Eco-Epidemiologic Aspects Of *Mannheimia Haemolytica In Egypt*

H.A. Kaoud[1], A.R. El-Dahshan, Manal.M. Zaki[1], [1] Shaaima [1] .A. Nasr[1]

Department of Veterinary Hygiene, Environmental Pollution and Management, Faculty of Veterinary Medicine, Cairo University, Giza 11221, Egypt. ka-oud@link.net

Abstract: The aim of this article was a trial to declare of the ecology of *Mannheimia haemolytica* (*P. haemolytica*) among farm animals in Egypt through the following :(1) Checking up the presumptive role of some environmental components in the indirect transmission of the infection. (2) Investigating survival and viability of the organism under simulated environmental conditions. Nasal swabs were collected from animals showed respiratory manifestations (cattle, buffaloes, sheep and goats) in some farms in different Governorates of Egypt. Soil and Air samples from animal houses were also obtained (A total of 435 samples). Results pointed out that; 27 isolates were recorded from 265 (10.18%) nasal swabs of herds manifested respiratory sings respiratory. 17 isolates were recorded from 100 examined air samples (17%) and 4 isolates were obtained from 70 examined soil samples (5.70%). There was a correlation between *Mannheimia. haemolytica* isolation from the nasal cavities and the dust content of the air (r =0.72) but it is not significant *(P < 0.05).* In the same time, there was a highly significant correlation between dust in the air and the respiratory manifestations affecting the animals (r = 0.82, *(P< 0.05).* It seems possible to conclude that the organism is not capable of surviving outside the animal's body for long time. *M. haemolytica* survived relatively longer in faeces than in tap-water under different incubation temperatures. There is a high significant correlation *(P < 0.05)* between the survival period of the organism and the soil moisture content especially in case of natural soil incubated at outdoor temperature.

[H.A. Kaoud, A.R. El-Dahshan, Manal. M. Zaki, Shaaima. A. Nasr. **Eco-Epidemiologic Aspects Of *Mannheimia Haemolytica In Egypt.*** Researcher. 2011;3(2):43-50]. (ISSN: 1553-9865).

Keywords: Eco-Epidemiologic - *Mannheimia haemolytica* (*P. haemolytica*)- respiratory manifestations.

1. Introduction

Mannheimia haemolytica is a well-known worldwide pathogen of ruminants and is considering a commensal of respiratory system of ruminants but it also can infect animals and causing bovine and ovine pneumonic pasteurellosis which is responsible for considerable economic losses to the cattle and sheep industries (Gilmour & Gilmour; 1989, Bowland & Shewen; 2000*).* Most species of *Mannheimia* are known as opportunistic pathogens (Al-Tarazi and Dagnall, 1997) and are frequently isolated from asymptomatic carriers (Gilmour et al; 1974, Bisgaard; 1993). In sheep, disease caused by *Mannheimia* species has mainly included pneumonia and septicaemia (Bisgaard; 1993).

Mannheimia haemolytica (Pasteurella haemolytica) has been recognized as the principal cause of death in case of pneumonic pasteurellosis affecting cattle, sheep and goats, and septicaemic pasteurellosis in sheep and goats (Jubb et al, 1993).

Respiratory disorders in animal production units in Egypt were reported to cause considerable losses due to lower productivity and severe deaths (Abdel Ghani et al., 1990; El- Battrawy, 1991 and Ismael et al., 1993), but the eco-epidemiologic role of *Mannheimia haemolytica* and its relative importance is still equivocal.

It is generally accepted that the principal source of infection is an endogenous one, therefore all the research was directed towards pathogenicity and immunity to *M. haemolytica*. Although a lot of success has been achieved, the organism still causes heavy losses to the livestock industry.

There are very few reports dealing with the survival and resistance of *M. haemolytica* outside the host's body. Mannheimia was extremely fragile when present in the air and survived best at high humidity and warm temperature (Gilmour et al, 1989). The aim of this article is a trial to declare of the ecology of *M. haemolytica* among farm animals in Egypt through the following: (1) Checking up the presumptive role of some environmental components in the indirect transmission of the infection. (2) Investigating survival and viability of the organism under simulated environmental conditions.

