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RISK FACTORS FOR LAMB AND KID MORTALITY IN SHEEP AND GOAT FARMS IN JORDAN

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Summary

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This study was conducted to identify the risk factors that are associated with neonatal mortality in lambs and kids in Jordan. The bacterial causes of mortality in lambs and kids were investigated. One hundred sheep and goat flocks were selected randomly from different areas of North Jordan at the beginning of the lambing season. The flocks were visited every other week to collect information and to take samples from freshly dead animals. By the end of the lambing season, flocks that had neonatal mortality rate ≥ 1.0% were considered as "case group" while flocks that had neonatal mortality rate less than 1.0% – as "control group". The results indicated that neonatal mortality rate (within 4 weeks of age), in lambs and kids, was 3.2%. However, the early neonatal mortality rate (within 48 hours of age) was 2.01% and represented 62.1% of the neonatal mortalities. The following risk factors were found to be associated with the neonatal mortality in lambs and kids: not separating the neonates from adult animals; not vaccinating dams against infectious diseases (pasteurellosis, colibacillosis and enterotoxemia); walking more than 5 km and starvation-mismothering exposure. The causes of neonatal mortality in lambs and kids were: diarrhea (59.75%), respiratory diseases (13.3%), unknown causes (12.34%), and accident (8.39%). Bacteria responsible for neonatal mortality were: *Escherichia coli, Pasteurella multocida, Clostridium perfringens* and *Staphylococcus aureus*. However, *E. coli* was the most frequent bacterial species identified as cause of neonatal mortality in lambs and kids and represented 63.4% of all bacterial isolates. The *E. coli* isolates belonged to 10 serogroups, the O44 and O26 being the most frequent isolates.

Key words: *E. coli*, Jordan. kids, lambs, neonatal mortality

INTRODUCTION

Perinatal lamb mortality is defined as losses occurring shortly before, during or within a week of birth (Haughey, 1991). It is the major cause in lowering productivity of sheep (Dennis, 1974). In South Africa, the average perinatal lamb mortality was estimated to be 10–12% (Cloete *et al.*, 1993). In Australia, the reported average mortality rates ranged from 5 to 23% (Dennis, 1974). Under normal conditions, the loss in Australia is 5 to 20%, with 80% of these occurring within the first few days of life (Moule, 1954). Neonatal lamb mortality is a complex problem, that may result from a variety of climatic, nutritional, management, infectious, genetic and other factors.

The objectives of the present study were to explore neonatal mortality problem in lambs and kids in Jordan and to identify the associated risk factors.

MATERIALS AND METHODS

Flocks and clinical observations

A total of 80 sheep flocks and 20 goat farms were randomly selected from different areas of Northern Jordan to include the Jordan Valley – 28 flocks, the plain and desert (Irbid & Mafraq) – 48 flocks and the Hill area (Ajlun & Bani-Kananah) – 24 flocks. These flocks were followed up from the beginning of the lambing season – October 2000 until the end of the lambing season – March 2001. The flocks were visited every two weeks to collect information about abortion, live births, neonatal mortality, accompanying clinical signs and management practices.

Bacteriological studies

Isolation of bacteria. All dead lambs and kids were collected and transported in an icebox and submitted for bacteriological examination in the Research Laboratory at the Faculty of Veterinary Medicine, Jordan University of Science and Technology. A total of 55 lambs and kids died at age less than 28 days were tested.

The obtained tissue specimens (spleen, heart, liver and lungs) were immersed in 96% alcohol and immediately flamed to decontaminate their outer surfaces. Small intestine was opened with sterile scissor and forceps and samples were taken from the jejunum-ileum part with lesions. Organ and intestinal samples were cultured on tryptic soy agar (TSA), 7% sheep blood agar (SBA) and MacConkey agar. All of the cultured plates were incubated aerobically and anaerobically by using anaerobic jar and a gasbag at 37° C for 24 to 72 h according to methods described by Quinn *et al.* (1994).

To obtain a pure bacterial culture, a single colony was carefully picked up and subcultured on tryptic soy broth (TSB) and incubated for 1–2 days then subcultured on SBA and TSA plates and incubated for 1–2 days.A stock culture from each isolate was stored at 4°C. For further identification of isolates, different biochemical tests were used according to the methods mentioned in the Clinical Veterinary Microbiology by Quinn *et al.* (1994).

