A preliminary study on the DNA-Vaccine for chicken protection against tick *Argas persicus* (Oken, 1818)

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ABSTRACT

The percent tick rejection upon feeding on DNA-immunised chicken had fluctuated between 74.64 ± 6.33 and 89.39 ± 3.15%. The reaction between the DNA-vaccinated chicken serum and the tick salivary gland proteins by enzyme linked immuno sorbent assay (ELISA) was positive and ranged between 0.190 ± 0.01 and 0.306± 0.012 absorbency units (AU) for the doses 200 and 800 µg DNA/kg chicken body weight, respectively. The check control was 0.139.± 0.017 AU. On the other hand, no positive reaction was detected by using the same chicken sera and Hyalomma dromedarii salivary gland proteins as non-specific antigen. There were positive reactions through ELISA against the gut proteins of A. pericus in sera collected after the first injection compared to the control group. After the second injection, the most significant effect was recorded for the vaccine DNA concentration 200 ug (0.375 ± 0.02 AU), then 1000 µg (0.269 ± 0.037 AU) and 600 µg (0.228 ± 0.011 AU). The concentration of 400 µg also increased the immune response of the chicken against the gut proteins after the fourth week, when the titration absorbance was 0.516 ± 0.014 AU with the control 0.077 ± 0.015 AU. The absence of non-specific reaction against the H. dromedarii gut protein proved that the antibodies in the serum of the DNA vaccinated chicken were specific for the A. persicus gut proteins. The electrophoretic pattern of the immunized chicken serum showed three new protein bands at the R_f 0.089, 0.0163 and 0.369 with molecular weights 225, 170 and 83 kDa, respectively. These protein bands indicated the development of the immune defense of the chicken against ticks.

***Key words**: Chicken tick, Argus persicus, Tick control, DNA-vaccine,*

INTRODUCTION

Protein-based immunization is at the basis of the well-established public health measure of vaccination. In 1993, a report of the World Bank concluded unambiguously that vaccination is the most cost-effective public health measure available.

Scientific progress is founded - more frequently than imagined - on methodological innovation. Thus, when the occasional revolution in vaccine methodology comes along, it is worth taking a serious look. New methods, in the hands of creative investigators, has led to new experimental approaches which give rise to new concepts and, occasionally, produce shifts in paradigms. Such a methodological leap would appear to have occurred with the advent of DNA-mediated immunization, now colloquially known as DNA vaccines (Whalen and Davis, 1995). Although the injection of DNA into tissues was originally reported in

the 1950s, the technology has gained more attention in recent years as a safe means of mimicking *in vivo* protein production normally associated with natural infection (Stasney *et al*., 1950). Nucleic acid or DNA inoculation is an important vaccination technique that delivers DNA constructs encoding specific immunogens directly into the host cell (Wolf *et al*., 1990; Tang *et al*., 1992; Tascon *et al*., 1996).

DNA or "genetic" vaccination (or as the World Health Organization suggested " nucleic acid vaccination) was now really and truly delivered into the scientific world. It may offer several potential advantages over traditional vaccination strategies such as whole-killed or live attenuated virus and recombinant protein-based vaccines. Since DNA vaccines are non replicating and the vaccine components are produced within the host cells, they can be constructed to function safely with the specificity of a subunit vaccine (Kim and Weiner, 2000). Vaccination based on DNA was applied in the field of veterinary protection. It has been shown that immunization of whole DNA libraries from pathogens can elicit a protective immune response against the pathogen (Barry *et al***.,** 1995; Manoutcharian *et al*., 1998; and Melby *et al.,* 2000). These libraries can then be easily fractionated and serially immunized as smaller and smaller library pools in order to eventually identify novel individual genes that stimulate immune protection. Immunization with an insect cDNA library may eventually allow for the identification of undiscovered vector antigen targets through such reductive immunization screening of the library.

Veterinary vaccines have their own set of desired characteristics. In addition to the obvious requirement for efficacy, vaccines for veterinary use has to be relatively inexpensive, stable under field conditions and easy to administer. These are the reasons for using genomic DNA to reduce expenses incurred by vaccination and handling. It is, therefore, our goal to process DNA vaccine against the chicken tick infestation.

