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EFFECT OF APITHERAPY ON DIMINISHING THE INCIDENCE OF PNEUMOENTERIC INFECTIONS IN BUFFALO CALVES

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SUMMARY

This study was designed to evaluate the prophylactic effect of bee venom apipuncture on diminishing the prevalence of pneumonenteric infections in newly born buffalo calves.

During the period of the study which lasted 6 weeks, a marked significant decrease of diarrhea and respiratory infections was observed in the bee venom treated calf group (n = 40) compared to the control group (n = 44). In the bee venom treated group 17.5% of the calves showed undifferentiated diarrhea, 15% suffered pneumonia while 67.5% of the calves remained healthy in contrast to the control calf group in which prevalence of diarrhea and pneumonia were 29.5% and 18.5% respectively, whereas 45.5% remained healthy. No deaths occurred in the bee venom treated group, on the other hand, three calves died in the control group.

The immunological investigation sh wed an improvement in the immune system as a result of bee venom apipuncture giving maxim m antibodies production represented by a significant increase of IgG level.

Accordingly we can conclude that bee venom could be used as a novel medication to improve the health condition and immunological status of buffalo calves without producing any side effects or allergic reactions.

INTRODUCTION

Respiratory and enteric infections represent a serious problem in buffalo calves farms. Approximately 76 - 80 % of the newborn calves get scour within the first 2 weeks of their life to decrease gradually until the 7th week (El-Garhy, 1982).

Various preventive and therapeutic approaches have been used to address the problem of non differentiated diseases in suckling calves. A standard preventive approach is to evaluate colostral management by reviewing procedures for feeding of colostrum and analyzing serum samples to detect failure of passive transfer. Other evaluative steps concentrate on treatment once disease de-

velops, which involves an assessment for disease detection methods and use of treatments consisting of antibiotics and electrolytes solutions.

Immunotherapy is another treatment module that has been actively investigated in recent years, especially that the relationships between low serum immunoglobulin concentration and incidence of diarrhea (Boyd et al., 1974, Fisher et al., 1976 and Fallon 1978) and pneumonia (Hurvell and Fey 1970, Thomas and Swan 1973 and Blom 1982) have been well established in calves.

Immunotherapy is based on non specific stimulation of the immune system. It doesn't involve recognition of specific antigen or targeting the immune response toward a specific pathogen but rather causes an overall immune response that hasten the recognition of foreign proteins, restimulating the normal immune response after a period of immunosuppression or increases anamnestic response after immunization or exposure to pathogens.

Apitherapy or bee venom therapy is one of these immunotherapy treatment modules under investigation. Simics (1994) reported that, bees have been appreciated for medicinal purposes as a treatment for chronic diseases and restoring the vitality of the body.

In the present work immunoglobulins classes (IgG, IgM, IgA, and IgE) were measured in newly born suckling buffalo calves following Bee Venom- apipuncture to study the effect of Abevac (A novel natural biological product that has been developed composed of Bee venom preparation which has special pharmacological activity) on serum immunoglobulin classes and their relationship with the susceptibility of the suckling buffalo calves to diarrhea and pneumonia.

MATERIAL AND METHODS

Animals

Eighty four male and female buffalo calves of age not more than one week and body weight 35 - 40 kg belonging to three different private farms in Giza governorate were the subject of this study. All the calves were born from mature buffaloes and remained with their dams for colostrum suckling. The calves were fed milk during the whole period of treatment which lasted for 6 weeks. All selected animals were assigned to non treated control (n = 44) and treated groups (n = 40). All animals were kept under the same managemental conditions with regards to nutrition, housing and necessary hygienic conditions. Animals were keenly and regularly observed and any ill-health conditions, abnormal signs or ailment were registered and clinically interpreted.

A-Non treated control group (n=44)

Some of the calves under investigation suffered from undifferentiated diarrhea manifested by greenish yellow to grey feces with consistency varying from watery to semi-solid. They endured as well from systemic illness (different degrees o dehydration, dullness, simple fever (40°C) and in appetence to milk). Other calves suffered fron pneumonia and showed fever (40°C), nasal dis charges, cough, dyspnea and respiratory distress. Diarrheic calves were given oral rehydrating solu tion (ORS, 500ml) every 4 hrs during the firs day, and on the second day ORS was given wit equal amount of milk. They were also treated b Amoxicillin trihydrate 15mg/kg B.wt. deep I/M injection, 1ml/10kg. B.wt. every 12 hrs for days.

