Dept. of Animal of Hygiene,
Fac. Vet. Med., Assiut University.

EPIDEMIOLOGICAL STUDIES OF BOVINE TUBERCULOSIS WITH SPECIAL REFERENCE TO TUBERCULOCIDAL EFFECT OF SOME DISINFECTANTS ON *M. BOVIS*

(With 4 Tables and 3 Figures)

By
S.A. MOHAMED

(Received at 31/12/2002)

دراسات وبائية على مرض السل البقري وتأثير بعض المطهرات على المسبب الميكروبي

سطوحي أحمد سطوحي

في هذا البحث تم فحص ٣٩٠١٤ حيوان في محافظة أسيوط باستخدام اختبار حساسية الجلد على مدى ١١ سنه. أظهرت نتائج البحث أن الأبقار كانت أكثر إصابة من الجاموس حيث بلغت ٠,١٨% بينما بلغت في الجاموس ٠,١٤%. كما شملت الدراسة تقييم بعض المطهرات على المسبب الميكروبي للمرض وأستخدم فيها ٦ أنواع مختلفة من المطهرات شائعة الإستعمال في المجال البيطرى (مركبات الأمونيا الرباعية, مركبات الأمونيا الرباعية مع الجلوترالدهيد, مركبات الفينول, مركبات اليود, مركبات الكلور وكذلك الفورمالين). أظهرت نتائج هذا الجزء أن الفورمالين ومركبات الفينول وكذلك الجلوترالدهيدات كانت أكثر تأثيرا على المسبب للمرض وأدت جميعها إلى قتل الميكروب بنسبة ١٠٠% عند أوقات متفاوتة. من ناحية أخرى فإن مركبات الأمونيا الرباعية واليود والكلور لم يكن لها أي تأثيرات تذكر إلا عند التركيزات العالية.

SUMMARY

An Intradermal tuberculin test survey was conducted on 39014 animals (24258 cattle and 14756 buffaloes) at Assiut province to determine the epidemiology of bovine tuberculosis. The obtained results showed that the prevalence rate of the disease was higher in cattle than in buffaloes. The prevalence rate was 0.18 and 0.14% for cattle and buffaloes, respectively. The overall mean prevalence rate of the disease was 0.17%.

In order to assess the ability of the commercially available disinfectants, 6 widely common disinfectants [quaternary ammonium compound (QAC), QAC with glutaraldhyde, Phenols, Iodine, Chlorine compounds as well as formaldehyde] were tested against *M. bovis*. All the products were prepared at three different concentrations including the manufacturer's recommended use. The results showed that the organism under test is sensitive to Tek-Trol (phenol), formaldehyde and glutaraldhyde. By using the recommended concentration of these germicides, *M. bovis* was completely destroyed after different time points. The quaternary ammonium compounds, Iodine, and chlorine compounds are not effective as a tuberculocidal agents.

Key words: *Epidemiological studies of bovine tuberculosis.*

INTRODUCTION

Bovine tuberculosis constitutes an important Zoonotic disease of worldwide distribution, causing significant economic losses when poorly controlled. The disease was classified as a list **B**, a disease which is considered to be of socio-economic or public health importance within countries and of significance to the international trade animals and animal products (Cousins 2001).

The progress, which had been made towards the total eradication of bovine tuberculosis, has changed the pattern of its present distribution. In USA, the disease was reduced from 4.9% on 1950 to 0.8% on 1990 (Sauert *et al.*, 1992). In Africa and Asia, tuberculosis is rare, where animals constantly living in open. Introduction of European breeds of cattle and the development of intensive agriculture, rapidly changed distribution of the disease in these areas (Alhaji, 1976). For instances, the prevalence rate of tuberculosis was 6.6% among native cattle in some areas of Kenya (Waddington, 1965) and up to 50% in Malawi (Waddington and Ellwood, 1972). In Egypt, General Organization of Veterinary Services (GOVS, 1992), reported that the Prevalence of bovine tuberculosis in cattle and buffaloes was high in certain Governorates as Alexandria (6.0% in 1991); Dakhalia was 9.6% (1992). However, the average prevalence rates were 0.8; 0.2; 0.77, and 0.71 % during 1989; 1990; 1991, and 1992, respectively.

Due to scare economic resources and limited technical infrastructures in the developing countries, bovine tuberculosis restricts cattle raising potentials and constitutes a risk for public health.