MATERIALS AND METHODS

DNA Extraction

DNA was extracted from freshly *Argas persicus* eggs according to the method described by El-Fiky (2003).

Chicken and DNA injection

Eighty 21-day-old spring chickens were kept at 25ºC and 70% RH for one week. They were divided into eight groups of 10 chickens each. Groups 1 –7 were injected intramascularly (im) with 50, 100, 200, 400, 600, 800 and 1000 µg extracted DNA, respectively. The 8th group was injected with the buffer as a control. The injection was repeated weekly for three weeks. After the fourth week, the tick feeding rejection test was carried out to determine the percent of tick feeding rejection after the immunization process.

Blood samples were collected before the beginning of the vaccination protocol and thereafter weekly before the DNA injection as well as after the fourth week. The samples were centrifuged at 1500 rpm and 5ºC for 15 min. The plasma were collected and kept refrigerated under -40ºC.

Protein analysis

The total plasma protein was determined using the method of Lowery *et al*. (1952). Gel electrophoresis of plasma proteins and its analysis were carried out according to El Kammah and Sayed (1999) and Sayed *et al*. (2001).

Monitoring of serum anti-tick protein in DNA vaccinated chicken

ELISA test described by Caponi and Migliorini (1999) was carried out to monitor the production of antibodies using both specific and non-specific antigens. The proteins extracted from the salivary gland and gut of *A. persicus* were characterized as specific antigen. Non-specific antigens were the proteins extracted from the salivary gland and gut of *Hyalomma dromedarii*.

RESULTS

Effect of DNA vaccination on chicken rejection of tick feeding

The percent rejection of feeding ticks on immunized chicken ranged between 74.64 ± 6.33 in response to DNA vaccination with 50 µg DNA/kg body weight and 89.39 ± 3.15 % in response to 100 µg DNA/kg chick body weight (Fig. 1).

Estimation of anti-tick protein by ELISA

Vaccination with DNA had no significant immune effect against the chicken ticks after the first, second and third injection. One week after the third injection and after tick feeding, the antibodies response was detected at a level relevant to the different DNA concentrations used. The highest antibody level was detected with injected DNA doses of 200 and 800 µg DNA, which gave 0.190 ± 0.01 and 0.306.± 0.012 absorbency unit (AU), respectively, against the check control (0.139.± 0.017 AU) (Table 1). ELISA test for non-specific reaction using the *Hyalomma dromedarii* salivary gland proteins against serum antibodies of the same chicken under investigation gave no positive reaction (Table 2).

The chicken serum tested against the proteins extracted from the gut of *A. pericus* show positive reactions after the first injection with all DNA concentrations compared to the control group. After the second DNA vaccine injection, the most significant effect was recorded from the DNA concentrations of 200 µg (0.375 ± 0.02), the 1000 µg (0.269 ± 0.037) and 600 µg (0.228 ± 0.011). The concentration of 400 µg also increased the immune response of the chicken against gut proteins after the fourth week, as titration absorbance was 0.516 ± 0.014 AU compared to the control which was 0.077 ± 0.015 AU (Table 3).

Immunized chicken plasma proteins

No significant differences were found between the control and the vaccinated chicken plasma protein. The electrophoretic pattern of the plasma proteins showed an increase in the protein content of the band with molecular weight (MW) 260 kDa (R_f 0.055), which increased by 42.33, 96.25, 148.84, 167.23, 264.55, 692.12 and 421.74% of the control band content after the vaccination with DNA concentrations 50, 100, 200, 400, 600, 800 and 1000 µg/kg body weight, respectively. The bands with MW 146 and 28 kDa also showed an increase in protein content with the exception of the 600 µg/kg where the protein content band decreased non significantly. The protein band with MW 138 kDa (R_f 0.219) increased in the 800 and 1000 µg/kg b.w. treatment by 157.84 and 162 % compared to the same protein band in the control group. A significant decrease was recorded in the protein band content (MW 104 and R_f 0.298), which decreased to 9.02 and 10.21%, in the plasma chicken vaccinated with DNA concentrations of 50 and 100 µg/kg b.w., respectively, compared to the control. In contrast, content of this band was increased over the control level through all other vacciations (Table 4).