Serotyping of isolated E. coli. Serotyping of O antigen of *E. coli* was carried out by means of technique described by Denka Seiken CO., LTD (Japan), using the available O antigen containing *E. coli* polyvalent sera:

- *E. coli* polyvalent serum 1 (O1, O26, O86a, O111, O119, O127 and O128).
- *E. coli* polyvalent serum 2 (O44, O55, O125, O126, O146 and O166).
- *E. coli* polyvalent serum 3 (O18, O114, O124, O151, O157, O128).
- *E. coli* polyvalent serum 4 (O6, O27, O78, O148, O159 and O 168).

When a positive reaction was observed with one of the polyvalent sera, then a slide agglutination test was done by using monovalent sera comprising the respective polyvalent serum, as described by the company.

Antibiotic susceptibility testing. Bacterial isolates were tested for antibiotic resistance. The Kirby Bauer technique was used. The Müller-Hinton agar medium (OXOID) was prepared in a uniform thickness (4 mm) in 90 mm diameter petri dishes. By using micropipette with sterile yellow tips, the overnight TSB culture was diluted 1:100 to obtain approximately 10^5 CFU/mL.

Paper disks impregnated with the following antibiotics were used: ampicillin, streptomycin, penicillin G, neomycin, gentamicin, bacitracin and cephradine.

Statistical analysis

The information collected in the questionnaire and laboratory results were coded and entered into SPSS computer program. The statistical tests used to analyze the data were chi-square test of independence, odds ratio and multiple logistic regression tests. Flocks that had neonatal mortality rate more than or equal to 1% were considered as "case group" while flocks that had neonatal mortality rate less than 1% were considered as "control group".

RESULTS

Neonatal mortality traits

The total number of sheep and goats in the 100 flocks studied was 18853 before lambing. The flock size ranged from 45 to 1200 animals (188.5±178.8; mean ± standard deviation). The total number of adult females was 14,427. It was found out that 50 sheep and goat flocks had neonatal mortality rate more than 1% and therefore, they were classified as "case group". The other 50 flocks with neonatal mortality rate less than 1% were classified as "control group". Therefore, 50% of sheep and goat flocks in Northern Jordan had a neonatal mortality problem. The overall neonatal mortality rate (within 4 weeks of age) in sheep and goat flocks was 3.2%. However, the early neonatal mortality rate (within 48 hours of age) was 2.01%. This showed that 62.8% of the neonatal mortalities occurred within 48 hours of life (Table 1). The neonatal mortality rate in the case group flocks ranged from 2% to 12% with a mean of 6%. The early neonatal mortality rate of these flocks was 4%. Thus, the early neonatal mortality represented 67% of all neonatal mortalities in the case group flocks. In the control group flocks, the neonatal mortality rate ranged from 0.0% to 0.7% with a mean of 0.4%. The early neonatal mortality rate of these flocks was 0.03%. Thus, early neonatal mortality represented only 7.5% of all neonatal mortalities in the control group flocks (Table 1). The abortion rates were 1.70% and 1.40% in the case group and the control group flocks respectively. The pregnancy rates were 93% and 97% in the case and control groups of flocks respectively (Table 1). There was no significant difference between the abortion or pregnancy rates in the case group and control group flocks ($P > 0.05$) by chi-square test. However, the mean flock size in the case

Table 1. Health indices of sheep and goat flocks in Northern Jordan, 2001

Health indices	Case flocks* (n=50)	Control flocks* (n=50)	Total flocks (n=100)
Flock size (mean)	235 *P*	142	188.53
Early neonatal mortality rate (%) **	4	0.03	2.01
Neonatal mortality rate (%) ***	6	0.4	3.20
Abortion rate (%)	1.7 *NS*	1.4	1.55
Pregnancy rate (%)	93 *NS*	97	95

*case flocks = flocks with neonatal mortality problem; control flocks = flocks without neonatal mortality problems; ** early neonatal mortality = mortality within 48 hours of age ; *** neonatal mortality rate = mortality within 28 days of age; *P* = significant difference ($P < 0.05$), NS= not significant ($P > 0.05$) vs controls.