Therefore, appropriate strategies to control the disease are of urgent need. A global method of testing and slaughtering all tuberculin-reactors could eradicate the infection, but it is rather difficult and execute. The high initial cost and elimination of a considerable number of cattle, without having replacement sources of tuberculosis cattle -free, represents serious constraints for this alternative. However, an increasing number of countries had virtually eradicated tuberculosis from their cattle population as UK, USA, Canada, Netherlands, Switzerland and Scandinavian countries (Collins, *et al.*, 1991).

M. bovis as well most of the acid-fast bacteria are known to be more resistant than other non-sporulating bacteria to the effects of acids, alkalis and detergents. *M.bovis*, has thick envelope of waxy material, making them extremely resistant to external influences (Maddock ,1933).

Although disinfection against TB bacilli has been tried and considered, there is a surprising lack of knowledge of the effects of some disinfectants on mycobacteria (Bradley *et al.*, 1991). Literatures includeing some disinfecting agents that had been tried and proved to have a significant germicidal action against TB (Collins, 1986; Band, 1990; Best *et al.*, 1990; Cole *et al.*, 1990; Rutala *et al.*, 1991; Berchieri & Barrow, 1996; Earnshaw & Lawrence, 1998; Erickson *et al.*, 2001 and Le 2002). Phenol (5%); formaldehyde is the most effective chemical against TB (Lotfy & Guindi, 1963). More information could be gained in this study that might add valuable knowledge to our hygienic armamentaria for controlling and ultimate eradication of the disease from the livestock.

The present study was conducted to determine the prevalence rate of tuberculosis among cattle and buffaloes over 11 years at Assiut province. Moreover, tuberculocidal effect of certain disinfectants commonly available in the veterinary field was also undertaken.

MATERIAL and METHODS

I- Epidemiological study:

Prevalence rate of bovine tuberculosis among animal collections, farms, and animal raised by farmers at the rural areas in different districts of Assiut province was determined over 11 years by single intradermal tuberculin test. However, 39014 animals were examined including 24258 cattle and 14756 buffaloes.

Procedures:

All procedures and precautions were conducted according to Boddie (1969); Kelly (1974), and Alhaji (1976) where the test was first applied as a screening test on all animals in the middle third of the left side of the neck. The hairs were firstly clipped and the area was cleaned with 70 % ethyl alcohol. The skin fold at the site of injection was measured with the caliper (Hinged type caliper with graduated quadrant) and then 0.3 ml of mammalian type PPD (1.5 mg/ml) was intradermally injected using intradermal tuberculin syringes. The results were obtained by re-measuring the skin fold 72 h after injection. The positive cases were slaughtered under veterinary supervision and the owners were compensated.

II- Tuberculocidal efficacy of some disinfectant:

I)- Disinfectants: Six chemical disinfectants were evaluated including:

1- **TH4** (12.5% quaternary ammonium compounds + 6.3% Glutaraldhyde).
2- **Quaternary Active Sterilizer** (25% quaternary ammonium compounds).
3- **Tek-Trol** (26 % phenolic compounds).
4- **Biocide-30** (2.85 % available iodine; 9.5 % phosphoric acid; 9.3 % sulphoric acid and 24.2 % non-ionic surfactants).
5- **Sod. Hypochlorite** (12 % available chlorine).
6- **Formalin** (36-40% formaldehyde gas in water).

Three concentrations of each compound were used including the recommended one by the manufacturer as well as higher and lower concentration.

II)- Tested strain:

Field strain of *M. bovis* was used in the present study. Bacteria were grown at 37 °C on Lowenstein Jensen slopes. A suspension was made by placing a loopful of bacteria from a culture on a Lowenstein Jensen slope into a bottle containing sterile physiological saline and stirring until an even suspension was obtained.

III)- Procedures:

Of each disinfectant, three tubes containing equal amounts of different concentrations were prepared. To each tube, 5 drops of the bacterial suspension were added and thoroughly mixed. From each concentration

and after the appropriate time interval (10, 30, 60, 90, 120 and 240 min.), a loopful was removed under aseptic conditions and streaked out on the surface of two McCarteny bottles of Dorset-egg media. The inoculated bottles were labeled and incubated together at 37°C with a control untreated TB suspension. After four weeks, the inoculated bottles were examined and the extent of growth in each was determined and recorded. Four plus (++++) indicate heavy growth while the good growth was given 3 plus (+++). Moreover, 2 plus (++) was given for few colonies while one plus (+) was given for a bottle having 1-2 colonies.