Four new bands were recorded in the electrophoretic patterns of the immunized plasma protein with molecular weights of 225, 220, 170, and 83 kDa (R_f 0.089, 0.098, 0.163 and 0.369, respectively). These new proteins may have been biosynthesized as a result of the expression of the injected DNA. These proteins may have been expressed tick proteins which played a role as antigens in the host blood and may build the vaccination function against *A. persicus*.

DISCUSSION

Anti-vector immunity was first demonstrated by Trager (1939) against the tick *Dermacentor variabilis* by animal immunization with homogenized tick extracts. Since then, only a few specific anti-vector molecular targets have been identified, and most of these targets are from ticks. Immunological targeting of tick midgut antigens has culminated in the commercial development of a recombinant protein vaccine against the cattle tick *Boophilus microplus* (Willadsen *et al.,* 1995). Also Sayed *et al.* (2001) isolated six protein fractions from the salivary gland of *A. persicas*, which were responsible for the chicken immunization against tick. However, the identification of one target tick antigen alone took 4 years to accomplish through the biochemical fractionation of kilograms of ticks down to microgram quantities of protein for serial vaccination and tick challenge studies (Willadsen *et al.,* 1989).

So the DNA vaccine technique is a suitable way to solve this problem. DNA immunization often stimulates potent cellular immunity in addition to humoral immunity against the immunogen, while protein immunization responses are often dominated by a humoral response (Gurunathan *et al*., 2000; Lai and Bennett., 1998; Robinson, *et al*., 1997).

DNA-based vaccination of chicken against *A. persicus* in the present study showed high rejection percent (about 74 – 89%) of the ticks after 4 hours being offered on vaccination ticks feeding. Foy *et al.* (2003) observed significantly increasing mortality among mosquitoes that were fed on either the *AgMuc1*- or the cDNA library-immunized mice compared to that of controls.

A. persicus DNA immunized chicken sera showed a higher content of IgG anti-gut protein than anti-salivary gland proteins. This indicates that through immunization with DNA extracted from *A. persicus* eggs, some proteins, which belong to the gut and salivary gland, were synthesized. In addition, the chicken immune system was activated due to these foreign proteins and produced antibodies against it. By analogy, Foy *et al*. (2003) showed that ELISA data were a measure of anti-midgut antibody quantity in the immune sera, which in mice immunized with mosquito DNA alone, are low enough to be mostly indistinguishable from preimmune sera. However, the ELISA data revealed high titers of anti-midgut protein antibody in mice boosted with midgut protein and higher quantities of midgut-binding IgG1 antibodies than IgG2a antibodies were observed in the same mice. Vaccination against the tick *Boophilus microplus* by two injections of DNA only induced very low immune responses in sheep (De Rose *et al.,* 1999).

REFERENCES

Barry, M. A., Lai, W. C., and Johnston, S. A. 1995. Protection against mycoplasma infection using expression-library immunization. Nature 377**:**632– 635.

Caponi, L. and Migliorini, P. (1999) ELISA Assay. In "Anti body usage in the lab" L. Caponi an P. Migliorini (Eds) Springer-Verlag. Berlin, Heidelberg, New York (1999) pp. 9-31.

De Rose, R., McKenna, R.V., Cobon, G., Tennent, J., Zakrzewski, H., Gale, K., Wood, P.R., Scheerlinck, J.P.Y. and Willadsen, P. (1999) Bm86 antigen induces a protective immune response against *Boophilus microplus* following DNA and protein vaccination in sheep. Vet. Immunol. Immunopathol. 71, 151-160.

El-Fiky Z. A. (2003). A simple and rapid method for mini preparation of high molecular weight DNA from certain acarines, bacteria and soybean. Insect Science and its Application, 23 (1): 51-57.

El Kammah, K. M. and Sayed, M. A. (1999). Biochemical fingerprints of egg and salivary gland proteins characterizing four common tick genera in Egypt. Arab. J. Biotech., 2 (2): 127-134.

