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**IMMUNOSUPPRESSIVE EFFECT OF SOME WATER
POLLUTANTS ON SHEEP VACCINATED WITH
BIVALENT LAMB DYSENTERY AND
PULPY KIDNEY VACCINES**
(With 6 tables)

By

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التأثير المثبط لبعض ملوثات الماء على الاستجابة المناعية للأغنام المحصنة
باللقاح الثنائي لمرض إسهال الحملان و الكلى الرخوة

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أجريت هذه الدراسة لاستكشاف تأثير بعض ملوثات الماء على الاستجابة المناعية للأغنام لمرضى إسهال الحملان و الكلى الرخوة . قبل هذه الدراسة أخذت عينات من الماء الذى تشرب منه الأغنام الموجودة بمزرعة كلية الزراعة بمشهور التى تمت بها الدراسة و أجرى التحليل الكيمائى لها و أسفر عن وجود نسبة عالية من النترات و النحاس و الذى شجع الباحثين على دراسة تأثير هذه المواد على الاستجابة المناعية للأغنام. أجريت هذه الدراسة على أربعة مجموعات متساوية من الأغنام ١٠ حيوانات لكل مجموعة و قد أخذت المجموعة الأولى نيترات الصوديوم، و المجموعة الثانية كبريتات نحاس و المجموعة الثالثة مخلوط بينهما و ذلك فى الماء بينما المجموعة الرابعة أخذت ماء بدون إضافة أى من هذه المواد و قد تم حساب عدد كرات الدم البيضاء و كذلك العدد التفرقى لكل من الليمفوسيت و النيتروفيل و البيروفيل الخ و وجد نقص معنوى أو غير معنوى فى هذه الخلايا. أيضا تمت دراسة بعض التغيرات البيوكيميائية فى المصل لبعض العناصر و الإنزيمات الخاصة بوظائف الكبد و الكلى و وجد بها تغير معنوى و غير معنوى فى أحيان أخرى مما يتضح منه أن هذه السموم الموجودة فى الماء من الممكن أن تؤثر على وظائف الكبد و الكلى. كما تم إثبات وجود أى أعراض مشابهة أو مرتبطة بالتسمم بالنحاس أو النترات على الأغنام. أيضا تمت دراسة الاستجابة المناعية فى الأغنام و وجد بها نقص معنوى و أحيانا شديد المعنوية فى مضادات السموم (B & E) خاصة المجموعة الأولى و الثالثة ووصل مضادات السم E إلى الصفر فى الشهر الرابع مما قد يستدل منه أن هذه الحيوانات تكون فى مرحلة خطره و عرضة للإصابة بالكوليمبترينوم نوع D . تم تسجيل نقص معنوى أو غير معنوى أحيانا فى

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أوزان هذه الحيوانات و أيضا تم تسجيل بعض الإصابات التي قد تكون ناشئة عن التثبيط المناعي للحيوانات المعاملة ، و خلاصة القول أنه يجب فحص الماء للتأكد من خلوه من الملوثات أو وجودها بالنسب العالمية المسموح بها قبل إجراء أى برنامج تحصينى و ذلك منعا لحدوث انخفاض أو تثبيط فى الاستجابة المناعية حتى يمكن السيطرة على الأمراض التي تحسن الحيوانات ضدها.

SUMMARY

The present study was conducted to explore the influence of water pollution on the efficacy of vaccination program. Samples of drinking water offered to sheep were collected where the concentrations of some pollutants were estimated. The results indicated pronounced high levels of nitrate and copper in water. These results were encouraging to investigate the effect of these toxicants on immune response of sheep vaccinated with bivalent lamb dysentery and pulpy kidney vaccines. Four equal groups of sheep were used in this experiment. These groups were offered drinking water containing sodium nitrate or copper sulphate or combination of sodium nitrate and copper sulphate in the first three treated groups respectively and the fourth group was kept as a control. The effect of these pollutants on total, differential leukocytic count and some serum parameters were recorded. Also the body weight gain and clinical manifestations were observed. The results of this investigations revealed that these chemicals suppressed the immune response of vaccinated sheep where unprotective level for Epsilon antitoxin titre was found in treated groups.

