

Dept. of Animal Hygiene and Zoonoses,
Fac. Vet. Med., Alex. Univ.

ROLE OF ENVIRONMENT IN OCCURRENCE OF CALF DIARRHOEA

(With 4 Tables)

By

A.A. DRAZ, M. SAMY* and A. BAUYMI**

*: Dept. of Animal Hygiene, Fac. Vet. Med., Zagazig Univ.

** : Dept. of Animal Hygiene, Fac. Vet. Med., Suez Canal Univ.

دور البيئة في حدوث اسهال العجول

عبد الماجد دراز ، محمود سامي ، أحمد بيومي

تم جمع عدد ١٤٨ عينة براز من عجول سليمة ظاهرياً (٨٠) و عجول مصابة بالإسهال (٦٨)، هواء (٤٥)، مياه (٣٠)، تربة (٣١)، مسحات جلدية (١٤٨) ومسحات من الحلمات (١٤٨) وتم فحصها بكتيريولوجياً. أظهرت النتائج تواجد ميكروب إشيريشيا كولاى والسالمونيلا (٧٠ و ٣,٧% على التوالي) ومن العجول المصابة بالإسهال (٧٩,٤ و ١٠,٣% على التوالي) بالإضافة الى انه تم عزل ميكروب الإشيريشيا كولاى والسالمونيلا من عينات الهواء (١٣,٣ و ٤,٤% على التوالي)، مياه (٧٠ و ٦,٦% على التوالي)، تربة (٧١ و ٢٥,٨% على التوالي)، مسحات جلدية (٥٠,٧ و ١٠,١% على التوالي) ومسحات الحلمات (٢٦,٣ و ٤,٧% على التوالي). هذا وقد تم التعرف على انواع السالمونيلا التي تم عزلها من العجول والبيئة المحيطة سيرولوجياً. كذلك تم عزل بعض البكتيريا السالبة والموجبة الجرام بنسب مختلفه من العجول والبيئة. هذا وقد لوحظ أن مطهر + TH4 له تأثير قاتل على ميكروب اشيريشيا كولاى عند تركيز ٠,٥ و ١% بعد ٢٠ و ١٠ دقائق عند التوالى وكذلك بالنسبة لميكروب السالمونيلا تيفيميوريم بعد ١٠ و ٥ دقائق على التوالي. بالإضافة إلى ذلك فإن الأيودوفور ٢% له تأثير قاتل على ميكروب الأشيريشيا كولاى عند تركيز ١% بعد ٢٠ دقيقة وبالنسبة لميكروب السالمونيلا تيفيميوريم عند تركيز ٠,٥ و ١% بعد ٢٠ و ١٠ دقائق على التوالي. وقد وجد أن البيئة تلعب دوراً كبيراً في الاحتفاظ ونقل الميكروبات المسببة للإسهال في العجول.

SUMMARY

A total of 148 faecal samples (80 from apparently healthy and 68 from diarrhetic calves), air (45), water (30), soil (31), dam coat (148) and teat swabs (148) were collected from a dairy farm and subjected for

bacteriological examination. The obtained results revealed that *E. coli* and Salmonella serotypes could be isolated from apparently healthy (70 and 3.7%) and diarrheic calves (79.4 and 10.3%). In addition, *E. coli* and Salmonellae were recovered from air (13.3 and 4.4%), water (70 and 6.6%), soil (71 and 25.8), dam coat (50.7 and 10.1%) and teat swabs (26.3 and 4.7%). Moreover, serotyping of isolated Salmonellae from calves and environment was carried out. Also, other Gram-negative and Gram-positive bacteria isolated from calves and environment were recorded. On the other hand, it was found that TH4+ is effective against *E. coli* (at concentrations of 0.5 and 1% after contact period of 20 minutes and 10 minutes respectively) and *Sal typhimurium* (at concentration of 0.5 and 1% after 10 and 5 minutes respectively). In addition, iodophor 2% is effective against *E. coli* (at a concentration of 1% after 20 minutes) and *Sal. typhimurium* (at concentrations of 0.5 and 1% after 20 and 10 minutes respectively). It can be concluded that the environment plays a dangerous role in maintaining the infectious agents responsible for diarrhoea in calves.