RESULTS

Results are summarized in 4 Tables and 3 Figures.

DISCUSSION

Tuberculosis is an important zoonotic disease of worldwide distribution. It is of importance for public health as well as for its detrimental effects on animal production. It is a disease of community life and poverty.

The use of intradermal tuberculin test procedures has been the basis for the detection of tuberculous animals in mass testing programs in the recent years. Cattle usually become hypersensitive and will react to the test within 3-4 weeks of infection by *M.bovis* (Francis, 1947).

Results in tables (1& 2) and figure 1, revealed that the prevalence rate of the disease was up to 0.82% and 0.24% in cattle and buffaloes, respectively. The results indicated that the disease is more spread in cattle than buffalos. The overall prevalence was 0.18 and 0.14 % in cattle and buffaloes, respectively. From this table, it is clear that the overall mean prevalence rate of bovine TB was 0.17%. The spread of tuberculosis in cattle and buffaloes depends on various factors, but mainly upon the close herding and housing of cattle necessitated by intensive husbandry. The distribution of infection, therefore, varies in different parts of the world, and also within each country, it varies from one area to another. In Egypt, General Organization for Veterinary Services (GOVS, 1992) showed that the prevalence of bovine tuberculosis in cattle is high in certain Governorates such as Alexandria (6% in 1990), Dakahlia and Behera was 9.6% and 14.06% during 1992,

respectively. The average prevalence was 0.08, 0.2, 0.77 and 0.71 in 1989, 1990, 1991 and 1992, respectively (GOVS, 1992).

The obtained results revealed that, prevalence rate of bovine TB is lower than those in Zambia where 165 (7.4%) animals were reactors out of 2226 tested cattle (Cook *et al.*, 1996). Moreover, prevalence rate of the disease was 13.2% and 51% of two different districts in Tanzania (Kazwala *et al.*, 2001). In France, among the 32,197 serologically-tested animals, the infection rate ranged between 2-13% (Le, 2002). In Argentina, a tuberculin survey on 20,000 animals showed that the average prevalence rate was 4.3% (Pan American Zoonoses center, 1988).

Great efforts were done to control the disease spreading. In Germany the disease was reduced from 35% (1950) to become herds free at 1962. Moreover, in Great Britain, cattle TB were 40; 18 and 0.06 % on 1934, 1946 and 1963, respectively (Bubbert *et al.* 1975).

The different epidemiological pictures of the disease between different countries could be attributed to different climatic zones, different management systems, sanitary measures as well the herd size (Kazawala *et al.*, 2001 and Kaneene *et al.*, 2002). Our study was focused on rural areas where animals constantly living in open, that are why the prevalence rate of the disease is low.

On the basis of tuberculocidal effect (Table 4 and figure 3), *M. bovis* is variably affected by disinfectants. From this table it is clear that Glutaraldhyde (TH4), Phenols (Tek-Trol) and formaldehyde are by far the most effective disinfectants under test. The results revealed all these disinfectants prevented *M.bovis* growth completely at different time points. There was no growth that could be detected after 30 minutes in case of formaldehyde. Moreover, at 2% formaldehyde, *M. bovis* required up to 90 minutes to be completely destroyed. In contrast, recommended concentration of TH4 and Tek-Trol prevented growth of the organism completely after 4 hours exposure. Similar results were obtained by Lotfy & Guindi (1963) and Peters & Spicher (1994). Early studies showed that *M.bovis* and all acid-fast bacilli are sensitive to phenolic compounds as well glutaraldhyde (Collins, 1986; Cole *et al.*, 1990; Broadley *et al.*, 1990; Russel, 1991; Rutala *et al.*, 1991, and Best *et al.* 2001). Moreover, Erickson *et al.*, 2001 found that the tuberculocidal activities of five commercially available glutaraldhyde-based disinfectants were so strong and all of them reduced the number of surviving mycobacterium by greater than five orders of magnitude.