INTRODUCTION

Environmental contamination of air, water, soil and food is well recognized a threat to the continued existence of many plants and animals community. The continuous use of nitrogenous fertilizers in agriculture is the major sources of nitrite and nitrate which have been to be present in a wide variety of food plants and drinking water in relatively high concentration (Walker, 1975 and Bogradi et al., 1991). Surface water stored in ponds and lakes may also contain dangerous amount of nitrate and nitrite because of natural nitrification or contamination by drainage from feed lots or cultivated lands. Animals may be exposed to copper through many ways , for examples copper sulfate as a therapy or growth promoters in veterinary field ; contamination of plants with fungicide spray containing copper, over dose with copper containing parasiticide drenches, drinking water contaminated by

copper sulfate used in snail eradication or too liberal ingestion of mineral mixtures containing copper.

Immune system as a target of chemical toxicant has recently gained concern and importance. A variety of chemicals, drugs and infectious agents can cause damage to lymphoid organs or to immune cells resulting in impairment of the immune response of the animals on antigenic stimulation. Such impairments would increase the animal's susceptibility to various infectious agents and also interfere in the outcome of vaccination programmes against these agents (Muneer, et al., 1988).

Enterotoxemia caused by *Clostridium* (CL) *perferingens* group affect sheep of all ages and at all times of the year. It causes heavy losses especially in flocks managed for lamb and mutton production (Radostits et al. 1994). Death from this organism due to absorption of toxins mainly Beta and Epsilon into circulation due to proliferation of the causative organism in the intestines.

The severity and acuteness of clostridial toxemia would make treatment of affected animals very difficult. Thus control by active immunization is of considerable importance and to be effective the immune system must be stimulated and not exposed to any factor affecting its efficacy on the formation of protective levels of antibody after vaccination. In this respect De Sain Blanquat et al. (1983) suggested that nitrate and nitrite impaired defense mechanisms of rats while Kahraman (1988) and Azoulay et al. (1987) found similar effect in mice. Moreover, Atef et al. (1991) concluded that nitrate and nitrite are environmental pollutants present in food and water and they may contribute to the etiology of liver and kidney diseases and problems related to failure of immunity in domestic fowls. Pocino et al. (1991) also recognized that, mice given excess of copper orally showed inhibition of the proliferative response to concanavalin (A).

Therefore, the objective of this study is to clarify the effect of the most predominant pollutant in examined water (namely nitrate and copper as found in this study) on the immune response of animals maintained on water with excessive level of such contaminants.

MATERIAL AND METHODS

A- Water analysis:

Samples of water offered in sheep farm (Fac. of Agriculture, Moshtohor) were collected where the levels of some toxic anions (e.g. ammonia, nitrite, nitrate, phosphate, sulphate) and some toxic cations e.g.

(copper, zinc, manganese and iron) were estimated according to the method in publications of World Health Organization Standards "WHO", (Anon, 1984).

B- Biological investigations

This study was conducted on 40 apparently healthy mixed breed sheep (3-5) months age. They belonged to sheep farm at Faculty of Agriculture, Moshtohor. The animals were allocated into four equal groups (each of ten). Water containing sodium nitrate, or copper sulphate or combination of both was offered as follows:

Group I (G1): offered water contained sodium nitrate in a dose of 1000 PPM daily for 2 months, the dose was selected after Seerley et al. (1991)

Group II (G2): consumed water with copper sulphate in a dose of 30 PPM daily for 2 months, after Pocino et al. (1991).

Group III (G3): taken water containing combination of sodium nitrate and copper sulphate together in the same doses and period previously mentioned.

Group IV (G4): kept as control given water without chemicals addition.

Treated water was offered to all investigated animals one month before and after vaccination with bivalent lamb dysentery and pulpy kidney vaccines kindly supply from "Vet. Sera and Vaccine Research Institute, Abassia, Cairo" (3 cm, S/C) at 1 month of the beginning of the experiment and a booster dose (2 cm, S/C) was given 2 months from the onset of the experiment for observation of any clinical signs.