2. Material and Methods

Materials

Nasal swabs were collected from animals showed respiratory manifestations (cattle, buffaloes, sheep and goats) in some farms in different Governorates of Egypt. Soil and Air samples from animal houses were also obtained (A total of 435 samples).

Sampling techniques

Nasal Swabs

Cotton-tipped, 15 cm long, sterile swabs (COPAN, ITALEE) were used. With the attempt to avoid picking contamination from the external nares, the swab was carefully inserted into each nostril, and then placed into transport media (brain heart infusion broth culture). The swabs were kept in an ice-box and taken to the laboratory.

Soil samples

A composite soil sample of approximately 1 kg was collected from each animal shed by taking numerous small samples from different sites of the floor. The samples were collected by means of shovels and placed into plastic bags. Two to five composite samples were collected from each farm or animal holding pen according to the method described by Zuberer (1994).

Air samples for dust estimation

Sample of Air within the animal houses were obtained by sucking air from different sites at the animal level, using 50 ml syringes in which a thin layer of non-absorbent cotton was placed to cover the head of the plunger and act as a filter.

Methods

Isolation of the organism

The swabs were removed from the transport media and inoculated onto nonselective bovine blood agar containing antibiotics (Becton-Dickinson) that inhibit growth of *non-Pasteurellaceae* (Jaworski *et al.*, 1993). Plates were incubated at 37 C in 5–10% carbon dioxide atmosphere and inspected after 24 and 48 hr of incubation. Based on morphology, suspect colonies were chosen for further identification. Bacterial isolates identified as *P. trehalosi* and *M. haemolytica* were evaluated for the presence of hemolysis on blood agar and were classified using biogrouping (Bisgaard and Mutters, 1986; Bisgaard *et al.*, 1986) and biovariant systems (Jaworski *et al.*, 1998).

Biochemical activity

All strains of *Mannheimia* ferment mannitol, glucose, maltose, sorbitol, and sucrose without gas production. Indole, urease, methyl blue (MB) and Voges-Proskauer (VP) reactions are negative. Catalase (almost always) and oxidase are positive. Typically they do not ferment trehalose. *Mannheimia* can be separated from genus *Pasteurella* by beeing not producing acid from D-mannose, from genus *Actinobacillus* (almost) by being urease negative, from genus *Haemophilus* by being mannitol positive and from genus *Lonepinella* by being VP negative (Angen *et al*., 2002).

Table1: Phenotypic characters separating existing species of *Mannheimia*

Characteristics	1.*M.haemolytica*	2.*M.glucosida*	3.*M.granulomatis*	4.*M.ruminalis*	5.*M.varigena*
B-Hemolysis(bovine blood)	+	+	_	_	+
Ornithine decarboxylase	_	d	_	_	d
L(+)Arabinose	_		_	_	+
D(+)Xylose	+	+	d	_	+
Meso-Inositol	d	+	d	_	d
D(-)Sorbitol	+	+	+		_
L(+)Rhammnose	_	_	_	_	d[b]
B-Glucosidase(NPG)	_	+	+	_	_b
Glycosides[c]	_	d	d	_	_b
α-Fucosidase	+	+	_		_b
B-Xylosidase(ONPX)	_	d	d	_	_

+, 90% or more of the strains positive within 1-2 days; (+), 90% or more of the strains positive within 3-14 days; -, 10% or less of the strains are positive within 14 days; d, 11-89% of the strains are positive. bstrains of Bisgaard Taxon 36 are positive. cGlycosides: cellobiose, esculin, amygdalin, arbutin, gentiobiose, and salicin.

Determination of dust in Air samples

The cotton filter was weighed before (Wb) and after (Wa) sampling and the dust content in the air was calculated as follows: Wa-Wb/50 x 10^6 = mg dust / m^3 of air.