Moreover, a phenol-based disinfectant with tuberculocidal claims, gave less than one order of magnitude reduction of the test organism. Moreover, Collins (1986) and Broadely *et al.* (1991) found that 2% alkaline glutaraldhyde sol (pH 8) was bactericidal against Mycobacteria within 20 minutes. The results in table 4 revealed that *M.bovis* is somewhat resistant to quaternary ammonium compounds (QAS), chlorine compound (Sod. Hypochlorite) and iodine compounds (Biocide-30). The CFU were drastically reduced only at high concentration of QAS and sod. Hypochlorite. On the other hand, the recommended concentration showed no tuberculocidal effect. Similar results were recorded before (Best *et al.*, 1990; Rutala *et al.*, 1991). Peters and Spicher (1994) found that quaternary ammonium compounds, even at higher concentrations, showed a totally insufficient efficiency to mycobacterium. Mycobacteria are resistant to acids, alkalis, quaternary ammonium compounds, non-ionic and anionic surface active agents (Croshaw, 1971; Russel, 1991). Many of these agents inhibit mycobacterial growth without killing them

Rutala *et al.* (1991) found that 100 ppm chlorine was not effective on either *M.tuberculosis* or *M. bovis* while 1000 ppm leads to complete inactivation on both. Best *et al.* (1990) mentioned that *M. tuberculosis* required higher concentration of available chlorine to achieve an effective level of disinfection. Concerning iodophore, Best *et al.* (1990) found that it has no tuberculocidal effect. In contrast, Rutala *et al.* (1991) found that iodophore achieved complete inactivation of *M. tuberculosis*.

The prevalence rate of bovine tuberculosis at Assiut province providing hopeful preliminary evidence that, eradication strategies are succeeding. One can safely conclude that testing and slaughtering of all positive reactive animals and applying a strict sanitation program and disinfection of the animal houses with the appropriate germicides will control disease spreading.

ACKNOWLEDGMENT

I'm grateful to Dr. Rafat Munier, Animal Health Institute, Assiut, for obtaining valuable data on the epidemiology of the disease.

REFERENCES

Alhaji, I. (1976): Bovine tuberculosis: A general review with special reference to Nigeria. The Vet. Bulletin, 46 (11): 829-841.

Band, D.E. (1990): The use of phenolic disinfectant in Animal husbandry. Int. Biodeterioration, 26 (2-4): 217-223.

Berchieri, J.R. and Barrow, P.A. (1996): The antibacterial effects for S. enteritidis phage type 4 of different chemical disinfectants and cleaning agents tested under different conditions. Avian Patholo., 25: 663-667.

Best, M.; Sattar, S.A.; Springthorpe, V.S.and Kennedy, M.E. (1990): Efficiencies of selected disinfectants against M. tuberculosis. J. Clin. Microbiol., 28 (10): 2234-2239.

Boddie, G.F. (1969): Diagnostic methods in Vet. Med., Great Brit., Edingburgh.

Brdadely, S.J.; Jenkins, P.A.; Furr, J.R. and Russel, A.D. (1991): Anti-mycobacterial activity of biocides. Letters in Appl. Microbiol., 13, 118-122.

Bubbert, W.T.; McCulloch, W.F. and Schonurrenberger, P.R. (1975): Disease transmitted from animals to man. 4th Ed. Charles Thomas Publisher, USA.

Cole, E.C,; Rutala, W.A.; Nessen, L.; Wannarmurker, N.S. and Weber, D.J. (1990): Effect of metodology, dilution, and exposure time on the tuberculocidal activity of glutaraldhyde-based disinfectants. Appl. Environ. Microbiol., 56 (6): 1813-1817.

Collins, C.H.; Yates, M.D., and Granges, J.M. (1991): A study of bovine strains of M. tuberculosis isolated from human in England. Tubercle, 62: 113-116.

Collins, F.M. (1986): Kinetics of the tuberculocidal responses by alkaline glutaraldhyde in solution and on an inert surface. J. Appl. Bact., 61 (1): 87-93.

Cook, A.J.; Tuchilo, L.M.; Buve, A.; Foster, S.D.; Godfray,-Fausett, P.; Pandey, G.S. and McAdary, K.P. (1996): Human and bovine tuberculosis in the Monze District of Zambia- A cross-sectional study. Br. Vet. J., 152 (1): 3-5.