Titration of toxin and antitoxin in this study was carried on using white Swiss mice of 15-25 gm body weight obtained from mice farm at Vet. Sera and Vaccine Research Institute, Abassia, Cairo.

All sheep were weighed just before and 2 month after beginning of the experiment.

Samples and analytical procedures:

A-Water samples:

These were collected from water supply of the respective sheep farm and quantitative estimation of ammonia, nitrite, nitrate, phosphate, sulphate,

copper, zinc, manganese and iron were done according to the method of the WHO (Anon. 1984).

B- Blood samples:

Samples of blood were collected from sheep on anticoagulant (heparin) for estimation of total and differential leukocytic counts (Kelly, 1984) and without anticoagulant and serum was then separated for estimation of antitoxin titer according to Gadalla et al., (1971).

Determination of some biochemical parameters were adopted as follows:

- Serum , total protein, albumin and globulin after Weichselbaum (1946) , Daumas et al., (1971).
Serum immunoglobulin IgM and IgG using single radial immunodiffusion kits (Kallested chaska, USA) , according to Pfeiffer et al., (1977).
- Serum, alkaline phosphatase was determined by using method of kind and king (1954)
- Serum Gamma glutamyl transferase (GGT) after Szasz (1969).
- Serum transaminases (GPT and GOT) were estimated by the methods of Reitman and Frankel (1957).
- Serum urea and creatinine were determined according to Houndan and Ropoport (1968) and Chaney and Morbach (1963) respectively
- Serum copper was estimated by atomic absorption after Fernandez and Kahn (1971).

C- Swabs:

Rectal, nasal and ocular swabs and pus from skin abscesses were taken aseptically from diseased sheep and subjected to bacteriological examination according to Cruickshank et al., (1975).

D- Skin scrapings:

These were collected from sheep with alopecia and examined parasitologically according to Kelly (1984).

RESULTS

Are presented at Tables 1, 2, 3, 4, 5, and 6.

DISCUSSION

It is well established that water pollution became one of the major problems facing public health. Steadily increasing flow of pollutants is discharged into natural water from different sources. Toxic contaminants of

water may directly produce their harmful effect or it may be indirectly predispose animals to infections by lowering immune status (Koller, 1982).

Concerning to our study, Table (1) indicated that, the examined water samples collected from water supply of sheep farm at the Fac. of Agric. at Moshtohor had some chemicals within the permissible limit of WHO (Anon, 1984). Non of the examined samples exceeded the permissible limit for sulphate, zinc and iron while 50% of samples exceeded the permissible limit of manganese and ammonia. It was quite clear that there was clear problem of pollution with nitrogenous substances where in addition to samples exceeding the permissible limit for ammonia it was found that 70% of the examined samples exceed the permissible limit of copper in water. The major source of nitrate contamination of water might be the agricultural application of nitrogen-based fertilizers and the subsequent runoff the surface waters or percolation to ground waters. These agricultural practices were used in the surroundings of the investigated localities. Bogradi et al., (1991) came to a similar conclusion. The higher concentration of copper might be due to its utilization in the component of some insecticides or due to the decaying effect of water pipes.

The results of total and differential leukocytic count are illustrated in table (2) where significant and highly significant reduction in total leukocyte (WBCs) and lymphocyte percentage occurred in G1 and G3 respectively while neutrophil percentage showed highly significant decrease in both G1 and G3 and significant reduction in G2. Monocyte and basophile percentage exerted significant increase in G1, while in G3 monocyte showed significant increase. The results concerning sheep having copper sulfate " Cu SO_4 " (G2 and G3) were nearly similar to that obtained by Dick and Dixon (1985) who recorded reduction of neutrophil and lymphocyte after exposure to chronic copper toxicity but were contradicted to that achieved by Arthington et al., (1995) who found that copper depletion or repletion did not affect neutrophil or lymphocyte function in growing beef heifers. Moreover the results disagreed with that obtained by Helmy et al., (1994) who recorded significant elevation of total leukocytic count in chickens supplemented with copper sulfate and Ishmael et al., (1972) who found that total WBCs was fluctuated slightly in the period before hemolysis in chronic copper toxicity in sheep. The reduction of the total WBCs and differential cell count of sheep having sod. Nitrate (G1 and G3), was also reported by Nikolov (1983) who recorded a decrease of leukocytic count in sheep fed forages containing nitrate but disagreed with the results of Zoporozhets (1986) who obtained