Determination of moisture content and pH of soil samples (Pepper et al 1995)

Upon arrival to the laboratory, each soil composite sample was mixed thoroughly and screened (2 mm mesh size) to remove large particles. Three replicates each of 25 g were used to determine the soil moisture content by drying at 105°C for 24 hr. Soil pH was determined in 0.01 M CaCl2 solution in three replicates of each dried soil sample by using a glass-electrode pH meter, where the soil salt ratio was 1:2 (w/w).

Survival of *M. haemolytica* under different environmental conditions

Survival of M. haemolvtica in Faeces and Tap-Water [(according to the method described by Plumb and Quintan (1986)]

Four duplicate samples of boiled tap-water (100 ml/each) and faeces (50 gm each) were sterilized by autoclaving at 121°C for 15 minutes. After cooling, three duplicate samples of each substrate were inoculated and thoroughly mixed with 5 ml of an 18-hour Brain-heart infusion broth culture *of M. haemolytica* ($2x10^8$ CFU/ml), while the fourth duplicate was kept as a control. One duplicate sample of water and faeces were kept at 4°C (Refrigerator), the other kept at room temperature (20-25°C) and the third was exposed to sun light (29-31°C). Each inoculated substrate was examined for the presence of viable *M. haemolytica* at 1.5, 3, 6, 12, 24, 48, 72, 96 and 120 hr, where post inoculation 0.1 ml of each water sample was aseptically withdrawn from each flask, spread on a blood agar plate and incubated overnight at 37°C. In case of faecal samples, 1 gm was taken aseptically from each flask, mixed thoroughly with 10 ml sterile distilled water in a test tube and left to stand for 10 minutes and then 0.1 ml of the supernatant was taken and spread on blood agar plate and incubated.

Viability of *M. haemolvtica* in soil (Wood 1973)

Two soil samples each of 500 g were placed in separate flasks and their moisture content was adjusted to approximately 40% and 60% by using distilled water. A third soil sample of 500 g was partially air-dried at room temperature until the moisture content reaches 10.9%. The pH of the three samples was determined by using a glass-electrode pH meter. Four sub-samples each of 50 g were prepared from each of the three soil samples of different moisture content, plus one sample from the stock soil and then sterilized by autoclaving at 121 °C for 15 min. After cooling, all the subsamples except one (control) were inoculated and thoroughly mixed with 5 ml of an 18-hour Brain-heart infusion broth culture *of M. haemolytica* ($2x10^8$ CFU/ml) and incubated at room temperature and out door exposed to sunlight (24ºC-31ºC). The samples were examined for the presence of viable organism at 12, 24, 48, 96 hours post-inoculation until no living organism was recovered. Approximately ,1 g was aseptically withdrawn from each flask and mixed well by vortexing in 10 ml sterilized distilled water in a test tube, and then left to stand for 10-15 min and then 0.1 ml of the supernatant was spread onto the surface of a blood agar plate and incubated at 37°C for 24 hrs.

3. Results

Prevalence of *M. haemolytica* among farm animals and their environment

The frequency of *M. haemolytica* isolation from nasal swabs in herds were 6.8% (4 out of 59),18 % (9 out 50),4% (2 out 50),11.10% (5 out 45) and 13.50% (7 out 52) in cattle, buffaloes, cattle & buffaloes, sheep and goats, respectively

Examination of 70 soil samples of different moisture content and hydrogen ion concentration collected from different animal enclosures found to be positive for *M. haemolytica* in range of 0 to 10% as shown in Table (2).

There was a positive linear correlation between *M. haemolytica* isolation from the soil and its PH-value, as well as a negative linear correlation between *M. haemolytica* isolation from the soil and its moisture content was found as shown in Fig.(1).

It was shown that increasing the amount of dust present in the air will significantly increase the frequency of isolation of *Mannheimia haemolytica* from nasal swabs where the percentage of *M. haemolytica* isolation was low in the farms with low dust level in air and high in the farms with high dust level Fig.(2). It is thought that the increase in dust in the air may affect *M. haemolytica* carrier rate in the animals' nasal cavities.