Foy, B. D., Magalhaes, T., Injera, W. E., Sutherland I., Devenport, M., Thanawastien, A., Ripley, Ca´rdenas-Freytag, D., L., and Beier, J. C. (2003) Induction of Mosquitocidal Activity in Mice Immunized with *Anophele gambiae* Midgut cDNA. Infection and Immunity, 71 (4): 2032–2040.

Gurunathan, S., Klinman, D. M., and Seder, R. A. (2000). DNA vaccines: immunology, application, and optimization. Annu. Rev. Immunol., 18**:** 927–974.

Kim, J. J. and Weiner, D. B. (2000). Optimization of DNA vaccines through the use of molecular adjuvants. In: DNA vaccines: methods and protocols (ed.) D.B.Lewrie and R.G. Whalen, Humana Press Inc., p. 205.

Lai, W. C., and Bennett, M. (1998). DNA vaccines. Crit. Rev. Immunol., 18: 449–484.

Lowery, O. H.; Rosebrough, N. J.; Farr, A. L. and Randall, R. J. (1952) Protein measurment with the folin phenol reagent. J. Biol. Chem. 193: 265-275.

Manoutcharian, K., Terrazas, L. I., Gevorkian, G., and Govezensky, T. (1998). Protection against marine cysticercosis using cDNA expression library immunization. Immunol. Lett., 62**:** 131–136.

Melby, P. C., Ogden, G. B., Flores, H. A., Zhao, W., Geldmacher, C., Biediger, N. M., Ahuja, S. K., Uranga, J., and Melendez, M. (2000). Identification of vaccine candidates for experimental visceral leishmaniasis by immunization with sequential fractions of a cDNA expression library. Infect. Immun., 68**:** 5595–5602.

Robinson, H. L., Ginsberg, H. S., Davis, H. L., Johnston, S. A., and Liu, M. A. (1997). The scientific future of DNA for immunization. American Academy of Microbiology critical issues colloquia, Washington, D.C.

Sayed, M. A., Habeeb, S. and El Kammah, K. M. (2001). Chicken immunological reaction to salivary gland proteins of *Argas persicus* (Oken) (Acari: Argasidae). Int. J. Acarol., 27 (2): 145-149.

Stasney, J, Cantarow, A. and Paschkis, K. E. (1950). Production of neoplasms by injection of fractions of mammalian neoplasms. Cancer Res., 11 777-782.

Tang, D., Devit, M. and Johnston, S. (1992). Genetic immunization is a simple method for elicting an immune response. Nature, 356: 152-154.

Tascon, R. E., Colston, M. J., Ragno, S., Stavropoulos, E., Gregory, D. and Lowrie, D. B. (1996). Vaccination against tuberculosis by DNA injection. Nature Med., 2: 888-892.

Trager, W. (1939). Acquired immunity to ticks. J. Parasitol., 25**:** 137–139.

Whalen, R. G. and Davis, H. L. (1995) DNA-mediated immunization and the energetic immune response to hepatitis B surface antigen. Clin Immunol Immunopathol;75 (1): 1-12.

Willadsen, P., Riding, G. A., McKenna, R. V., Kemp, D. H., Tellam, R. L., Nielsen, J. N., Lahnstein, J., Cobon, G. S., and Gough, J. M. (1989). Immunologic control of a parasitic arthropod. Identification of a protective antigen from *Boophilus microplus*. J. Immunol., 143**:** 1346–1351.

Willadsen, P., Bird, P., Cobon, G. S., and Hungerford, J. (1995). Commercialisation of a recombinant vaccine against *Boophilus microplus*. Parasitology, 110**:** 543-550.

Wolf, J. A., Malone, R. W., Williams, P., Chong, W., Acsadi, G., Jani, A. and Felgner, P. L. (1990). Direct gene transfer into mouse muscle *in vivo*. Science, 27: 1465-1468.