Leukocytosis in cattle fed on grass containing nitrate and nitrite due to excessive application of fertilizer. Moreover the present results were nearly justified by the prior work of Hanaa Hegazy (1991) in poultry.

Concerning the serum biochemical parameters, table (3) showed a significant or highly significant reduction in the levels of total protein, albumin, globulin and some of globulin fractions (IgG and IgM). In the same time the concentrations of some serum enzymes e.g. (alkaline phosphatase "ALP", gamma glutamyl transferase (GGT) showed higher levels in treated groups at various periods of experiment. Such results might indicate some disturbance in liver functions due to these pollutants. Similar results were interpreted by Ishmael et al. (1971) who observed some morphological and histopathological changes in liver parenchyma several weeks before signs of copper toxicity in sheep. As regard to the levels of IgG and IgM, their decreased values might be due to the decrease in total protein as suggested by Coria and Mc Clurkin (1978) or might be attributed to lymphopenia as recorded in the present study.

The present results concerning serum enzymes were previously noticed in prior work of Todd (1969) and Ismael et al. (1972) who recorded marked increase in level of serum enzymes immediately or several weeks before episodes of hemolysis in chronic copper toxicity in sheep. Moreover, these results are in accordance with that obtained by Gopinath and Howell (1975), Gracy et al. (1976) and Hidirglov et al. (1984) who recorded elevation in some serum enzymes in sheep with chronic copper toxicity. However Atef et al. (1991) found changes in Egypt in chickens after addition of sodium nitrate or nitrite and Helmy et al. (1994) who recorded elevation of serum alanine amino transferase and alkaline phosphatase in chickens supplemented by Cu SO_4 .

Values of urea showed significant increase at 2 months post vaccination (m.p.v) in G1 while in G3 it revealed significant and highly significant increase at 1.m and 2. months post water drinking (m.p.w.d.) Creatinine had significant elevation only in G3 at 2.m.p.w.d. These changes are nearly similar to that recorded by Atef et al. (1991) in chickens. However Neda et al. (1986) found a slight increase of serum urea in sows given a large dose of sodium nitrate.

Serum copper showed significant increase at 2.m.p.w.d. in G2 and G3. This striking findings fitted closely with findings of McCosker (1968) and Ishmael et al. (1972) who reported that blood copper level remain within normal level until 1-2 day before hemolysis or might be elevated several days before hemolysis due to chronic copper toxicity in sheep.

Regarding to results of toxin-antitoxin neutralization test (Table 4), the behaviour of antitoxin titer after vaccination in G4 (control group) is similar to that recorded by Fathia Shafie (1992) and Makhareta (1992). The titer in G1 showed significant and highly significant reduction for B-antitoxin and highly significant decrease for E-antitoxin from 1-4 month post vaccination (m.p.v.). The titer in G2 revealed significant decrease o B and E-antitoxin, while the titer in G3 showed highly significant decrease in both B and E antitoxin from 1-4 m.p.v. These results indicate that sod. nitrate or copper sulfate may impaired the immune response of immunized sheep by lowering their antitoxin level and titer steadily reduced in treated groups in a faster manner reaching to non protective level at 4 m.p.v. for E. antitoxin in G3 while in other sheep it was sufficient to protect it. Betatoxin (B) appears to be the most significant toxic fraction produced by *C. perferingens* type B and C while Epsilon (E) was proved to be the most significant toxic fraction produced by *C. Perferingens* type D (Willis, 1977 and Sneath et al. 1986). In this respect Sterne et al., (1962) concluded that about 0.5 IU/ml serum for E-antitoxin were considered to be at risk, while sheep with titers of 0.1-1 IU/ml serum were suggested as poor responders to vaccination. In this investigation antitoxin titer for E.toxin reached to zero at 4 m.p.v. in G3, it can be considered that sheep treated by combination of sod. nitrate and copper sulfate are poor responder to vaccination, this might be due to potentiation of these chemicals to each other. The behaviour of present results are similar to results obtained by De Saint Blanquat et al. (1983) in rats, Azoulay et al. (1987), Kahraman, (1988) and Eisenstein et al. (1994) in mice and Atef et al. (1991) in domestic fowls. However, Ward et al. (1993) declared that, supplementation of copper for growing steer feed diets did not affect antibody production at 7 or 77 days post supplementation. Furthermore Haverkos (1987) suggested that, the use of large quantities of nitrate inhalants in humans was considered as promoter for kaposi's sarcoma.