Survival of *M. haemolytica* in cattle manure and tap water

The survival rate of *M. haemolytica* in cattle manure incubated at 4°C, 20°C (room temperature) and 23-26°C (outdoor temperature exposed to sunlight) was 72 hr, 24 hr and 12 hr, respectively, while it survived for 12 hr, 6 hr and 1.5 hr in tap water incubated at 4°C, 20°C (room temperature) and 23-26°C (outdoor temperature exposed to sunlight), respectively (Fig. 3-a and Fig.3-b). *M. haemolytica* survived relatively longer in faeces than in tap water under different incubation temperatures.

Table (2): Prevalence of *M. haemolytica* among farm animals and their environment.

M.haemolytica isolations from nasal swabs	Soil samples and/or floor			Air samples	
	Mean moisture content %	Mean PH value	*M. haemolytica* isolations	Dust in Air mg/m^3	*M.haemolytica* isolations from Air
4/59 (6.80) ‡ Cattle	16.70	7.20	1/20 (5)†	22	3/20 (15)
9/50(18.0) Buffaloes	19.60	6.90	0/5 (0)	115	6/20 (30)
2/50 (4.0) Cattle& Buffaloes	18.20	7.50	2/20 (10)	28	1/20 (5)
5/45(11.10) Sheep	15.50	7.80	0/13 (0)	130	3/20 (15)
7/52 (13.50) Goats	14.60	7.50	1/12(8.33)	152	4/20 (20)

†; No. of isolations / no. of animals or examined samples (% of positives).
‡; No. of affected animals / total no. of animals present (% of affected animals).

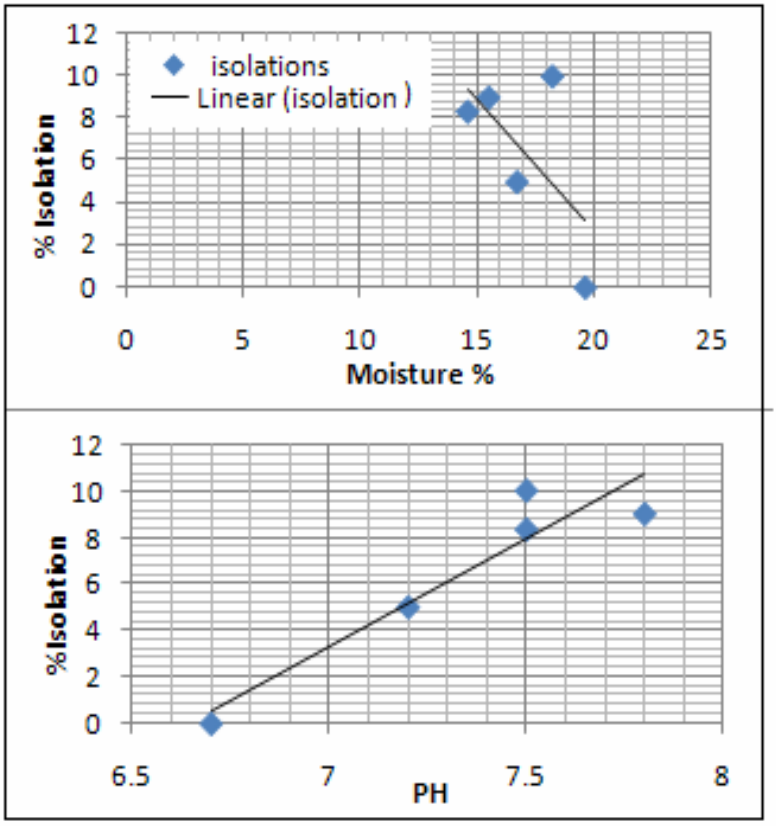

Fig. (1): Relationship between PH-values, moisture content and frequency of *M. haemolytica* isolation from soil (correlation coefficient).