Croshaw, B. (1971): The destruction of mycobacteria. In: Inhibition and destruction of the microbial cell. ED. Hugo, W.B. pp. 429-449. London: Academic Press.

Cousins, D.V. (2001): M. bovis infection and control in domestic livestock. Rev. Sci. Tech., 20 (1): 71-85.

Earnshaw, A.M. and Lawrence, L.M. (1998): Sensitivity to commercial disinfectants and the occurrence of plasmids within various Listeria monocytogenes genotypes isolated from poultry products and the poultry processing environment. J. Appl. Microbiol., 84: 642-648.

Erickson, B.D.; Campbell, W.L. and Cerniglia, C.E. (2001): A rapid method for determining the tuberculocidal activity of liquid chemical germicides. Curr. Microbiol. 43 (2): 79-82.

Francis, J. (1947): Bovine tuberculosis. Staples Press, Ltd, London.

GOVS (1992): General organization for the Vet. services, Ministry of Agriculture, Egypt.

Hanson, P.J.; Chadwick, M.V.; Gaya, H. and Collins, J.V. (1993): A study of glutaraldhyde disinfection of fibreoptic bronchoscopes experimentally contaminated with M. tuberculosis. J. Hosp. Infect., 25 (2): 145-147.

Kaneene, J.B.; Bruning-Fann, C.S.; Granger, C.M.; Miller, R. and Porter-Spalding, B.A. (2002): Environmental and farm management factors associated with tuberculosis on cattle farms in northeastern Michigan. J.Am.Vet. Med. Ass., 221 (6): 837-842.

Kazawala, R.R.; Kambarage, D.M.; Doborn, C.J.; Nyange, J.; Jiwa, S.F. and Sharp, J.M. (2001): Risk factors associated with the occurrence of bovine tuberculosis in cattle in the southern highlands of Tanzania. Vet. Res. Commun., 25(8): 609-614.

Kelly, W.R. (1974): Vet. clinical diagnosis, 2nd ED. Bailliere, Tindall, London.

Le, G.D.; Calavas, D.; Brank, M.; Citti, C.; Rosengartner, R.; Bezille, P. and Poumarat, F. (2002): Serological prevalence of M. bovis infection in suckling beef cattle in France. Vet. Rec., 150 (9): 268-273.

Lotfy, O. and Guindi, S.M. (1963): A study on the differential resistance in-vitro of the human, bovine and avian tubercle bacilli to disinfectants. 4th Arab Annual Vet. Congress, pp.143-149.

Maddock, E.G.G. (1933): Studies on the survival time of the bovine tubercle bacillus in soil, dung and on grass. J. Hyg., 33: 103-117.

Pan American Zoonoses Center (PAHO/WHO) (1988): The status of Bovine tuberculosis in Latin America and the Caribbean. Special publication, No.8, Martinez, Argentina, pp. 7-18.

Peters, J. and Spicher , G. (1994): Model tests for effectiveness assay of disinfectants on surfaces. III. Dependence of test results on the type of substances and the test microbes (Staph. aureus and M. terrae). Zentralbl Hyg. Umweltmed, 195 (2): 97-110.

Russel, A.D. (1991): Mycobactericidal agents. In. principles and practice of disinfection, preservation and sterilization , 2nd ED.Russel,A.D.; Hugo, W.B. and Ayliffe, G.A.J. Oxford: Blackwell Scientific publications.

Rutala, W.A.; Cole, E.C.; Wannamaker, N.S. and Weber, D.J. (1991): Inactivation of Mycobacterium tuberculosis and M. bovis by 14 hospital disinfectants. Am. J. Med., 91 (3B): 2675-2715.

Sauert, J.; Jolis, R.; Ausina, V.; Castro, E. and Coronudella, R. (1992): Human tuberculosis due to M. bovis. Tubercle and lung disease, 73: 388-391.

Waddington, F.G. (1965): Observation on tuberculin sensitivity in cattle in Kenya. Br. Vet. J., 121: 319-331.

Waddington, F.G. and Ellwood, D.C. (1972): An experiment to challenge the resistance to tuberculosis in BCG vaccinated cattle in Malawi. Br. Vet. J., 128: 541-552.

