Archives of Razi Institute, Vol. 77, No. 3 (2022) 1207-1210

DOI: 10.22092/ARI.2022.358759.2303

Original Article

Ovine Pasteurellosis Vaccine: Assessment of the Protective Antibody Titer and Recognition of the Prevailing Serotypes

Qasim, M. T[1], Mahdi Mohammed Alakkam, E[2], Mahdi Mohammed, M[3], Hachim, S. K[4, 5], Sabah Jabr, H[6 *], Emad Izzat, S[7], Mohammed, K. A[8], Hamood, S. A[9], Shnain Ali, M[10], Najd Obaid, F[11], Mohsin Al-Dhalemi, D[12]

1. Department of Anesthesia, College of Health and Medical Technology, Al-Ayen University, Thi-Qar, Iraq
2. Specialist Obstetrician and Gynecologist, Al-Forat General Hospital, Department of Obstetrics and Gynecology, Baghdad, Iraq
3. Al-Manara College FOR Medical Sciences, Maysan, Iraq
4. Medical Laboratory Techniques Department, Al-Farahidi University, Baghdad, Iraq
5. Medical Laboratory Techniques Department, Al-Turath University College, Baghdad, Iraq
6. Anesthesia Techniques Department, Al-Mustaqbal University College, Babylon, Iraq
7. Al-Nisour University College, Baghdad, Iraq
8. Department of Medical Physics, Hilla University College, Babylon, Iraq
9. Al-Esraa University College, Baghdad, Iraq
10. Department of Dentistry, Al-Zahrawi University College, Karbala, Iraq
11. Altoosi University College, Najaf, Iraq
12. The Islamic University, Najaf, Iraq

Received 24 December 2019; Accepted 15 May 2019
Corresponding Author: huda.sabah@mustaqbal-college.edu.iq

Abstract

Sheep husbandry is considered one of the most important activities in the socio-economic development in the Middle East region, especially in Iraq and Islamic Republic of Iran (IRI). Therefore this study was designed to evaluate the level of ovine pasteurellosis vaccine protective antibody titer and identification of the prevailing serotypes in Iraq (Basrah, Baghdad, Tikrit, Mosul, Erbil). The vaccine was made from *pasteurella multosida* Bio-type A and the serotypes of *Mannheimia haemolytica.* This investigation was performed from September 2021 to January 2022, in Iraq. Sheep blood sera samples were obtained from control unvaccinated and vaccinated sheep after 14, 21 and 28 days post vaccination. The results showed that out of 319 sheep blood sera samples which were evaluated using indirect Haemagglutination (IHA) test to detect *Mannheimia haemolytica* serotypes, the high prevalence (100 %) of *M. haemolytica* A2 was found in all the five study regions area, while 96.5 % was *M. haemolytica* A7 and 88.1 % was *M. haemolytica* A1. The level of antibody titer was measured by specific serum antibody titer of *pasteurella multosida* Bio-type A. The results revealed that out of 268 vaccinated blood sera samples the overall antibody titer were 12 (3.8 %), 16 (5%) and 17 (5.3 %) for protective antibody titer of 1:160, 1:80 and 1:40 respectively and for antibody titer of 1:20 were 15 (4.7%) and for antibody titer of 1:10 were 17 (5.3 %), whereas the antibody titer in the control group was 4 (7.8 %). The result of this study indicated that the vaccine administered has limited protective power against *pasteurella multocida* Bio-type A which lead to researchers for further study on identification of specific strain of *pasteurella multosida* and development of multivalent vaccine including the most prevalent *pasteurella* serotypes.

Keywords: Sheep, Ovine Pasteurellosis Vaccine, Serotypes, Antibody Titer

1. Introduction

Sheep husbandry is considered one of the most important activities in the socio-economic development in the Middle East region, especially in Iraq and Islamic Republic of Iran (IRI). In Iraq and IRI sheep plays pivotal role to their national economy. In general sheep supply more than 45% of the domestic meat consumption (1, 2). The importance of sheep husbandry has several aspects between other livestock species. In fact sheep produce several animal products such as meat, dairy products, and wool, also their skin used for leather production. Therefore, sheep husbandry can generate direct cash income from exports of live animal, meat, and skin (3). Due to the traditional breeding and management programs, and prevailing diseases the potential of sheep husbandry has not been efficiently exploited. It is well documented that ovine *pasteurellosis* is one of the most economically significant sheep infectious diseases that warrant control. Due to the high mortality rate, reduction in live weight gain, and poor food conversion ratio, ovine pasteurellosis lead to great economic loss. *Mannheimia haemolytica* and *Pasteurella multocida* are categorized as the most important respiratory pathogens affecting sheep in Iraq (1-3).

Different environmental effectors such as heat stress, overcrowding, violent weather condition, poor housing, and ventilation have been identified as noxious physical and physiological stressors which can trigger ovine pasteurellosis (4). Many commercial vaccines have been developed to control ovine pasteurellosis (5). However, it is scientifically approved that each vaccine induced uncertain levels of protection in response to different bacteria/virus strains in different circumstances.