Pneumonic calves were treated by potentiated sulfonamides (25mg/kg I/V or I/M every 24 hr for 5 days), bisolvon ampoules I/M (2 ampoules daily for 2 days) and AD3E I/M injection once every week

B- Treated groups (n=40)

Abevac vials: contain 1 mg bee venom in the form of lyophilized powder manufactured by the Egyptian organization for biological products and vaccines (VACSERA), the powder is dissolved in 2 ml of distilled water

Certain accupoints were selected for Abevac injections, the calves were injected I/D on GV-1 (Jiao-chao) at the indentation between the base of the tail and the anus and ST-25 (Hai-men) about 1 cm lateral to the umbilicus (Choi et al., 2003).

This apitherapy was carried out every second day for 6 weeks, beginning with an initial dose of 0.2 ml of the dissolved powder, followed by a maintenance dose of 0.4 ml; each dose was divided for both injection sites. Rectal temperature was measured and the injection areas were checked for adverse reactions every day during the treatment period.

Some calves of this group were slightly diarrheic while others showed bilateral serous nasal discharges without signs of systemic reactions (normal milk appetite and normal body temperature). Neither oral nor parentral antimicrobial therapy was administered.

Samples

Blood samples were obtained from the jugular vein of each calf in clean centrifuge tubes without anticoagulants, just before treatment and at the end of every week during the test period 6 weeks). Sera were separated by centrifugation and stored at -20°C until assayed. The sera were clear and free from hemolyses.

Determination of serum immunoglobulin classes

Serum IgG, IgM, IgA levels were measured by single radial immunodiffusion technique (Mancini et al., 1965 and Fahy and Mackelvey 1965) using specific plates for IgG and IgM and IgA (Diffu-plat, Biochientifica S.A., Argentina). Ring diameter was measured after 22 hrs (IgM, and IgA) and 18 hr (IgG) of incubation at room temperature, according to the manufacture's instructions.

Serum immunoglobulins E levels were determined according to WHO, (1994)

Statistical analysis

The obtained results were processed statistically according to SPSS, where mean value and standard error were presented. Comparison between groups for significance was done using T test.

RESULTS

The results revealed that 13 (29.5%) calf of the control group suffered from undifferentiated diarrhea, 8 (18.2%) suffered from pneumonia while 20 (45.5%) remained healthy. Two (4.5%) diarrheic and 1(2.3%) pneumonic calf died.

In the Abevac treated group 7 (17.5%) suffered from undifferentiated diarrhea, 6 (15%) suffered from pneumonia, while 27 (67.5%) of the calves remained healthy throughout the study period without occurrence of deaths (table 1).

Table (1) Diarrhea, pneumonia and deaths in control and Abevac treated groups

Animal groups	Diarrheic calves		Pneumonic calves		Healthy		Deaths	
	No.	%	No.	%	No.	%	No.	%
Control group (n=44)	13	29.5	8	18.2	20	45.5	3	6.8
Abevac treated group (n=40)	7	17.5	6	15	27	67.5	0.0	0.0

Table 2 Serum IgG (mg %) concentrations in control and Abevac treated groups

	Control group	Abevac treated group
Before treatment	1629.17 ± 69.12	1621.06 ± 58.12
W1	1607.11 ± 71.52	1628.13 ± 63.27
W2	1577.19 ± 63.22	1729.12 ± 71.25*
W3	1543.25 ± 72.31	1699.18 ± 67.32*
W4	1521.21 ± 64.29	1737.29 ±73.12**
W5	1497.16 ± 39.12	1817.79 ±64.25**
W6	1409.37 ± 43.13	1803.23 ± 61.33**