Table 1: Prevalence rate of tuberculin tested cattle

Year	Tuberculin-tested cattle				
	NO. of examined animals	Positive cases	Suspected cases	Negative cases	Prevalence rate
1989	3153	0	0	3153	0.0
1990	2274	7	0	2267	0.30
1991	1899	0	0	1899	0.0
1992	2072	17	6	2049	0.82
1993	2494	0	0	2494	0.0
1994	2890	9	2	2879	0.31
1995	2764	6	0	2758	0.1
1996	1597	1	0	1596	0.0
1997	1673	0	0	1673	0.32
1998	1255	4	0	1251	0.0
1999	2187	0	0	2187	0
Total	24258	44	8	24214	0.18

Table 2: Prevalence of tuberculin tested buffaloes.

Year	Tuberculin-tested buffaloes				
	NO. of examined animals	Positive cases	Suspected cases	Negative cases	Prevalence rate
1989	1463	1	0	1462	0.1
1990	2070	3	0	2067	0.14
1991	1503	1	0	1502	0.1
1992	1217	0	0	1217	0.0
1993	1669	0	0	1669	0.0
1994	1172	0	0	1172	0.0
1995	2389	4	0	2385	0.17
1996	825	2	0	823	0.24
1997	855	0	0	855	0.0
1998	914	10	0	904	1.1
1999	679	0	0	679	0.0
Total	14756	21	0	14735	0.14

Table 3: Overall prevalence rate of tuberculin-tested animals.

Year	Species				Exam. animals	Total positive	Overall prevalence rate (%)
	Cattle		Buffaloes				
	No.	Positive	NO.	Positive			
1989	3153	0	1463	1	4616	1	0.02
1990	2274	7	2070	3	4344	10	0.23
1991	1899	0	1503	1	3402	10	0.03
1992	2072	17	1217	0	3289	17	0.52
1993	2494	0	1669	0	4163	0	0.00
1994	2890	9	1172	0	4062	9	0.22
1995	2764	6	2389	4	5153	10	0.19
1996	1597	1	825	2	2422	3	0.12
1997	1673	0	855	0	2528	0	0.00
1998	1255	4	914	10	2169	14	0.65
1999	2187	0	679	0	2866	0	0.00
Total	24258	44	14756	21	39014	65	0.17

Table 4: Tuberculocidal effect of some disinfectants on *M.bovis*.

Disinfectant	Conc.	Time/min.					
		10	30	60	90	120	240
TH4	1:100 (1.0%)	++	++	++	+	+	-
	1:200 (0.5%)**	++	++	++	++	++	-
	1:300 (0.3%)	++++	+++	+++	++	++	++
QAS	1:250 (0.4%)	++	++	++	++	++	++
	1:500 (0.2%)**	+++	+++	+++	+++	++	++
	1:750 (0.13%)	++++	++++	++++	++++	+++	+++
TEK-TROL	1:125 (0.8%)	++	++	++	++	-	-
	1:250 (0.4%)**	++	++	++	++	++	-
	1:500 (0.2%)	++++	+++	+++	+++	++	++
Biocide-30	1:300 (0.3%)	++++	++++	+++	++	++	++
	1:400 (0.25%)**	++++	++++	++++	+++	+++	+++
	1:500 (0.2%)	++++	++++	++++	++++	++++	++++
S.hypochlorite	200ppm	++++	+++	+++	++	++	+
	100ppm*	++++	++++	++++	++	++	++
	50ppm	++++	++++	++++	++++	++++	++++
Formalin	10%	-	-	-	-	-	-
	5%**	+	-	-	-	-	-
	2%	++	++	+	+	-	-

