68.

Biberstein, E. L. & D. C. Hirsh, 1999. *Pasteurella*. In: *Veterinary Microbiology*, eds D. C. Hirsh & Y. C. Zee, Blackwell Science Inc., p. 135.

Cameron, C. M., M. M. Engelbrecht & A. S. M. Vermeulen, 1978. The serological and immunological relationships of types A and D of *Pasteurella multocida* to field isolates from sheep. *Onderstepoort Journal of Veterinary Research*, **45**, 215–220.

Cappuccino, J. G. & N. Sherman, 2001. Microbiology: A Laboratory Manual, eds J. G. Cappuccino & N. Sherman, State University of New York, Rockland Community College.

Chandrasekaran, S., K. Hizat, Z. Saad, M. Y. Johara & P. C. Yeap, 1991. Evaluation of combined *Pasteurella* vaccines in control of sheep pneumonia. *British Veterinary Journal*, **147**, 437–443.

Christensen, H., M. Bisgaard, Ø. Angen, W. Frederiksen & J. E. Olsen, 2005. Characterization of sucrose-negative *Pasteurella multocida* variants, including isolates from large-cat bite wounds. *Journal of Clinical Microbiology*, **43**, 259–270.

Christensen, J. P., K. D. Petersen, H. C. Hansen & M. Bisgaard, 1999. Occurrence of fowl cholera in Danish wild birds, and poultry production. Possible connections. *Dansk Veterinaertidsskrift*, **82**, 342–346.

Chung, Y. J., Y. Zhang & B. Adler, 1998. The capsule biosynthetic locus of *Pasteurella multocida* A:1. *FEMS Microbiology Letters*, **166**, 289–296.

Dahi, C., A. Bermin, J. P. Hristensen, M. Bisgaard, A. P. Muhairawa, K. M. D. Petersen, J. S. D. Poulsen & A. L. Jensen, 2002. The effect of concurrent infection with *Pasteurella multocida* and *Ascaridia gallion* free range chickens. *Veterinary Microbiology*, **86**, 313–324.

De Jong, M. F., 1999. Progressive and non-progressive atrophic rhinitis. In: *Diseases of Swine*, 8th edn, eds B. E. Straw, S. D'Allaire, W. L. Mengeling& D. J. Taylor, Iowa State University Press, Ames, Iowa, pp. 355–384.

Deressa, A., Y. Asfaw, B. Lubke, M. W. Kyule, G. Tefera & K. H. Zessin, 2010. Molecular detection of *Pasteurella multocida* and *Mannheimia haemolytica* in sheep respiratory infections in Ethiopia. *The International Journal of Applied Research in Veterinary Medicine*, **8**, 101–108.

Diker, K. S., M. Akan & R. Haziroglu, 1994. Antimicrobial susceptibility of *Pasteurella haemolytica* and *Pasteurella multocida* isolated from pneumonic ovine lungs. *The Veterinary Record*, **134**, 597–598.

Doutre, M. P. & P. Perreau, 1981. *Pasteurella*and *Mycoplasma argininic*arriers in healthy sheep in Senegal. *Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux*, **34**, 365–368.

Dziva, F., A. P. Muhairwa, M. Bisgaard & H. Christensen, 2007. Diagnostic and typing options for investigating diseases associated with *Pasteurella multocida*. *Veterinary Microbiology*, **128**, 1–22.

Erian, W., B. Katlan & O. B. Babah, 2011. Drought vulnerability in the Arab region. Case study- drought in Syria. Ten years of scarce water (2000–2010). Arab Center for the Studies of Arid Zones and Dry Lands (ACSAD) and United Nations, Secretariat of the International Strategy for Disaster Reduction (ISDR).

FAOSTAT, 2007. http://faostat.fao.org/. FAOSTAT © FAO Statistics Division 2007, (Date last accessed 23 August 2007).

Ganiere, J. P., F. Escande, G. Andre & M. Larrat, 1993. Characterization of *Pasteurella* from gingival scrapings of dogs and cats. *Comparative Immunology, Microbiology and Infectious Diseases*, **16**, 77–85.

Gilmour, N. J. L. & J. S. Gilmour, 1989. Pasteurellosis of sheep. In: *Pasteurella and Pasteurellosis*, eds C. Adlam & J. M. Rutter, Academic Press, London, pp. 223–262.

Hancock, R. D., L. C. B. Fallavena & L. A. O. Riberio, 1991. Pneumonic pasteurellosis due to *Pasteurellamultocida*in a flock of lambs in Brazil. *The Veterinary Record*, **128**, 154–155.

Harper, M., J. D. Boyce & B. Adler, 2006. *Pasteurella multocida* pathogenesis: 125 years after Pasteur. *FEMS Microbiology Letters*, **265**, 1–10.

Jamaludin, R., P. J. Blackall, M. F. Hansen, S. Humphrey & M. Styles, 2005. Phenotypic and genotypic characterization of *Pasteurella multocida* isolated from pigs at slaughter in New Zealand. *New Zealand Veterinary Journal*, **53**, 203–207.

Kärber, G., 1931. Beitragzurkollektiven Behand lung pharmakologischer Reihenversuche; *Archiv fur Experimentelle Pathologie und Pharmakologie*, **162**, 480–487.

Lacasta, D., L. M. F. Mayayo, J. J. Ramos & M. D. Heras, 2008. Influence of climatic factors on the development of Pneumonia in lambs. *Small Ruminant Research*, **80**, 28–32.

Mutters, R., H. Christensen & M. Bisgaard, 2005. Genus I. *Pasteurella* Trevisan 1887. 94^{AL} Nom. Cons. Opin. 13, Jud. Comm. 1954b, 153. In: *Bergey's Manual of Systematic Bacteriology*, eds D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity, Springer, New York, pp. 857–866.

Odugbo, M. O., L. E. Odama, J. U. Umoh & A. G. Lamorde, 2006. *Pasteurella multocida* pneumonic infection in sheep: Prevalence, clinical and pathological studies. *Small Ruminant Research*, **66**, 273–277.

Townsend, K. M., J. D. Boyce, J. Y. Chung, A. J., Frost & B. Adler, 2001. Genetic organization of *Pasteurella multocida* cap loci and development of a multiplex capsular PCR typing system. *Journal of Clinical Microbiology*, **39**, 924–929.

Umesh, D., P. S. Lonkar, C. P. Srivastava & P. S. K. Bhagwan, 1994. An outbreak of pneumonia due to *Pasteurella multocida* in sheep. *Indian Veterinary Journal*, **71**, 1163–1167.

Viera, F. J. B., F. J. T. Trigo & L. J. Mexa, 1993. Serotypes of *Pasteurella haemolytica* and *Pasteurella multocida* isolated from ovine and caprine lungs. *Veterinaria México*, **24**, 107–112.

Watson, P. J. & R. L. Davis, 2002. Outbreak of *Pasteurella multocida* septicaemia in neonatal lambs. *The Veterinary Record*, **151**, 420–422.

Younan, M., H. Schmid & E. Hellmann, 1988. Species identification and serotyping (capsular antigen) of *Pasteurella* strains from sheep flocks in South Germany and in Syria. *Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene. Series A: Medical Microbiology, Infectious Diseases, Virology, Parasitology*, **270**, 98–108.

Paper received 31.10.2019; accepted for publication 20.01.2020

Correspondence:

Hussam Al-haj Ali, D.V.M, PhD
Department of Molecular Biology
and Biotechnology,
Atomic Energy Commission of Syria (AECS),
P.O. Box: 6091, Damascus, Syria,
Fax: +963 11 6112289
e-mail : ascientific@aec.org.sy