*: Significant at $P < 0.05$
**: Significant at $P < 0.01$

Fig 1 Serum IgG (mg %) concentrations in control and Abevac treated groups

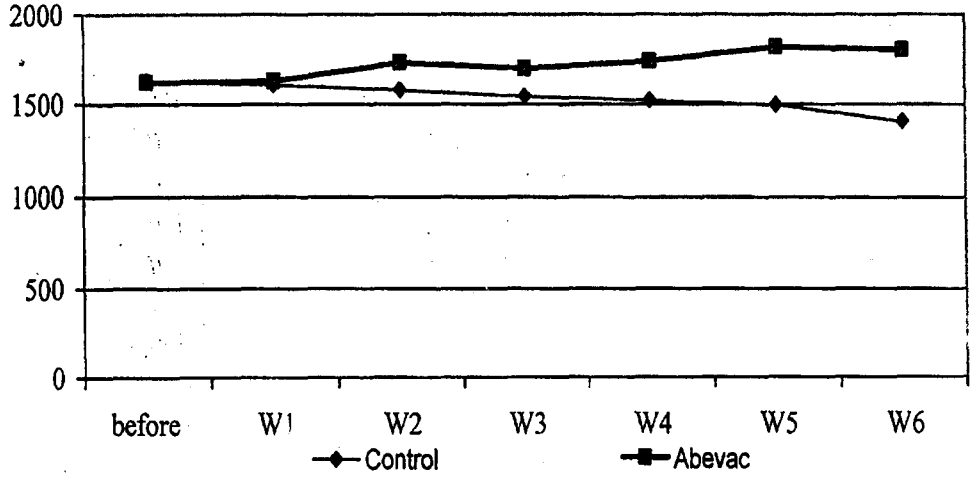


Table 3 Serum IgM (mg %) concentrations in control and Abevac treated groups

	Control group	Abevac treated group
Before treatment	179.06 ± 13.29	184.22 ± 16.26
W1	178.09 ± 16.07	182.19 ± 17.21
W2	178.31 ± 19.17	181.13 ± 18.29
W3	176.27 ± 13.26	179.24 ± 18.30
W4	175.22 ± 17.21	175.60 ± 15.67
W5	174.21 ± 19.31	169.36 ± 19.16
W6	172.36 ± 14.36	168.50 ± 16.70

*: Significant at $P < 0.05$

Fig. 2 Serum IgM (mg %) concentrations in antibiotic control and Abevac treated groups

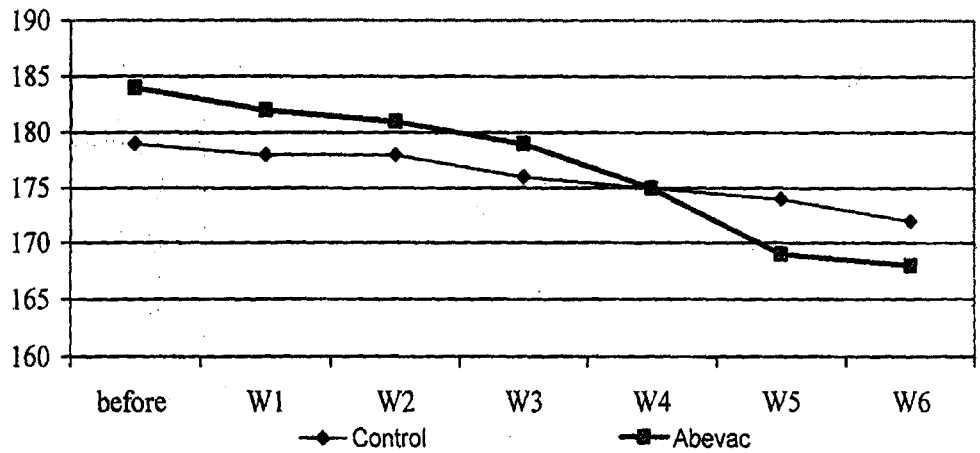


Table 4 Serum IgA (mg %) concentrations in control and Abevac treated groups

	Control group	Abevac treated group
Before treatment	21.06 ± 2.10	22.12 ± 2.00
W1	20.90 ± 2.13	22.23 ± 2.19
W2	20.93 ± 2.11	23.06 ± 1.87
W3	20.82 ± 2.17	23.01 ± 1.98
W4	21.03 ± 2.12	23.17 ± 1.93
W5	21.08 ± 1.97	23.07 ± 1.98
W6	21.09 ± 1.95	23.09 ± 1.91