Table 2. Chi-square test for the association between risk factors and neonatal mortality in lambs and kids in northern Jordan, 2001

Risk factor	Case flocks (n=50)			Control flocks (n=50)			Chi-square	P value
	Present		Absent	Present		Absent		
	n	%	n	n	%	n		
No separation of neonate	38	76	12	14	28	36	23.07	0.000
Walking more than 5 km.	27	54	23	11	22	39	10.86	0.002
Starvation-mismothering	37	74	13	21	42	29	10.51	0.002
No vaccination of pregnant dams *	40	80	10	25	50	25	9.89	0.003
No vaccination of neonates **	32	64	18	19	38	31	6.76	0.016
Truck transportation	22	44	28	11	22	39	5.47	0.033
Flock type*** (sheep or goat)	42	84	8	38	76	12	1	0.454
Using hormone sponge	17	34	33	20	40	30	0.386	0.679

* vaccination against colibacillosis, pasteurellosis and enterotoxemia; ** vaccination against enterotoxemia; *** sheep = present, goat = absent.

group flocks was significantly higher (t-test, $P<0.05$) than the mean flock size of the control group.

There was no association (chi-square test, $P>0.05$) between the type of the flock (sheep or goat) and neonatal mortality problem (Table 2). Therefore, we gathered together the sheep and goat flocks in further statistical tests. According to the analysis by chi-square test, 6 factors were found to be significantly associated with neonatal mortality problem in sheep and goats in this study (Table 2). These factors were:

- No separation of the neonate from other animals (chi-square=23.07, $P<0.05$). This risk factor increased the accidents and the contamination of the environment of neonates.
- Walking more than 5 kilometers (chi-square = 10.87, $P<0.05$). This practice is not uncommon in Jordan. We found out that 54% of the case group flocks and 22% of the control group flocks walk for long distance.
- Starvation-mismothering exposure (SME) (chi-square = 10.5, $P <0.05$).
- No vaccination of dams during pregnancy period (chi-square = 9.89, $P <0.05$). The vaccines, given to pregnant dams, were against colibacillosis, pasteurellosis and enterotoxemia.
- No vaccination of neonates, (chi-square = 6.76, $P <0.05$). The vaccine,

which is given to neonates in Jordan, is against enterotoxemia.

- Crowded truck transportation (chi-square = 5.47, P <0.05). Moving sheep and goat flocks from one place to another is practiced in Jordan due to shortage of pasture and drinking water. It was found that 44% of the case group flocks and 22% of the control group flocks had been transported by truck.

The use of hormone sponges in the case group flocks was not associated with neonatal mortality problem of sheep and goats (chi-square = 0.386, P>0.05). The rate of using hormone sponges was 34% in the case group flocks and 40% in the control group flocks.

The odds ratio for the factors, which are significantly associated with neonatal mortality problem and the 95% confidence interval for the odds ratio are presented in Table 3.

The final step of logistic regression analysis and the significant risk factors associated with neonatal mortality in lambs and kids are presented in Table 4. According to the results obtained by this statistical test, four risk factors were found to be associated with the neonatal mortality in lambs and kids in Northern Jordan. They are: 1. No separation of neonates from adults. 2. Walking more than 5 km. 3. Starvation-mismothering exposure (SME) and 4. Not vaccinating dams against infectious diseases (colibacillosis, pasteurellosis and enterotoxemia) (Table 4).

Causes of mortalities by clinical symptoms

The dominating clinical symptoms, accompanying the cases of neonatal mortalities in lambs and kids, in Northern Jordan, are presented in Table 5. Out of 405 neonatal mortalities of the lambs and kids, 59.75% were associated with diarrhea, 13.33% – with respiratory symptoms,

Table 3. Odds ratio of the risk factors for neonatal mortality in lambs and kids in Northern Jordan, 2001

Risk factors	Case flocks (n=50)			Control flocks (n=50)			Odds ratio (OR)	95% C.I.* for OR	
	Present		Absent	Present		Absent			
	n	%	n	n	%	n		Lower	Upper
No separation of neonate	38	76	12	14	28	36	8.14	5.40	12.8
Walking more than 5 km	27	54	23	11	22	39	4.16	2.83	6.11
Starvation-mismothering	40	80	10	25	50	25	4.0	2.68	5.97
No vaccination of pregnant dam**	37	74	13	21	42	29	3.93	2.73	5.65
No vaccination of neonate ***	32	64	18	19	38	31	2.90	2.07	4.05
Truck transportation	22	44	28	11	22	39	2.78	1.89	4.09

* C.I. = confidence intervals; ** vaccination against colibacillosis, pasteurellosis and enterotoxemia; *** vaccination against enterotoxemia.