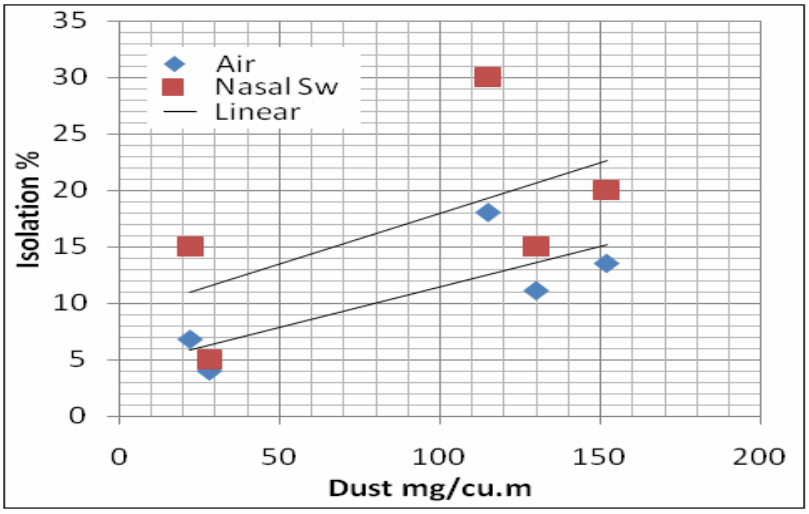

Fig. (2): Relationship between air dust and frequency of *M. haemolytica* isolation from nasal swabs and indoor air. There was a correlation between *M. haemolytica* isolation from the nasal cavities of animals in the present study and the dust content of the air (r =0.72) but it is not significant (P < 0.05). On the contrary, there was a highly significant correlation between dust in the air and the respiratory manifestations affecting the animals (r = 0.82, P< 0.05).

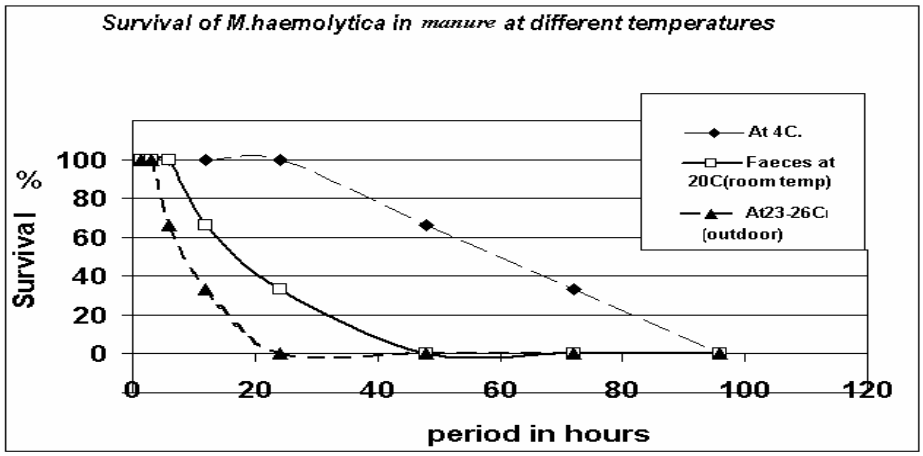

Fig. (3-a): Survival rate of *M. haemolytica* in cattle manure.

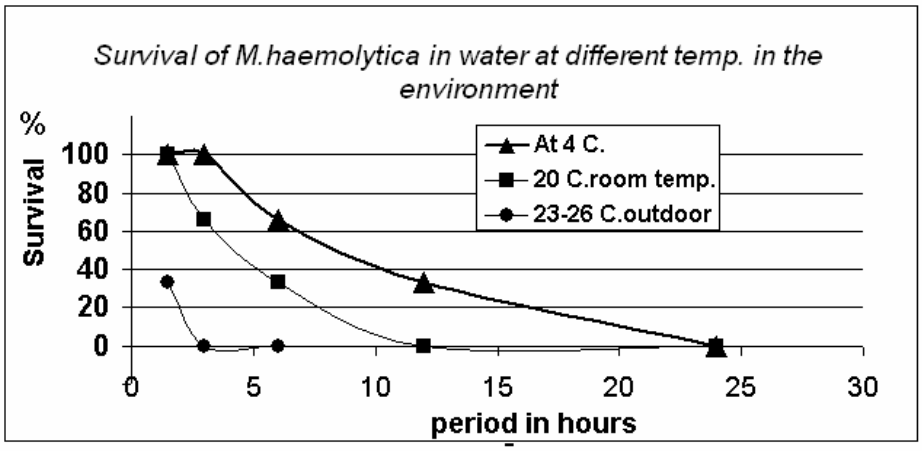

Fig. (3 -b): Survival rate of *M. haemolytica* in tap water. .