*: Significant at $P < 0.05$
**: Significant at $P < 0.01$

Fig. 3 Serum IgA concentrations in control group and Abevac treated group

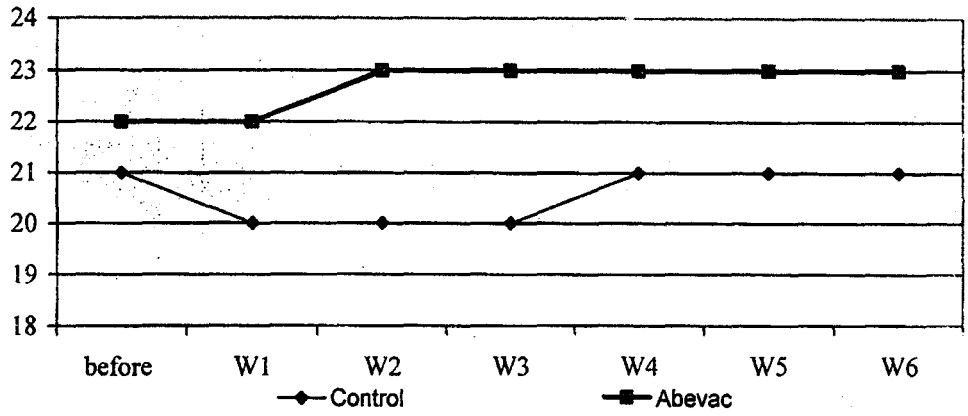
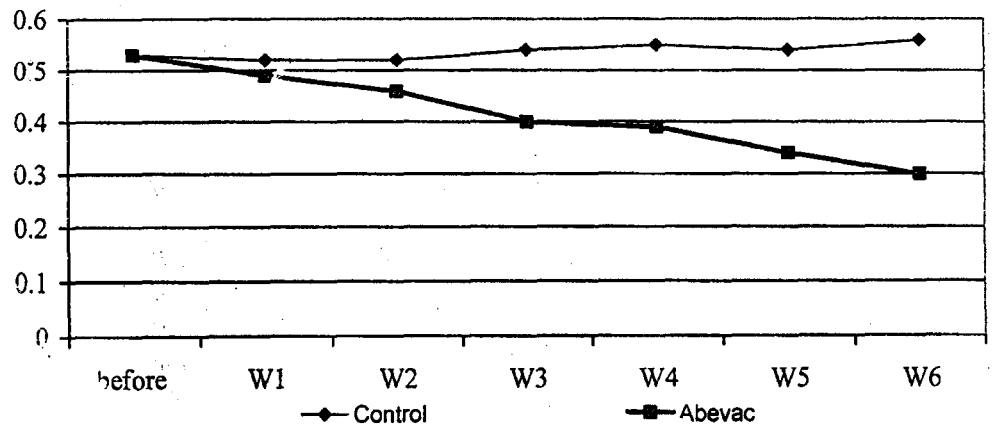


Table 5 Serum IgE (Iu/ml) concentrations in control and Abevac treated groups

	Control group	Abevac treated group
Before treatment	0.53 ± 0.07	0.53 ± 0.07
W1	0.52 ± 0.06	0.49 ± 0.05
W2	052 ± 0.06	0.46 ± 0.09
W3	0.54 ± 0.07	0.40 ± 0.03
W4	0.55 ± 0.03	0.39 ± 0.02*
W5	0.54 ± 0.08	0.34 ± 0.04*
W6	0.56 ± 0.09	0.30 ± 0.06**

*: Significant at $P < 0.05$
**: Significant at $P < 0.01$

Fig. 4 Serum IgE concentrations control group and Abevac treated group



Immunological parameters

Serum IgG level showed a slight significant increase (1729.12 ± 71.25 mg/dl) 2 weeks from the beginning of the trial ($P < 0.05$), followed by a moderate significant increase (1737.29 ± 73.12 mg/dl) 4 weeks and (1803.23 ± 61.33 mg/dl) 6 weeks from the beginning of bee venom injections (Table 2 fig. 1).