Table 4. The final logistic regression of risk factors associated with neonatal mortality of lambs and kids in Northern Jordan, 2001

Risk factors	Odds ratio	Level of significance (P value)	95% Confidence interval	
			Lower	Upper
No separation of neonate	3.6	0.000	1.23	10.8
Walking more than 5 km	4.9	0.004	1.68	14.8
SME*	3.6	0.019	1.23	10.8
No dam vaccination**	2.92	0.040	1.005	8.81

* starvation- mismothering exposure (SME); ** vaccination against colibacillosis, pasteurellosis and enterotoxemia.

Table 5. Clinical symptoms recorded in neonatal mortalities of lambs and kids in Northern Jordan, 2001

Symptoms	Number	%
Diarrhea	242	59.75
Respiratory	54	13.33
Unknown causes	50	12.35
Respiratory symptoms and diarrhea	25	6.17
Various, due to an accident	34	8.40
Total	405	100.00

Table 6. Distribution of 60 pathogenic bacteria isolated from 55 neonatal lambs and kids mortalities in Northern Jordan, 2001

Isolated bacteria	Number	%
E.coli	38	63.4
Pasteurella multocida	14	23.6
Clostridium perfringens	5	8.3
Staphylococcus aureus	3	5.0
Total	60	100.0

8.39% – with accidents and 6.17% – with combination of respiratory symptoms and diarrhea. The cause of 12.34% of lethal cases was unknown and mostly reported as sudden death.

Results from bacteriological analyses

Isolated bacterial species. Laboratory analyses of bacterial pathogens involved in neonatal mortality in lambs and kids are presented in Table 6. From 55 neonatal lambs and kids mortalities, examined bacteriologically, 60 pathogenic bacteria were isolated as followed: *E. coli* – 38 (63.4%), *Pasteurella multocida* – 14 (23.3%), *Clostridium perfringens* – 5 (8.3%) (not been typed), and *Staphylococcus aureus* – 3 (5%) .

Serogroups of E. coli. Out of the 38 *E. coli* isolates, 24 strains were O-

serogroupable and 14 were not typed (NT). The O serogroups identified in descending order were: O44 (5), O26 (5), O125, O144, O126, O142, O111, O128 (2 each) and O119, O55 (1 each).

Resistance to antibiotics. It was found that all strains of *E. coli* (100%) were resistant to penicillin, 95% – to bacitracin, 76% – to tetracycline, 74% – to streptomycin, 50% – to ampicillin, 44% – to neomycin, 35.8% – to cephradine and 8% – to gentamicin. In this study, the 14 isolated strains of *P. multocida* were resistant to bacitracin, 21% – to gentamicin and ampicillin, 14% – to gentamicin and cephradine, 7% – to neomycin and tetracycline and all isolated strains of *P. multocida* were sensitive to penicillin.