Our results revealed that none of the copper supplemented sheep showed signs of hemolysis or other signs related to acute or chronic copper toxicosis, these observations are similar to results of Ishmael et al. (1971). Moreover, Todd (1969) considered that chronic copper toxicity had two distinct phases, in the first phase, copper would accumulate in the liver over a period of weeks or months and clinical signs were absent.

The absence of clinical signs related to toxicity by sodium nitrate or copper sulphate together with the results of blood pictures and some biochemical changes might be due to the presence of a certain degree of subclinical toxicity. An explanation of this was offered by Hill and Williams (1965) who reported that lamb receiving a high experimental intake of copper had shown no signs related to copper toxicity.

With respect to body weight gain, (Table 5) showed significant decrease of body weight in G1 and G3. These data agreed with that observed by Atef et al. (1991) in chickens supplemented with sodium nitrate or nitrite and Helmy et al. (1994) in chicken supplemented by copper sulfate. In this respect dietary nitrate or nitrite had been reported to depress growth in swine (Toilet et al. 1960); in cattle (Weichenthal et al. 1963) and in sheep (Goodrich et al. 1964).

The adverse effects of nitrate on growth, liver, kidney and immunity could be attributed to oxidation of important iron containing enzymes as the cytochromes responsible for cellular respiration and other oxidation reduction processes (Wood 1980). Another explanation could be based on destruction of retinal (Adams, et al. 1966) essential for normal growth and immunological functions (Aitken, 1982) or on interference with thyroid hormones which regulate cell metabolism and growth (Bloomfield et al. 1961).

Some affections were recorded in this study during clinical observation (Table 6) and the total number of these affections were 9, 7, 13 and 5 cases in G1, G2, G3 and G4 respectively. The results of bacteriological examination revealed isolation of Staph. Sp., Strept. Sp., Salmonella sp. As predominant bacteria beside micrococci, Serratia sp. and anthracoids from affected sheep. Sarcoptic mites were detected from sheep manifested alopecia. These affection began nearly at 3 m. post beginning of experiment and might occur as a result of a state of immunosuppression permitting secondary infection to ensue. This interpretation was previously achieved by Muneer et al., (1988). Clinical cases contributed to clostridium infection were not appeared on the experimental sheep during this study.

In conclusion, the presences of sodium nitrate or copper sulfate or both in drinking water decreased or suppressed the immune response of vaccinated sheep and might produce unprotective titer after short time of vaccination in comparison with control. This interferes with vaccination programmes and may increase the susceptibility of animals to secondary infection and also leads to a decrease in body weight gain. So, attention should be paid for water analysis before vaccination and avoidance of drinking water.

containing these agents in levels higher than permissible limits to obtain maximum protective immune response.