In spite of vaccination programs against pasteurellosis using killed P. multocida biotype A, there have been reports on the high rate of morbidity and mortality associated with ovine pasteurellosis in Iraq. To the best of the author's knowledge, there have not been previously published studies about the effectiveness of the ovine pasteurellosis vaccine given and identification of the dominant serotypes of pasteurellosis in Iraq.

Therefore, this study was designed to investigate the following issues: 1) identifying prevailing serotype of ovine pasteurellosis in Iraq, 2) to evaluate the effectiveness of ovine *pasteurella* vaccine applied in Iraq in developing protective antibody titer.

2. Materials and Methods

2.1. Animals and General Considerations

The current research was performed in different regions and cities in Iraq (Basrah, Baghdad, Tikrit, Mosul, Erbil).

The animals in this study were Hamdani (n=62, unvaccinated=10), Karadi (n=63, unvaccinated=11), Awassi (63, unvaccinated=10), Naeimi (n=65, unvaccinated=10), and Arabi (n=66, unvaccinated=10) breeds from both sex and within the average age of 7-10 months (45 kg $\leq$ animal weight). Killed pasteurellosis vaccine (HEMOPAST-B, VETAL, Turkey) containing *P. multocida* and *Mannheimia haemolytica* produced by the VETAL veterinary health company, Turkey was used. The vaccine was administered to sheep above six month of age, around lateral cervical vertebrate through sub-cutaneous (SC) route.

2.2. Study Design and Blood Sampling

Blood samples were collected randomly using plain vacutainer tubes in order to identify the prevailing serotype of ovine pasteurellosis and for antibody titer evaluation. Then the blood samples were centrifuged at 3000 rpm for 15 min to separate the serum. Thereafter, the collected serum samples at - 20ºC were transferred to the laboratory for more evaluations as follows. According to the OIE recommendation for sero-typing and antibody titer evaluation the Indirect Haemagglutination (IHA) test was used (6).

2.3. Statistical Analysis

Statistical analysis was conducted with the SAS system software version (version 9.00; Cary, NC, U) for windows and prevalence of ovine pasteurellosis serotype was determined using the frequency procedure

for the chi-square (χ^2) fisher exact test. A value of $P \leq 0.05$ was used as an indicative for statistically significant difference.

3. Results and Discussion

From a total 319 blood sera samples, 100 % *M. haemolytica* serotype A2 was isolated followed by 96.5% and 88.1 % of *M. haemolytica* serotype A7 and *M. haemolytica* serotype A1 respectively there was no statistically significant difference ($P>0.05$) on the proportion of *Mannheimia haemolityca* serotypes. The recorded data showed that most of the investigated animals were positives to all serotype of *M. haemolytica* in each of study area (Table 1). The present study showed high prevalence of *M. haemolytica* serotype A2 followed by A7 which is comparable with previous study reported by Ahmed (7) who showed that the serotype A2 is the most prevalent in Iraq.

Table1. Proportion of *M. haemolitica* serotypes

Study areas	***Mannheimia haemolityca* serotypes**		
	A1 N (%)	**A2 N (%)**	**A7 N (%)**
Basrah	102 (89.5)	114 (100)	111 (97.4)
Baghdad	45 (86.54)	52 (100)	45 (86.54)
Tikrit	46 (93.9)	49 (100)	49 (100)
Mosul	38 (71.7)	53 (100)	53 (100)
Erbil	47 (92.2)	51(100)	49 (96.1)
Total	87.2	100	96.2

The protective level of *pasteurella* vaccine effectiveness were evaluated by measuring specific serum antibody titers produced against *pasteurella multocida* Bio-type A in sheep. The level of protective antibody titer (1: 40 and 1: 20) in the control group was 4 (7.8 %), while after vaccination of a total 268 blood sera samples the overall detection rate were 12 (3.8 %), 16 (5%) and 17 (5.3 %) for protective antibody titers level 1:160, 1:80 and 1:40 respectively and for antibody titer 1:20 and 1:10 were found 15 (4.7%) and 17 (5.3 %) correspondingly (Table 2). Specific antibody titers (1:160, 1:80 and 1:40) in vaccinated animals were higher than the corresponding titers in the control group (1: 40 and 1: 20) as described by Akan, Öncel (8) and Tesfaw, Jenberie (9). The recorded data also revealed that the vaccine induced significant antibody response against *pasteurella multocida* for 45 (16.7%) vaccinated sheep after 14, 21 and 28 days post vaccination over unvaccinated sheep 7.8 % However, the development of antibody was decreased in most of vaccinated sheep over all sampling days.

The vaccinated sera samples from Basrah city showed relatively higher antibody titer than other region in Iraq (table 2) and there was an increase in specific antibody titers on 28 day followed by 21 day post vaccination, compared to 14 day post vaccination for vaccinated sera antibody titers (1:160, 1:80 and 1:40).