Keywords: Occurrence, Calf, Diarrhoea.

INTRODUCTION

Environment surrounding calves constitutes the main source of pathogenic and potentially pathogenic microorganisms. Such pathogens are involved as causative agents in calf diarrhea.

Environmental conditions in the intensively confined houses can markedly influence the incidence of the disease that may be higher if large number of animals are allowed to house in unhygienic conditions (Quigley, 1995).

Roy and Twenouth (1972) recorded that the enteric diseases was basically a result of alteration of gastric and intestinal function to less than the optimum and its clinical manifestation as an infectious disease depend on the age of the calf and the balance between its immunological and microbiological environment.

Boylan (1982) reported that under most farm conditions eliminating infectious agents from the environment was difficult, but good management procedures could aid in maintaining a level of environmental contamination that was less than critical.

Infection may be transmitted directly from dams or infected calves or indirectly through contaminated food and water, feeding and watering utensils or a build up of infection in the calf pen (Williams *et al.*, 1975).

In Egypt, several studies have been carried out on environmental pathogens (Abd-El-Karim, 1971; Hamoud *et al.*, 1978; Marzouk *et al.* 1980; Mowafi *et al.* 1980; Bashandy *et al.*, 1983; Mostafa, 1984; Soliman, 1984 and El-Masry, 1989 & 1996).

The aim of this work is to determine the environmental sources with certain pathogens as causative agents for diarrhea in calves and to study the efficacy of two disinfectants on the viability of some bacterial isolates recovered from diarrheic calves to assess preventive measure to avoid transmission of infection.

MATERIAL and METHODS

This study was carried out in a farm belonging to Beheira province. Two hundred and forty cows with 151 calves were included in this farm. Cows were housed in open yard system with dirty floor supplied with a parlour. Calves were kept in a calf pen and grouped by age in three pens. Disposal of manure was carried out mechanically.

2.1. Faecal samples:

A total of 148 faecal samples were collected from apparently healthy (80) and diarrheic calves (68). Each sample was divided into 2 parts, the first part was inoculated into nutrient broth and the second into selenite F broth. After incubation, plating was carried out on the selective media.

2.2. Environmental samples:

2.2.1. Air samples:

Forty five air samples were collected from the investigated cow house using liquid impinger (Brachman *et al.*, 1964). Each sample was inoculated into Selenite F broth and nutrient broth. After incubation, plating was carried out using blood agar, Salmonella Shigella agar and Salt mannitol agar and the plates were incubated at 37 °C for 48 hours.

2.2.2. Water samples:

Thirty drinking water samples each of 200 ml were filtered through 0.45 µm membrane filter. Each membrane was cultured on the previously mentioned media.

2.2.3. Soil samples:

Thirty one soil samples each of 20 gm were collected aseptically by a sterile spatula and transferred to a sterile brown glass bottle and placed in 100 ml sterile distilled water and mixed on a mechanical shaker for one hour and left undisturbed for 10 minutes to allow the large particles to settle. Ten ml of the supernatant were added to 90 ml of nutrient broth and Selenite F broth. After incubation, 0.1 ml from each was taken immediately and plated on previously mentioned agar media.

2.2.4. Dam coat swabs:

One hundred and forty eight skin swabs were collected from dams of apparently healthy (80) and diarrheic calves (68). Sterile swabs moistened with sterile broth were rubbed on the skin of hind quarters and perineal region.

2.2.5. Teat swabs:

One hundred and forty eight teat swabs were collected from dams of apparently healthy (80) and diarrhea calves (68) according to Rendos *et al.* (1975). Coat and teat swabs were inoculated into sterile test tubes containing nutrient broth and plated on previously mentioned agar media.