On the other hand the control group showed a serum IgG value of 1577.19 ± 63.22 mg/dl after 2 weeks, 1521.21 ± 64.29 mg/dl after 4 weeks and 1409.37 ± 43.13 mg/dl after 6 weeks.

Serum IgM value (table 3 & fig. 2) showed a non significant decrease in the buffalo calves treated with bee venom while Serum IgA (table 4 & fig. 3) showed a non-significant increase within the same group through out the trial period

Serum IgE (table 5 & fig 4) showed a non significant decrease after 2 weeks followed by a slight significant decrease 4 weeks later ($P < 0.05$) reaching a moderate significant decrease ($P < 0.01$) 6 weeks from bee venom injections. The estimated values were 0.64 ± 0.09 IU/ml after 2 weeks, 0.39 ± 0.02 IU/ml after 4 weeks and 0.30 ± 0.06 IU/ml after 6 weeks in the trial calves group compared to 0.52 ± 0.06 IU/ml, 0.55 ± 0.03 IU/ml, and 0.56 ± 0.09 IU/ml after 2, 4, and 6 weeks respectively in the control calves group.

DISCUSSION

Since newly born calves are neurologically well developed but physiologically immature they are vulnerable to several infectious agents. However in the last years in the calf industry, outbreaks of diarrhea or pneumonia tend to be sporadic with low morbidity and mortality rates. Various preventive and therapeutic approaches have been used to address the problem of non differentiated diseases in suckling calves. One of the novel treatments in the animal field is the application of bee venom therapy which is the part of apitherapy which utilizes bee venom in the treatment of health conditions. Apitherapy has been used in human beings (Lin, 1987, Feng, 1989) and in pigs (Hwang and Jenkins, 1988 and Lin et al., 1988) for the purpose of diarrhea treatment.

One of the studies involving human beings was performed by Lin, (1987) in which 86.8% of 170 children suffering from infantile diarrhea, recovered and even chronic diarrhea that showed resistance against previous treatments with traditional Chinese or western medicines was reduced using apitherapy (Feng, 1989). On the other hand Choi et al., (2003) concluded from the trial they designed to examine the therapeutic effect of honeybee venom in piglets (n = 47) with bacterial diarrhea that bee venom therapy was effective in controlling bacterial diarrhea in preweaning piglets up to 93.6%.

The purpose of the present study was to shed some light on the ability of bee venom to alleviate the clinical signs of diarrhea and respiratory diseases of newly born buffalo calves or to prevent them during the maintenance of bee venom therapy.

In the bee venom treated group only 17.5% of the calves showed undifferentiated diarrhea, 15% suffered from pneumonia, while 67.5% of the calves remained healthy in contrast to the control calf group in which prevalence of diarrhea and pneumonia was 29.5% and 18.5% respectively where-

as 45.5% remained healthy. No deaths occurred in the bee venom treated group, while three calves died in the control group. From the later we could conclude that injection of bee venom has effectively lowered the incidence of diarrhea and respiratory infections in calves.

Apitherapy does not only possess a strong antibacterial, antifungal and radioprotective effect (Aretmove 1958) but is also known to induce an immunomodulating effect (Roger et al., 1992). It could enhance the release of endogenous opoid peptides. This compound may not only boost the immune system but also reduces the intestinal response to the enterotoxins (Hwang and Jenkins, 1988).

Stimulation of the immune system could be clearly seen from the significant boost in the total level of serum IgG in bee venom injected calves along with a significant decrease in serum IgE 6 weeks from the beginning of the study when compared to the control calves.

Increase in the total level of serum IgG could be interpreted as a direct immunological reaction to Phospholipase A2 (PLA2) which represents the major antigen and allergen in the bee venom constituents (Kagey-Sabotka, et al., 1976 and M¸ller et al., 1995).

Normally, as an initial response to bee sting, low-affinity IgG1 anti-PLA_2 antibodies are elicited (Aalberse, et al., 1983). Repeated exposure to bee venom generates high-affinity IgG4 anti-PLA antibodies (Carballido, et al., 1993 and Lucas, 1990). Akdis et al., (1996) demonstrated that increased IL-10 production at the beginning of bee venom immunotherapy. IL-10 was mostly produced by PLA-specific T cells, and maintain by B cells and monocytes. IL-10 has a direct c mitogenic effect on B cells and promotes B c antibody production (Stites et al., 1997). The creased IL-10 production also accounts fo change in PLA-specific IgE and IgG4 ratio in vor of IgG4 (Akdis et al., 1996).