Viability of the organism in soil:

Table (3) and Fig. (4) depicts the survival rate of the organism in experimental soils of different moisture content incubated at two temperature regimes. The organism survived in the moist soil (60% moisture content) for 72 hr and 48 hr when incubated at room temperature (25 °C) and outdoor temperature (29-31 °C), respectively, while it survived in the dry soil (10.9% moisture content) only for 24 hr and 12 hr at the same incubation temperatures. The results showed that there is a high significance correlation ($P < 0.05$) between the survival period of the organism and the soil moisture content especially in case of natural soil incubated at outdoor temperature.

Table (4): Effect of soil moisture content & temperature .on the survival *M. haemolytica* in soil:

Soil		Incubation temp.	Survival time in hours				
Moist	pH value		12	24	48	72	96
10.9%	7.2	Room temp. 25°C	++	+	-	-	-
		Outdoor temp. 29-31°C	++	-	-	-	-
40%	7.8	Room temp. 25°C	+++	++	+	-	-
		Outdoor temp. 29-31°C	+++	+	-	-	-
60%	7.6	Room temp. 25°C	+++	+++	++	+	-
		Outdoor temp. 29-31°C	+++	++	+	-	-

- = No growth; += low growth; ++= moderate ; +++=heavy growth

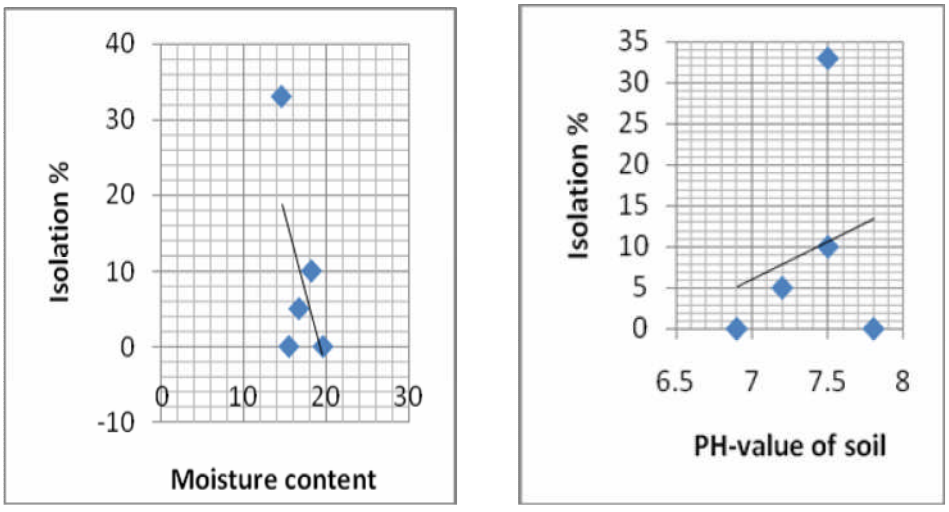

Figure (4): The survival % of *M. haemolytica* in soil at different moisture content, PH and temperature

4. Discussion:

Prevalence of *M. haemolytica* among farm animals and their environment

Nasal swabs were collected from animals showed respiratory manifestations (cattle, buffaloes, sheep and goats) in some farms in different Governorates of Egypt. Table (2) indicated that, 27 isolates of *M. haemolytica* were reported out of 256 of animals suffered of respiratory manifestations (10.18%). These results are in accordance with the results recorded by Poulsen et al., (2006) where a total of 57 *Mannheimia* isolates were obtained from nasal swabs of apparently healthy sheep in Norway.