2.3. Identification of bacteria was carried out according to Edward and Ewing (1972) and Murry *et al.* (1984).

2.4. Serological identification of isolated *Salmonella* spp. was carried out according to Kauffmann (1978).

2.5. Efficacy of TH4+ and Iodophor on the viability of *E. coli* and *Salmonella typhimurium*:

The bactericidal effects of TH4+ (composed of Quaternary ammonium compounds and Glutaraldehyde as well as plant extracts) and Iodophor 2 % were assessed at on *E. coli* and *Salmonella typhimurium* concentrations of 0.5 and 1 % after contact periods of 5, 10 and 20 minutes.

RESULTS and DISCUSSION

Table (1) revealed that *E. coli* was isolated from faecal matter of apparently healthy calves at percentage of 70 %. This indicates that *E. coli* is considered to be a normal inhabitant of the intestinal tract. Under stress factors the organisms becomes pathogenic causing calf diarrhea. A finding is supported by Amstutz (1965) who reported that feeding and management errors were frequently associated with calf diarrhea.

In addition, *E. coli* could be isolated from diarrheic calves at percentage of 79.4% (Table 1). This result is supported by that reported by Anon (1964), Soliman (1984), El-Masry (1989), Hozain (1990), Hafiz et al. (1994), Salib (1995) and El-Masry (1996).

E. coli was recovered from air, water and soil of calf pen at percentage of 13.3, 70 and 71 % respectively (Table 2). Moreover, *E. coli* could be isolated from dam coat and teat swabs at percentage of 50.7 and 26.3 % respectively (Table 2). These results are supported by those Johansen (1972), Fiser and Svtasvsky (1973), Rendos et al. (1975), Eberhart (1977), Mowafi et al. (1980), Zakarya et al. (1980), Bashandy et al. (1983), Mostafa (1984), Soliman (1984), Asma et al. (1996), El-Masry (1989 & 1996).

The difference in isolation rate of *E. coli* may be attributed to environmental and managerial conditions (build up of infection in calf house, the farm dirt and the adverse climatic changes) as reported by Acres (1985) and Hinton et al. (1994).

Salmonellae were isolated from apparently healthy and diarrheic calves at percentage of 3.7 and 10.3% respectively (Table 1). In addition, Salmonellae could be recovered from air, water, soil, dam coat and teat swabs at percentage of 4.4, 6.6, 25.8, 10.1 and 4.7 % respectively (Table 2). Similarly, Salmonellae could be isolated from calves and environment by Abd-El-Karim (1971), Osborne et al. (1977), Bulgin et al. (1982), El-Sayed (1987), Mettias (1987), Saad (1993), Asma et al. (1996) and El-Masry (1996).

Serological identification of Salmonellae isolated from calves and environment revealed that *Sal. typhimurium* could be isolated from apparently healthy and diarrheic calves at percentage of 2.9 and 9.7% respectively. Also, *Sal. enteritidis* was isolated from diarrheic calves at a percentage of 2.4% and *Sal. dublin* was recovered from apparently healthy and diarrheic calves at percentage of 1.4 and 4.8% respectively. In addition, *Sal. typhimurium* was isolated from air, water, dam coat and teat swabs at percentage of 2.2, 6.6, 12.9, 3.4 and 2% respectively. The respective values for *Sal. enteritidis* were 0.0, 0.0, 6.4, 2.7 and 0.7%. Moreover, *Sal. dublin* and *Sal. newport* were recovered from dam coat (3.2 and 3.2%) and teat swabs (1.3 and 0.7%), Table (3).

It was found that *Sal. typhimurium* was the most common serotype isolated from calves and environment causing diarrhea in calves as reported by Hurd et al. (1994). The survival of infective agents, the presence of rats and mice and the management practices may change the

balance between the calf and its microbial flora especially *Salmonella* as recorded by Watts and Wall (1952), Abd-El-Karim (1971), Williams et al. (1975) and Roy (1980).