** recommended conc. By the manufacturer

Fig. (1): Prevalence rate of tuberculosis among cattle and buffalos

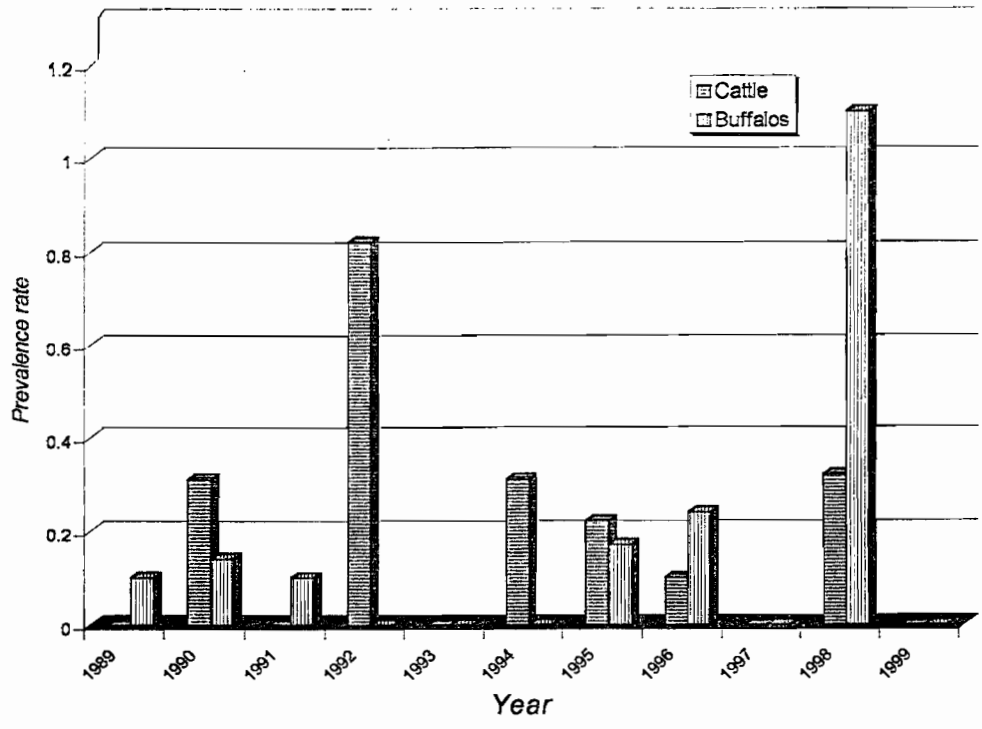

Fig. (2): Overall prevalence rate among tested animals

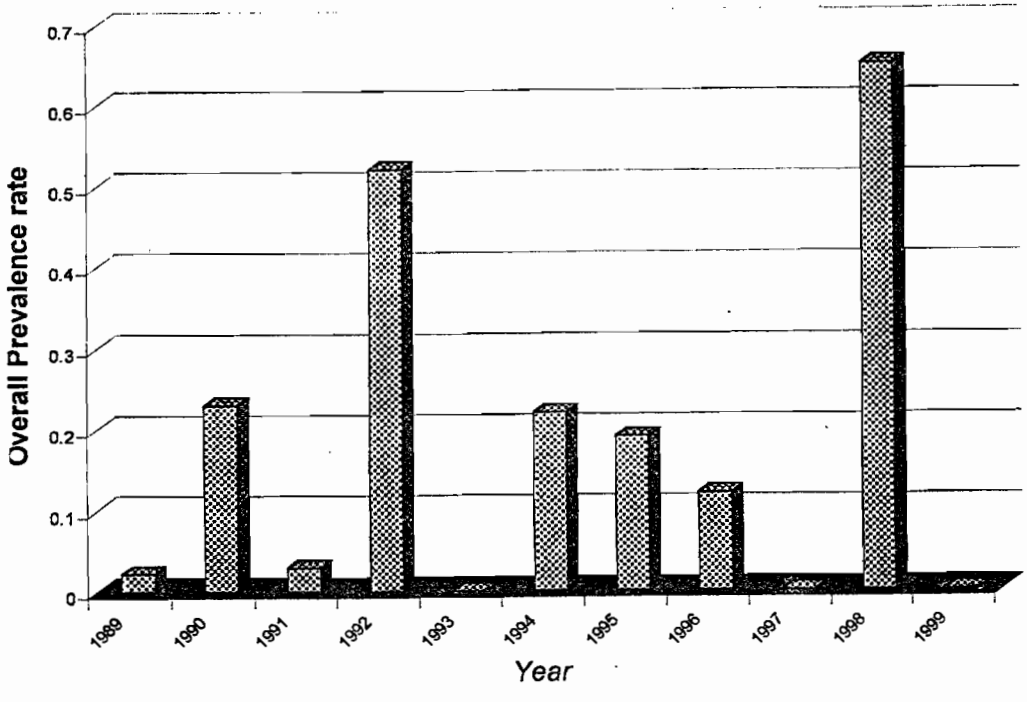

Fig. (3): Tuberculocidal effect of recommended concentration of sel disinfectant on *M. bovis*.