Decrease in Serum IgE could be explained by triggering of Th1 cytokine IFN- as a result of i venom immunotherapy (Secrist, et al., 1993, tori et al., 2000, von Garnier et al., 2000). T secreted IFN-7 lowers the IgE production (St et al., 1997). As previously reported by Mül (1994) and Akdis et al., (1996) decrease in I production means decrease in systemic aller reaction.

The results of this study therefore encourage u recommend the use of intradermal bee venom lution injections as a natural immunomodulato give immune protection against enteric and piratory diseases which are considered the n important influencing factors in neonatal mortalities.

REFERENCES

Aalberse, R.C., R. van der Gaag, and J. van Leuwen (1 Serologic aspects of IgG4 antibodies. I.†Prolonged munization results in an IgG4-restricted response. J munol. 130: 722-726

Akdis, C. A., M. Akdis, T. Blesken, D. Wymann, S. S kan, U. Muller, K. Blaser. 1996. Epitope-specific T tolerance to phospholipase A2 in bee venom imm therapy and recovery by IL-2 and IL-15 in vitro. J. Invest. 98:1676.

Artemov, N.M. (1958). The physiological proofs of api apy. Bee venom actsas a cholinolytic agent in the Th. International apicultural congress, Rome, Italy

Astori, M., C. von Garnier, A. Kettner, N. Dufour, G. radin, F. Spertini. 2000. Inducing tolerance by in

sal administration of long peptides in naive and primed CBA/J mice. J. Immunol. 165:3497

Blom, Y.B. (1982). The relationship between serum immunoglobulins values and incidence of respiratory disease and enteritis in calves. Nord. Veterinaer., Med., 34:276 -281

Boyd, J.M., Baker, J.R., and Legland.(1974). Neonatal diarrhea in calves. Vet.Rec.,95: 310-313

Carballido, J.M., N. Carballido-Perrig, M.K. K‰gi, R.H. Meloen, B. W¸thrich, C.H. Heusser, and K. Blaser (1993) T cell epitope specificity in human allergic and non-allergic subjects to bee venom phospholipase A2. J. Immunol. 150: 3582-3591

Choi, S.H, Cho, S.K., Kang, S.S., Bae, C.S ., Bai, Y.H ., Lee, S.H ., and Pak, S.C . (2003) . Effect of apitherapy in piglets with preweaning diarrhea. Am. J. Chin. Med.; 31(2):321-6

ElGarhy,M.M.(1982).Studies on the digestive troubles of the newly born calves.Ph.D. Thesis, Fac.Vet.Med. Cairo University.

Fahy,J.L., and Mackelvey,E.M.(1965). Quantitative determination of seum immunoglobulin in antibody agar plates. J. Imm.,94: 84-90

Fallon, R.J. (1978). The effect of immunoglobulins levels on calf performance and methods of artificially feeding colostrums to the newborn calf. Ann. Rech. Vet., 9: 347-352

Feng,W.L.(1989).Acupuncture treatment for 30 cases of infantile chronic diarrhea. J. Tradit.Med.9:106-107

Fisher, E.W., Martinez, A.A.,Trainin, Z. and Meirom, R. (1976). Studies of neonatal calf diarrhea.IV serum and faecal immunoglobulins in neonatal salmonellosis. Br.Vet.J., 132:39-49

Hurvell,B., and Fey, H. (1970).Comparative studies on the gamma globulin level in sera of market calves in relation to their health. Acta. Vet. Scand., 11:341-360

Hwang, Y.C., and Jenkins,E.M (1988). Effect of acupuncture on young pigs with induced enteropathogenic Escherichia coli diarrhea. Am.J. Vet. Res.49: 1641-1643

Kagey-Sabotka, A., R.M. Franklin, N.F. Adkinson Jr., M. Valentine, H. Bauer, and L.M. Lichtenstein (1976) Allergy to insect sting. II. Phospholipase A: the major allergen in honey bee venom. J. Allergy Clin. Immunol. 57: 29-40