The results recorded in Table (1) revealed isolation of *Klebsiella pneumoniae* (5 and 11.8%), *Pseudomonas aeruginosa* (6.2 and 10.3%), *Shigella* (1.2 and 0.0%), *Proteus* spp. (8.7 and 19.1%), *Arizona* (2.5 and 0.0%), *Citrobacter* (2.5 and 1.5%), *Enterobacter* (2.5 and 1.5%), *Staph. aureus* (6.24 and 13.2%) and *Strept. faecalis* (2.5 and 7.3%) from healthy and diarrheic calves respectively. These results are coinciding with those reported by El-Amrousi et al. (1972), Ahmed (1975), Farid et al. (1979), Mettias (1987), Mnatsakanov et al. (1991), Salib (1995) and El-Masry (1996).

Results presented in Table (2) showed that *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Shigella*, *Proteus* spp., *Arizona*, *Citrobacter*, *Enterobacter*, *Providencia*, *Staph aureus* and *Strept. faecalis* could be isolated from air, water, soil, dam coat and teat swabs of examined animals at various percentages. These results are supported by those obtained by Abd-El-Karim (1971), Brander (1973), Knittel (1975), Rendos et al. (1975), Mowafi et al. (1980), Marzouk et al. (1980), Zakarya et al. (1980), Soliman (1984) and El-Masry (1989 & 1996). Such organisms may have a role in inducing enteritis in calves under certain circumstances (Topley and Wilson, 1975; Buxton and Fraser, 1977).

The results indicated that TH4+ is effective against *E. coli* (at concentrations of 0.5 and 1% after contact period of 20 and 10 minutes respectively) and *Sal. typhimurium* (at concentrations of 0.5 and 1% after 10 and 5 minutes respectively). In addition, iodophor 2% is effective against *E. coli* (at concentration of 1% after 20 minutes) and *Sal. typhimurium* (at concentrations of 0.5 and 1% after 20 and 10 minutes respectively), Table (4).

From the previous results, it can be concluded that the environment plays a dangerous role in maintaining the infectious agents responsible for diarrhea in calves especially *E. coli* and *Salmonella*. So, to avoid infection by environmental pathogens, good hygienic measures should be applied including adequate ventilation, proper housing, hygienic disposal of sewage, thorough cleaning and disinfection of calf houses as well as proper management of calves and dams.

REFERENCES

- Abd-El-Karim, A.M. (1971): "The incidence of animal infections in the soil of animal dwellings." Egypt. Vet. J., Fac. Vet. Med., Cairo Univ., Vol. XIX No. 19: 23-44.*
- Acres, S.D. (1985): "Enterotoxigenic Escherichia coli infection in newborn calves." J. Dairy Sci., 68(1): 229-256.*
- Ahmed, S.A. (1975): "Studies on bacterial flora of scouring newborn buffalo-calves in Egypt." Ph.D. Thesis, Fac. Vet. Med., Cairo Univ.*
- Amstutz, H.E. (1965): "Occurrence and etiology of infectious calf diarrhoea." J.V.A.M., 146(12): 1360-1363.*
- Anon (1964): "Veterinary investigation service. An analysis of the results of postmortem examination of calves." Vet. Rec., 72: 1240-1247.*
- Asma, O.A.; Abd-El-Wahed, Z.H.; Kohli, K.H. and El-Sheikh, A.R. (1996): "Some studies on clinical, hematological and biochemical changes in diarrheic neonatal buffalo calves with reference to hygienic conditions." Assiut Vet. Med. J., 35(69): 91-101.*
- Bashandy, E.Y.; Badawy, E.M. and El-Agrab, H.M. (1983): "The effect of the in vitro disinfection on the microbial density of water in poultry farms." Alex. J. Vet. Sci., 1(1): 167-172.*
- Boylan, C.G. (1982): "Protecting newborn calves from Escherichia coli scours." Vet. Med. Small Anim. Clin. P. 807-809.*
- Brachman, P.S.; Ehrlich, R.; Eichenwald, H.F.; Gabelli, V.J.; Kethly, T.W.; Madin, S.H.; Maltman, J.R.; Middlebrook, G.; Morton, J.D.; Silver, I.H. and Wolfe, E.K. (1964): "Standard sampler for assay of airborne microorganisms." Science, 144: 1295.*
- Brander, G.C. (1973): "Dairy herd environment and the control of mastitis." Vet. Rec., 92(12): 501-506.*
- Bulgin, M.S.; Anderson, B.C.; Ward, A.C.S. and Evermann, J.F. (1982): "Infectious agents associated with neonatal calf disease in south western Idaho and eastern Oregon." J. Am. Vet. Med. Assoc., 180(10): 1222-1226.*
- Buxton, A. and Fraser, G. (1977): "Animal microbiology." Vol. I. Immunology, bacteriology, mycology, diseases of fish and laboratory methods. Blackwell Scientific Publications.*
- Eberhart, R.J. (1977): "Proceedings of Seminar in Mastitis Control." International Dairy Federation, Document No. 85.*