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Table (1): Statistical analysis of the chemical constituents of water samples collected from sheep farm.

| Chemical Constituents | Concentration M \pm S.E. | Permissible limit (PPM) | No. of samples exceeding (P.L.) | Frequency |
|-----------------------|-------------------------------|----------------------------|------------------------------------|-----------|
| Ammonia | 0.71 \pm 1.9 | 0.5 | 5 | 50 % |
| Nitrite | 2.31 \pm 1.4 | 0.0 | 0.0 | 0.0 % |
| Nitrate | 110.13 \pm 10.8 | 45 | 7.0 | 70 % |
| Phosphate | 4.14 \pm 1.2 | 0.0 | 0.0 | 0.0 % |
| Sulphate | 120.52 \pm 20.3 | 200 | 0.0 | 0.0 % |
| Copper | 2.51 \pm 1.1 | 1.00 | 6.0 | 60 % |
| Zinc | 1.91 \pm 0.03 | 5.00 | 0.0 | 0.0 % |
| Manganese | 0.35 \pm 0.05 | 0.10 | 5.0 | 50 % |
| Iron | 0.09 \pm 0.003 | 0.30 | 0.0 | 0.0 % |

P.L. according to Anon (1984).

Table (2): Mean values of total and differential leukocytic count of treated and control sheep at 2 m. post beginning of experiment.

| Groups Item | G1 sod.nitrate treated group | G2 copper sulfate treated group | G3 combination | G4 control |
|--------------------------|------------------------------------|---------------------------------------|--------------------|------------------|
| TWBCs $10^3/\text{mm}^3$ | 7.52 \pm 0.55* | 8.68 \pm 0.13 | 6.85 \pm 0.62** | 9.1 \pm 0.044 |
| Lymphocyte % | 54.83 \pm 0.41* | 55.78 \pm 0.24 | 52.13 \pm 0.37** | 56.1 \pm 0.39 |
| Neutrophil % | 34.81 \pm 0.38** | 35.87 \pm 0.36* | 32.67 \pm 0.43** | 37.16 \pm 0.49 |
| Monocyte % | 2.71 \pm 0.46* | 1.81 \pm 0.32 | 2.98 \pm 0.63* | 1.32 \pm 0.34 |
| Basophil % | 2.61 \pm 0.28* | 1.51 \pm 0.35 | 1.76 \pm 0.33 | 1.22 \pm 0.48 |
| Eosinophil % | 4.75 \pm 0.41 | 3.83 \pm 0.37 | 4.51 \pm 0.28 | 4.6 \pm 0.43 |

TWBCs: Total white blood cells

*Significant at $P < 0.05$

**Highly significant at $P < 0.01$

Table (3): Mean values of different parameters in sheep given sod. nitrate, copper sulphate or combination in comparison to control.

| Parameters | Duration after water drinking in | | | | | | | |
|------------------|----------------------------------|-------------|--------------|------------|------------------|-------------|--------------|------------|
| | G1 (Sod. nitrate) | | G2 (Cu, Sod) | | G3 (Combination) | | G4 (Control) | |
| Groups | 1.m.p.w.d | 2.m.p.w.d | 1.m.p.w.d | 2.m.p.w.d | 1.m.p.w.d | 2.m.p.w.d | 1.m.p.w.d | 2.m.p.w.d |
| T.P gm/dL | 6.3±0.35* | 5.7±0.26** | 6.89±0.31 | 6.28±0.44* | 5.8±0.13** | 5.1±0.43** | 7.92±0.52 | 7.80±0.41 |
| Alb gm/dL | 1.8±0.43 | 1.4±0.41* | 2.14±0.64 | 1.86±0.31 | 1.6±0.37* | 1.2±0.22** | 2.81±0.34 | 2.78±0.38 |
| Glob gm/dL | 4.5±0.36 | 4.3±0.26 | 4.79±0.48 | 4.42±0.36 | 4.0±0.35* | 3.9±0.41* | 5.11±0.37 | 5.02±0.44 |
| Alp. U/L | 158.3±6.8* | 171.9±4.6** | 134.8±3.3 | 136.7±5.5 | 181.4±3.9** | 192.1±3.4** | 130.2±5.1 | 128.4±4.5 |
| GGT U/L | 30.9±0.71* | 35.8±0.66** | 29.4±0.86 | 32.1±0.94* | 33.5±0.35** | 38.4±0.67** | 28.1±0.9 | 29.01±0.7 |
| GPT U/L | 42.5±2.3 | 48.3±2.0** | 40.3±1.1 | 43.2±2.0 | 44.8±1.8 | 51.1±2.7** | 38.5±2.4 | 39.4±2.1 |
| GOT U/L | 273.9±3.5 | 283.4±4.01* | 275.6±1.9 | 280.9±3.3* | 283.4±2.8* | 293.3±3.9** | 270.5±4.5 | 272.1±1.3 |
| Urea mg/dL | 22.6±1.4 | 24.7±1.5* | 23.2±1.1 | 23.4±1.5 | 24.9±1.0* | 29.4±1.8** | 20.3±1.3 | 19.5±1.7 |
| Creatinine mg/dL | 2.2±0.64 | 3.1±0.39 | 2.0±0.54 | 2.6±0.62 | 2.4±0.65 | 3.6±0.64* | 1.8±0.87 | 1.9±0.74 |
| Copper µg/dL | 135.9±2.2 | 137.8±2.4 | 139.6±2.1 | 143.2±1.8* | 138.9±1.9 | 144.5±2.1* | 135.2±2.3 | 138.5±1.75 |
| IgG mg/ml | 17.1±1.32 | 16.81±1.91 | 17.88±1.64 | 17.69±2.1 | 16.94±1.37 | 14.1±1.18* | 18.75±1.51 | 17.33±1.25 |
| IgM mg/ml | 2.23±0.35 | 2.14±0.35 | 2.28±0.46 | 2.24±0.57 | 2.12±0.65 | 1.35±0.38* | 2.37±0.81 | 2.61±0.45 |