Lin, J.H., Lo, Y.Y., Wang, N.S., Lai,S.G. Kung and Chan, W.W.(1988) . Control of preweaning diarrhea in piglets by acupuncture and Chinese medicine medicine. Am. Chin.Med. 16:75-80

Lin, Y,C.(1987). Observation of therapeutic effects of acupuncture in 170 cases of infant diarrhea. J. Tradit. Chin. Med. 7:203-204

Lucas, A.H. (1990) IgG subclass restricted immune responses to allergens. Semin. Immunopathol. 12: 385-420

Mancini, G., Carbonara, A.O., and Heremans,J.F. (1965).Immunochemical quantitation of antigen by single radial immunodiffusion. Immunochemistry, 2:235-242

Mih·ly Simics,(2001).Bee venom therapy. Copyright © 1998-2001 by Mih·ly Simics. Apitronic Services, 9611 No. 4 Road, Richmond, B.C., Canada, V7A 2Z1, HYPERLINK "http://www.beevenom.net/" http:// www.beevenom.net/

Muller, U.R. (1994)/ Insect venom allergy. Ther, Umsch. Jan, 51(1):38-44

Müller, U.R., T. Dudler, T. Schneider, R. Crameri, H. Fischer, D. Skrbic, R. Maibach, K. Blaser, and M. Suter (1995) Type 1†skin reactivity to native and recombinant phospholipase A2 from honey bee venom is similar. J. Allergy Clin. Immunol. 96: 395-402

Roger, P.A., Schoen, A.M., and Limehouse, J. (1992). Acupuncture for immune mediated disorders.Literature review and clinical application. Probl.Vet.Med. 4:162-193

Secrist, H., C.J. Chelen, Y. Wen, J.D. Marshall, and D.T. Umetsu (1993) Allergen immunotherapy decreases interleukin 4†production in CD4 T cells from allergic individuals. J. Exp. Med. 178: 2123-2130

Stites, D. P., Terr, A. I. and Parslow, T. G. (1997). Medical immunology 9th ed Middle east edition Appelton & Lange Norwalk, Connecticut/San Mateo California

Thomas, L.H., and Swan, R.G.(1973). Influence of colostrums on the incidence of calf pneumonia. Vet. Rec.,92:454-455.

von Garnier, C., M. Astori, A. Kettner, N. Dufour, C. Heusser, G. Corradin, F. Spertini. 2000. Allergen-derived long peptide immunotherapy doown-reggulates specific IgE response and protects from anaphylaxis. Eur. J. Immunol. 30:1638.

World Health Organizatioon (1994): Expert committee on biological standardization. WHO. Tech. Rep. Ser. 1994, 840:7.

تاثير العلاج بسم النحل على معدل الأصابات التنفسية و المعوية فى عجول الجاموس حديثى الولادة

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هذه الدراسة صممت لتقييم التأثير الوقائي لحقن سم النحل على الأقلال من معدل الأصابات التنفسية و المعوية فى عجول الجاموس حديثى الولادة. اثناء فترة الدراسة التى امتدت لمدة ستة اسابيع لوحظ انخفاض معدل حالات الأسهال والأصابات التنفسية فى مجموعة العجول الجاموس المحقونة بسم النحل (40 عجل) مقارنة بعجول المجموعة الضابطة (44 عجل)

سجلت17.5% حالة أسهال و15% أصابة تنفسية فى عجول الجاموس المحقونة بسم النحل بألاضافة الى عدم نفوق اى من العجول فى هده المجموعة مقارنة بالمجموعة الضابطة حيث سجلت29.5% حالة أسهال و18.5 % حالة أصابة تنفسية بينما نفق ثلاثة منها طوال فترة التجربة

تبين من الفحص المناعى تحسن فى مناعة العجول الجاموس المحقونة بسم النحل فى زيادة معنوية فى مستوى الاجسام المضادة من نوع (ج) مقارنة بالمجموعة الضابطة

فقد تبين ان حقن سم النحل يعتبرعلاج حديث لرفع المناعة فى عجول حديثى الولادة و تحسين الحالة الصحية بدون اى اعراض جانبية او حساسية