- Edward, P.R. and Ewing, W.H. (1972): "Identification of Enterobacteriaceae." 3rd Ed. Burgeys Publishing Co. Minneapolis.*
- El-Amrousi, S.; Nafie, E.K.; El-Rehewi, M. and Abdel-Mottilb (1972): "Studies on enteritis in buffalo-calves in Assiut." J.Egypt. Vet. Med. Assoc., 31: 219-225.*
- El-Masry, M.A.L. (1989): "Dairy house hygiene in relation to incidence of mastitis." M.V.Sc. Fac. Vet. Med., Zagazig Univ.*
- El-Masry, M.A.L. (1996): "Some epidemiological studies on diarrhoea in calves." Ph.D., Fac. Vet. Med., Moshtohor, Zagazig Univ., Benha Branch.*
- El-Sayed, A.S.A. (1987): "The role of Enterobacteriaceae group in enteritis of buffalo calves." M.V.Sc. Thesis, Fac. Vet. Med., Cairo Univ.*
- Farid, A.F.; Lotfi, Z.S. and Abd-El-Malek, A.S. (1979): "Some observations on biochemical behaviour of Coliform cultures." J. Egypt. Vet. Med. Assoc., 39(2): 127-131.*
- Fiser, A. and Svitasvsky, K. (1973): "Occurrence of haemolytic strains of *E. coli* and Staphylococci in the air of large premises for pigs and dairy cows in the course of year." *Wissenschaftliche Zeitschriftliche Reiche*, 22(5): 508-514.*
- Hafiz, M.A.H.; Khan, A.; Khan, M.Z.; Sabri, M.A. and Naz., N.A. (1994): "Bacteriology of neonatal calf diarrhoea in buffaloes and cattle." *Buffalo J.*, 10(2): 177-183.*
- Hamoud, M.M.; Metwally, M.A.; Shouman, M.T. and El-Nassan, A.A. (1978): "Viability of some important animal pathogens in different types of soli." *Egypt. Vet. Med. J.*, XXVI(26): 139-148.*
- Hinton, M.; Allen, V. and Linton, A.H. (1994): "The effect of the management of calves on the prevalence of antibiotic-resistant strains of *Escherichia coli* in their faeces." *Letters in Appl. Microbiol.*, 19(4): 197-200.*
- Hozain, S.H.S. (1990): "Studies on some disease problems in buffalo-calves fed on milk replacers in Egypt." M.V.Sc. Thesis, Fac. Vet. Med., Cairo Univ.*
- Hurd, H.S.; Hancock, D.D.; Thomas, L.A. and Wells, S.J. (1994): "Salmonella prevalence in U.S. dairy calves and a comparison of active and passive surveillance systems." *Intern. Society for Anim. Hyg.*, FS30 - FS33.*