DISCUSSION

In Jordan, the neonatal mortality rates of sheep and goats for the years 1990–1998 ranged from 1.4% to 5% with a mean of 3.2% according to the Annual Reports of Veterinary Department, Ministry of Agriculture (Anonymous, 1999). In this study, the observed neonatal mortality rate of lambs and kids of 3.2% was in agreement with the above data. The early neonatal mortality rate (within 48 hours of life) observed by us was 2.01%, and the early neonatal mortalities represented 62.1% of the total neonatal mortalities. The results are similar to those described by Duchet-Suchaux *et al.* (1982) who reported that most lambs' deaths occur within 48 hours of life and they are also in agreement with the study of Dalton *et al.* (1980) who reported that most lambs' deaths occur during the first 3 days of life. Otesile *et al.* (1991) reported that 67.2% of all neonatal deaths occurred during the first week of life. In this study lambs and kids were at higher risk of dying during the first 4 weeks if they were not being separated from adult animals, walking for a distances more than 5 km, if experienced SME, if dams were not vaccinated during pregnancy against *E. coli*, enterotoxemia and pasteurellosis, if the neonates were not vaccinated against enterotoxemia and submitted to crowded truck transportation. These 6 risk factors were identified by chi-square test when tested separately. However, when all potential risk factors were tested together by the logistic regression analysis which identify and discard not truly risk factors, 2 factors were discarded (no vaccination of neonates and exposure to crowded transportation) whereas the other 4 risk factors were confirmed. These results are in agreement with the result of Bekele *et al.*, (1992) who reported that health management interventions as foster mothering, vaccination with polyvalent vaccine against pateurellosis and clostridial infection prevented neonatal lamb mortality. The SME was one of the risk factors associated with neonatal mortality similar to the result described by McFarlane (1961), Dennis (1974). Barlow *et al.* (1987), Gama *et al.* (1991), Bekele *et al.*, (1992), Aldomy (1995) and Mugerwa *et al.* (2000). They found that SME is one of the major causes of deaths in lambs and kids.

Other major causes of deaths in lambs and kids are pneumonia, diarrhea and congenital malformation (Yapi *et al.*, 1990). Neonatal diarrhea in lambs is considered an important cause of lamb mortality Smith *et al.* (1975). In our studies diarrhea was the main sign accompanying the death in about 60% of lambs and kids mortalities. This result is higher than the result reported by Aldomy (1995), who found out that 46.6% of the lambs and kids died within the neonatal period in Jordan had diarrhea. A high percentage of

lambs' deaths caused by diarrhea, but less than reported in this study, was found by Ahmad *et al.* (2000) who reported that 42.86% of lambs showed signs of diarrhea and died later. Also, studies described by Hodgson (1994), Morris and Sojka (1985) reported that diarrhea of lambs is responsible for high rate of mortality in lambs.

In this study, the second important cause of neonatal mortality in lambs and kids were respiratory diseases. This result is in agreement with the findings of Mugerwa *et al.* (2000) who reported that respiratory infections represented 54% of causes for neonatal lamb mortality and with the results of Ndamukong (1985), Njau (1988) and Gama *et al.* (1991). According to their data, the respiratory problem is the major cause of neonatal mortality.

Bacterial pathogens are responsible for high rates of mortality in lambs. Traditionally, *E. coli* has been considered an important cause of diarrhea in lambs (Hodgson, 1994; Morris & Sojka, 1985). In this study *E. coli* was the main bacterial cause of neonatal mortality. The percentage of *E. coli* isolated was 63.4% of all bacterial isolates. This is similar to the result obtained by Fejes *et al.*, (1990), Morris & Sojka (1985) and Hodgson (1994) who reported that *E. coli* has been considered as an important cause of diarrhea in lambs.

Another pathogenic bacteria, which was also associated with neonatal mortality in this study, was *Cl. perfringens* (that had not been typed) which represented 8.3% of the bacterial causes of neonatal mortalities in lambs and kids. A similar result was reported by Aldomy (1995).

Other bacterial cause associated with lambs and kids mortality was *P. multocida* – 23.3% of all bacterial isolates in cases of lamb and kid mortality. This rate is higher than that found out by Aldomy (1995) in Jordan, but similar to the rate determined by Malone *et al.* (1985) – 24.8% of the deaths in lambs. Also, Macleod *et al.*, (1983) reported that *E. coli* and *Pasteurella* spp. are highly responsible for lambs mortality in North Ireland, as in our studies.

The frequency of resistance to antibiotics in *E. coli* (with the exception to penicillin and bacitracin because of the natural resistance to them) and *P. multocida* isolated from domestic animals has been increased markedly due to excessive use of antibiotics. Although neomycin and cephradine are not commonly used in animals, the level of resistance of *E. coli* to these antibiotics was relatively high. This is also indicative of excessive use of antibiotics.

Our results revealed that a high percentage of *E. coli* strains was resistant to the 10 antibiotics tested. Although the number of tested strains was relatively small, the results corresponded to these of Blanco *et al.*, (1996). Also, our findings about the sensitivity of P. *multocida* are similar to the results described by Berman & Hirsh (1978); Fales *et al.* (1982) and Quinn *et al.* (1994).

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