In the present study the percentage of *M. haemolytica* isolation was low in the farms with low dust level in air and high in the farms with high dust level Fig. (1), there was a correlation between *M. haemolytica* isolation from the nasal cavities of animals in the present study and the dust content of the air (r =0.72) but it is not significant *(P < 0.05)* as shown in Table (2) and Fig. (2) .On the contrary, there was a highly significant correlation between dust in the air and the respiratory manifestations affecting the animals (r = 0.82, *P< 0.05*)

These results may indicate that there was some sort of shedding of the organism to the environment, and the organism cannot multiply in the soil, and might be fragile in aerosol and its survival highest in warm humid air (> 16.5°C, > 69.8% RH) and lowest in dry air (30% RH) (Gilmour et al; 1989).

It seems possible to conclude that the organism is not capable of surviving outside the animal's body for long time. The dust content of the air is one of the environmental predisposing factors that permit the colonization of the microorganism in the respiratory tract by *P. haemolytica and* thus promoting the development of pneumonic pasteurellosis (Webster, 1970).

Survival of *M. haemolytica* in the environment *Manure* and *Tap water:*

The survival rate of *M. haemolytica* in cattle manure incubated at 4°C, 20°C (room temperature) and 23-26°C (outdoor temperature exposed to sunlight) was 72 hr, 24 hr and 12 hr, respectively, while it survived for 12 hr, 6 hr and 1.5 hr in tap water incubated at 4°C, 20°C (room temperature) and 23-26°C (outdoor temperature exposed to sunlight), respectively (Fig. 2). *M. haemolytica* survived relatively longer in manure than in tap water under different incubation temperatures. It is evident that the exposure to sunlight had the most effect on the viability of the organism, while incubation at 4°C had the least effect. The longer survival of the organism in faeces may be attributed to the profuse availability of organic matter (Wray and Thompson, 1971).

Viability of the organism in soil*:*

Table (4) and Figure (4) depicts the survival rate of the organism in experimental soil of different moisture content incubated at two temperature regimes. The organism survived in the moist soil (60% moisture content) for 72 hr and 48 hr when incubated at room temperature (25 °C) and outdoor temperature exposed to sunlight (29-31 °C), respectively, while it survived in the dry soil (10.90% moisture content) only for 24 hr and 12 hr at the same incubation temperatures(25 °C and 29-31 °C) . The results showed that there is a high significant correlation *(P < 0.05)* between the survival period of the organism and the soil moisture content especially in case of natural soil incubated at outdoor temperature.

Buxton and Fraser; (1977) recorded that there is none of the *pasteurellae* was especially resistant to adverse conditions. However, Wilson and Miles (1984) found that exposure of *pasteurellae* on solid media to sunlight for 3-4 hr was lethal for the organisms.

It is evident that the exposure to sunlight had the most effect on the viability of the organism, while incubation at 4°C had the least effect. The longer survival of the organism in manure may be attributed to the profuse availability of organic matter in manure. It is possible to conclude that the organism is not capable of surviving outside the animal's body for long time. Dust content of the air permits the colonization of the respiratory tract by *M. haemolytica*, thus promoting the development of pneumonic *pasteurellosis.*

References

1. -Abdel- Ghani, M; El-Seedy, F. R.; Shokry, S. and Raid, E. M. (1990): Incidence and bacterial causes of buffalo calves mortality with respiratory disorders. Vet. Med. J. Giza 38: 233-243.
2. -Al-Tarazi,Y.H.M. and Dagnall,G.J.R. (1997): Nasal carriage of *Pasteurella haemolytica* serotypes by sheep and goats in Jordan. Trop. Animal Health Prod., 29:177–179
3. -Angen O, Mutters R, Caugant DA, (2002). Int J Syst Bacteriol 49:67-86).
4. -Bisgaard M: Ecology and Significance of Pasteurellaceae in Animals (1993): Zentralbl Bakteriol, 279:7-26.
5. -Bisgaard, M. and Mutters, R. (1986): Re-investigations of selected bovine and ovine strains previously classified as *Pasteurella haemolytica* and description of some new taxa