- Johansen, E. (1972): "Sensitivity to antibiotics of Coliform bacteria from mastitis and the environment." Vet. Bull., 43(8).*
- Kauffmann, F. (1978): "Kauffmann-white scheme." WHO BO/27, 1, Rev. 2.*
- Knittel, M.D. (1975): "Occurrence of *Klebsiella pneumoniae* in surface waters." Appl. Microbiol., 29: 595-597.*
- Marzouk, M.A.; Mowafi, L.A. and Zakarya, A.H. (1980): "Studies in commensal microorganisms inhabiting bovine skin in Sharkia Governorate." J. Egypt. Vet. Med. Assoc., 40(1): 2-10.*
- Mettias, K.N. (1987): "Studies on enteritis in buffalo calves and its control by application of some vaccines." Ph.D. Thesis, Fac. Vet. Med., Cairo Univ.*
- Mnatsakanov, S.T.; Mezhlumyan, A.A. and Vartanyan, G.G. (1991): "Adhesion of Enterobacteriaceae isolated from cattle farms." Biologicheski Zhurnal Armenii, 41(2): 151-154.*
- Mostafa, L.A.R. (1984): "Studies on hygienic conditions in some dairy farms." Ph.D. Thesis, Fac. Vet. Med., Cairo Univ.*
- Mowafi, L.E.; Marzouk, M.A.; Zakarya, A.H. and El-Olemy, G. (1980): "Soil as a reservoir of some pathogenic agents in Sharkia Governorate." J. Egypt. Vet. Med. Assoc., 40(2): 1-2.*
- Murry, R.G.E.; Brenner, D.J.; Bryant, M.P.; Holt, J.G.; Krieg, N.R.; Moulder, J.M.; Norbert, P.H.A.; Staky, J.T.; Lapage, S.P.; Hans Lautro, P.; John Liston and Niven, C.V. (1984): "Bergeys Manual of Systemic Bacteriology." 8th Ed., Williams and Wilkins Co., Baltimore.*
- Osborne, A.D.; Linton, A.H.; Pearson, H.I. and Shimeld, C. (1977): "Epidemiology of Salmonella infection in calves." The source of calfhood infection by *Salmonella dublin*." Vet. Rec., 101(26-27): 513-516.*
- Quigley, J.D.; Martin, K.R.; Bemis, D.A.; Potgieter, L.N.D.; Reinemeyer, C.R.; Dowlen, H.H. and Lamar, K.C. (1995): "Effects of housing and colostrum feeding on serum immunoglobulins, growth and faecal scours of Jersey calves." J. Dairy Sci., 78: 893-901.*
- Rendos, J.J.; Eberhart, R.J. and Kesler, E.M. (1975): "Microbial populations of teat ends of dairy cows and bedding materials." J. Dairy Sci., 58(10): 1492-1500.*
- Roy, J.H.B. (1980): "Factors affecting susceptibility of calves to disease." J. Dairy Sci., 63(4): 650-654.*

- Roy, J.H.B. and Twenouth, J.H. (1972): "Nutrition and enteric disease in calves." In Proceedings of the Nutrition Society, 31(1): 53-60.
- Saad, M.K. (1993): "Salmonellosis in newborn calves in a closed dairy farm." Vet. Med. J. Giza, 41(1): 43-45.
- Salib, O.R. (1995): "Studies on colibacillosis vaccines in calves." Ph.D. Thesis, Fac. Vet. Med., Cairo Univ.
- Soliman, A.M. (1984): "Hygienic studies on neonatal mortalities in calves." Ph.D. Thesis, Fac. Vet. Med., Cairo Univ.
- Topley, W.C. and Wilson, G.S. (1975): "Principles of bacteriology, virology and immunity." 6th Ed. Vol. II, Baltimore, The Williams and Wilkins.
- Watts, P.S. and Wall, M. (1952): Aust. Vet. J., 28: 165-168 (Cited after Ashoub, 1991: "Viability of some animal non-viral infective agents of veterinary health importance in water used for livestock." Ph.D. Thesis, Fac. Vet. Med., Moshtohor, Benha Branch, Zagazig Univ.).
- Williams, M.R.; Spooner, R.L. and Thomas, L.H. (1975): "Quantitative studies on bovine immunoglobulins." Vet. Rec., 96: 81-84.
- Zakarya, A.H.; Marzouk, M.A. and Mowafi, L.E. (1980): "Studies on the air pollution in animal dwellings in Sharkia Governorate." J. Egypt. Vet. Med. Assoc., 40: 2-9.

Table (1): Bacteria isolated from fecal samples of examined calves.