m.p.w.d: month post water drinking with Cu Sod or sod. nitrate or both

* Significant at P<0.05

**Highly significant at P<0.01

Table (4): Mean of antitoxin titer of sheep after vaccination at different periods.

| M.P.V Before | G1 | | G2 | | G3 | | G4 | |
|--------------|------------|------------|-----------|-----------|------------|-----------|-----------|----------|
| | B* | E** | B | E | B | E | B | E |
| M1 | 3.4±0.81* | 0.0** | 4.5±0.73 | 3.1±0.58* | 1.3±0.41** | 0.0** | 6.2±0.84 | 4.8±0.62 |
| M2 | 6.7±0.53** | 3.6±0.71** | 9.6±0.88* | 5.5±0.7* | 3.5±0.85** | 1.5±0.4** | 12.8±0.94 | 7.5±0.53 |
| M3 | 4.5±0.62** | 2.8±0.96** | 7.8±0.63* | 4.1±0.55* | 2.6±0.54** | 1.0±0.3** | 10.5±0.85 | 6.4±0.76 |
| M4 | 2.4±1.01** | 1.0±0.72** | 8.7±0.57* | 3.5±0.88 | 1.4±0.39** | 0.0 | 8.5±0.57 | 4.8±0.81 |

B: Beta toxin

E: Epitoxin

* Significant at P<0.05

**Highly significant at P<0.01

M.P.V = Month post vaccination

Table (5): Effect of some water pollutants on body weight of sheep.

| Groups | Body weight | |
|--------|--|-----------------------------|
| | Before beginning of experiment (M±S.E) | 2.m.post experiment (M±S.E) |
| G1 | 28.8±0.73 | 32.4±0.85* |
| G2 | 27.5±0.61 | 33.7±0.77 |
| G3 | 29.4±0.84 | 31.3±0.91* |
| G4 | 28.2±0.66 | 34.5±0.75 |

* Significant at P<0.05

Table (6): Some affections recorded during clinical observation of sheep at 2. m to 4.m post beginning of experiment.

| Affections | Number of affected animals in: | | | |
|-------------------|--------------------------------|------------|------------|------------|
| | G1 (10) | G2 (10) | G3 (10) | G4 (10) |
| Respiratory signs | 2 | 3 | 4 | 1 |
| Diarrhoea | 3 | 0 | 3 | 2 |
| Alopecia | 1 | 0 | 2 | 0 |
| Skin abscess | 2 | 2 | 3 | 2 |
| Conjunctivitis | 1 | 2 | 1 | 0 |
| Total | 9 | 7 | 13 | 5 |