within the *Pasteurella haemolytica*-complex. Acta Pathologica, Microbiologica et Immunologica Scandinavica, Section B, Microbiology 94: 185–193.
6. -Bisgaard, M., Phillips, J. E. & Mannheim, W. (1986). Characterization and identifcation of bovine and ovine Pasteurellaceae isolated from the oral cavity and rumen of apparently normal cattle and sheep. Acta Pathol Microbiol Immunol Scand Sect B 94, 9±17.
7. -Bowland, S. L., and P. E. Shewen. (2000): Bovine respiratory disease: commercial vaccines currently available in Canada. Can . Vet. J. 41:33-48.
8. -Buxton,A. and Fraser, G. (1977): Animal Microbiology. 1st .ed.,Vol. 1. Blackwell Scientific Publication. Oxford, London, Edinburgh, Melbourne, pp. 126- 127.
9. -El- Battrawy, N. E. E. (1991): Bacteriological studies on respiratory diseases in buffalo- calves. M.V.Sc. Thesis. Dept. of Microbiology. Fac. of Vet. Med. Cairo University.
10. -Gilmour, N. J. L. andJ. S. Gilmour (1989): Pasteurellosis of sheep. In: Pasteurella and Pasteurellosis. eds. C. Adiam and J. M. Rutter. pp. 223- 262. San Diago Academic Press.
11. -Gilmour N.J.L., Thompson D.A, and Fraser J. (1974): The recovery of *Pasteurella haemolytica* from the tonsils of adult sheep. Res Vet Sci, 17:413-414
12. -Gilmour, M. L; Wathes, C M. and Taylor, F. G. R. (1989): The airborne survival of *Pasteurella haemolytica* and its deposition in and clearance from the mouse lung. Vet. Microbiol. 21: 363-375.
13. -Ismail, M.; El- Jakee, J.; Attia, S. A.; Nagwa Ata and Shoukry, S. (1993): Bacterial causes of respiratory disorders in Buffalo- calves in Egypt. Vet. Med. J. Giza. 41: 95- 99.
14. -Jaworski, D.L.Hunter, and A. C. S. Ward. 1998. Biovariants of isolates of Pasteurella from domestic and wild ruminants. Journal of Veterinary Diagnostic Investigation 10: 49–55.
15. -Jaworski M.D., Ward A.C., Hunter D.L., Wesley I.V. (1993): Use of DNA analysis of *Pasteurella haemolytica* biotype T isolates to monitor transmission in bighorn sheep (*Ovis canadensis canadensis*). J. Clin. Microbiol., 31, 831–835.
16. -Jubb, K. V. K.; Kennedy, P. C. and Paluer, N. (1993): Pathology of Domestic Animals. 4th ed. Vol. 2 pp. 632-638. Academic Press, Inc.
17. -Plumb, J.A. and Quinlan, E.E. (1986): Survival of *Edwardesiella ictaluri* in Pond water and Bottom Mud. The Progressive Fish-Culturist 1986; 48: 212-214
18. -Poulsen L.L, Reinert T.M., Sand, R.L.Magne Bisgaard, Christensen H., Olsen J.E, Stuen S. and Bojesen A.M.(2006): Occurrence of *Mannheimia haemolytic* spp. in apparently healthy sheep in Norway. Acta Veterinaria Scandinavica, 48:19doi:10.1186/1751-0147-48 19.
19. -Webster,A.J.E (1970): Resistance to infectious disease ,Eds.R.H.Dunlop and H.W.Moon.Saskatoon,Saskatoon Modern Press.pp.61.
20. -Wood R.L.(1973): Survival of *Erysipelothrix rhusiopathiae* in soil under various environmental conditions.Cornell Vet.63:390-410.
21. -Wray C. and Thompson,D.A (1971): Serotypes of *Pasteurella haemolytica* isolated from calves.Br.Vet.J.127:56-57 (Supplement).
22. -Zuberer D.A.(1994):In:Methods of soil analysis. Part2. Microbiological and biochemical properties. SSSA Book series no.5 eds.R.W.Waever; J.S.Angle and P.S. Bottonley.soil science society of America Inc.

12/2/2010