Isolates	Apparantly healthy calves		Diarrheic calves	
	+ ve	%	+ ve	%
Gram negative				
- <i>E. coli</i>	56	70	54	79.4
- Salmonella	3	3.7	7	10.3
- <i>Klebsiella pneumoniae</i>	4	5	8	11.8
- <i>Pseudomonas aeruginosa</i>	5	6.2	7	10.3
- Shigella spp.	1	1.2	--	0.0
- Proteus spp.	7	8.7	13	0.0
- Arizona spp.	2	2.5	--	0.0
- Citrobacter	2	2.5	1	1.5
- Enterobacter	2	2.5	1	1.5
Gram positive				
- <i>Staphylococcus aureus</i>	5	6.2	9	13.2
- <i>Streptococcus faecalis</i>	2	2.5	5	7.3

No. of examined apparantly healthy calves = 80.

No. of examined diarrheic calves = 68.

Table (2): Bacteria isolated from environment of calves, dam coat and teat swabs:

Isolates	Air		Water		Soil		Dam coat		Teat swab	
	+ ve	%	+ ve	%	+ ve	%	+ ve	%	+ ve	%
Gram negative										
- <i>E. coli</i>	6	13.3	21	70	22	71	75	50.7	39	26.3
- <i>Salmonella</i>	2	4.4	2	6.6	8	25.8	15	10.1	7	4.7
- <i>Klebsiella pneumoniae</i>	2	4.4	6	20	3	9.7	14	9.4	8	5.4
- <i>Pseudomonas aeruginosa</i>	5	11.1	3	10	6	19.3	15	10.1	10	6.7
- <i>Shigella</i> spp.	--	0.0	1	3.3	1	3.2	--	0.0	--	0.0
- <i>Proteus</i> spp.	8	17.8	6	20	9	29	2	1.3	11	7.4
- <i>Arizona</i> spp.	--	0.0	1	3.3	1	3.2	--	0.0	--	0.0
- <i>Citrobacter</i>	--	0.0	4	13.3	1	3.2	6	4	2	1.3
- <i>Enterobacter</i>	1	2.2	3	10	2	6.4	5	3.4	4	2.7
- <i>Providencia</i>	1	2.2	3	10	2	6.4	--	0.0	4	2.7
Gram positive										
- <i>Staphylococcus aureus</i>	--	0.0	3	10	9	29	4	2.7	2	1.3
- <i>Streptococcus faecalis</i>	2	4.4	4	13.3	11	35.5	7	4.7	6	4

No. of examined air samples = 45

No. of examined water samples = 30

No. of examined soil samples = 31

No. of examined dam coat samples = 148

No. of examined teat swab samples = 148

Table (3): Salmonella serotypes isolated from calves, environment, dam coat and teat swabs:

Salmonella serotypes	Apparently healthy calves		Diarrheic calves		Air		Water		Soil		Dam coat		Teat swab	
	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%
<i>Salmonella typhimurium</i>	2	2.9	4	9.7	1	2.2	2	6.6	4	12.9	5	3.4	3	2
<i>Salmonella enteritidis</i>	--	0.0	1	2.4	--	0.0	--	0.0	2	6.4	4	2.7	1	0.7
<i>Salmonella dublin</i>	1	1.4	2	4.8	--	0.0	--	0.0	1	3.2	3	2	2	1.3
<i>Salmonella newport</i>	--	0.0	--	0.0	--	0.0	--	0.0	1	3.2	3	2	1	0.7

Table (4): Efficacy of TH4+ and Iodophor against *E. coli* and *Salmonella typhimurium*:

Disinfectants	Concentration of disinfectant	<i>E. coli</i>						<i>Salmonella typhimurium</i>					
		5 min.		10 min.		20 min.		5 min.		10 min.		20 min.	
TH4+	0.5 %	+	+	-	-	-	-	+	-	-	-	-	-
	1 %	+	-	-	-	-	-	-	-	-	-	-	-
Iodophor	0.5 %	+	+	+	+	+	+	+	+	+	+	+	-
	1 %	+	+	+	+	+	+	+	+	+	+	+	-