The results showed that the low level of antibody titers (1:20 and 1:10) where the rate increase on day 21 post vaccination than 14 day and there was titer decrease on the day 28 for vaccinated sera antibody titers 1:20. Several previous studies investigated the efficacy of vaccination against *P. multocida* and found that the vaccination against *P. multocida* was not effective (10, 11) and argued that the limited protection of specific antibody against *P. multocida* via vaccination was probably, because *P. multocida* strains did not share universal antigens, and many of them proposed that the finding of a new strain with wide spectrum of antigens would help solve this problem.

Despite the identification of the serotype of ovine pasteurellosis was not inclusive of all serotypes, *M. haemolytica* serotype A2, *M. haemolytica* serotype A7 and *M. haemolytica* serotype A1 were identified respectively in order of importance. The vaccine applied against *pasteurella multocida* Bio-type A were found less effective in developing protective antibody, this can be perhaps that the *pasteurella multocida* serotype of the study area may not much with the vaccine applied and yet *M. haemolytica* vaccine is not available in the country. Therefore, the development of a multivalent vaccine using the most prevalent *Pasteurella* serotypes derived from different geographical origins will help to effectively prevent the disease.

Table 2. Overall specific antibody titer in response to *pasteurella multocida* Bio-type A

Titer Level	Basrah %			4 other region %			Control %
	Day 14	Day 21	Day 28	Day 14	Day 21	Day 28	-
1:160	6.52	2.78	9.38	-	5.77	3.77	-
1:80	10.87	11.1	3.13	-	3.85	7.55	-
1:40	4.35	8.33	9.38	2.04	7.69	7.55	1.96
1:20	6.52	5.56	3.13	2.04	11.54	3.77	5.82
1:10	6.52	8.33	3.13	6.12	1.92	11.32	-

Authors' Contribution

Study concept and design: M. T. Q. and S. A. H.
Acquisition of data: E. M. M. A. and M. S. A.
Analysis and interpretation of data: M. M. M.
Drafting of the manuscript: S. K. H.
Critical revision of the manuscript for important intellectual content: H. S. J. and F. N. O.
Statistical analysis: S. E. I.
Administrative, technical, and material support: K. A. M. and D. M. A.

Ethics

Approval for the research study was obtained from the Al-Mustaqbal University College, Babylon, Iraq ethics board.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Alemneh T, Tewodros A. Sheep and goats pasteurellosis: Isolation, identification, biochemical characterization and prevalence determination in Fogera Woreda, Ethiopia. J Cell Anim Biol. 2016;10(4):22-9.
2. Nejiban ZS, Al-Amery MA. Clinical and diagnostic study of sheep Pneumonic pasteurellosis in Basrah, Iraq. Al-Qadisiyah J Vet Med Sci. 2018;17(1):1-5.
3. Radostits OM, Gay CC, Blood DC, Hinchcliff KW, McKenzie R. Clínica Veterinária: um tratado de doenças dos bovinos, ovinos, suínos, caprinos e eqüinos: Guanabara koogan; 2002.
4. Sahay S, Shome R, Sankarasubramanian J, Vishnu US, Prajapati A, Natesan K, et al. Insights into the genome sequence of ovine Pasteurella multocida type A strain associated with pneumonic pasteurellosis. Small Rumin Res. 2018;169:167-75.
5. Wilson BA, Ho M. Pasteurella multocida: from zoonosis to cellular microbiology. Clin Microbiol Rev. 2013;26(3):631-55.
6. Benkirane A, De Alwis M. Haemorrhagic septicaemia, its significance, prevention and control in Asia. Vet Med. 2002;47(8):234-40.
7. Ahmed WA. Evaluation of Cross Protective Efficacy of Commercial Vaccines against Mannheimia haemolytica in Mice: Waffa A. Ahmed, Asmaa H. Abdullah, Ansam K. Mohammed and Roua J. Mohammed. Iraqi J Vet Med. 2019;43(1):165-70.
8. Akan M, Öncel T, Sareyyüpoğlu B, Hazıroğlu R, Tel OY, Cantekin Z. Vaccination studies of lambs against experimental Mannheimia (Pasteurella) haemolytica infection. Small Rumin Res. 2006;65(1-2):44-50.
9. Tesfaw L, Jenberie S, Sori H, Sisay T, Negussie H. Efficacy of Mannheimia haemolytica A2, A7, and A2 and A7 combined expressing iron regulated outer membrane protein as a vaccine against intratracheal challenge exposure in sheep. Afr J Microbiol Res. 2014;8(11):1237-44.
10. Chandrasekaran S, Hizat K, Saad Z, Johara M, Yeap P. Evaluation of combined Pasteurella vaccines in control of sheep pneumonia. Br Vet J. 1991;147(5):437-43.
11. Cameron CM, Bester FJ. The inefficacy of polivalent Pasteurella multocida vaccines for sheep. Onderstepoort J Vet Res. 1983;50(2):101-4.