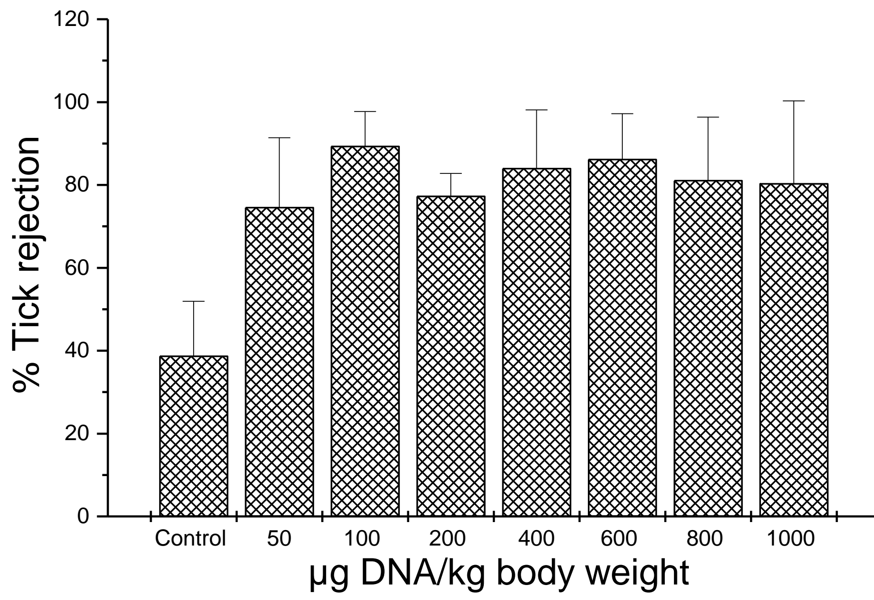

Fig (1): Percent Argas persicus ticks rejection after chicken immunization with three weekly doses of A. persicus egg DNA intramascular injections.

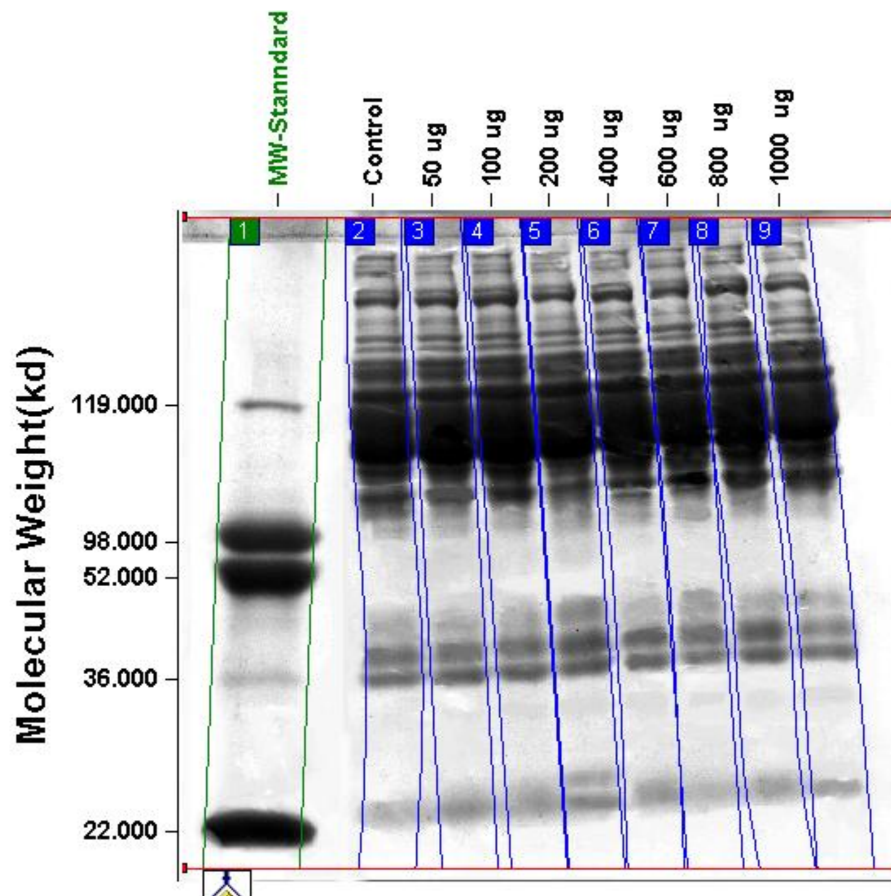

Fig. (2) Electrophoretic pattern of chicken plasma after the third injection with DNA.

Table (1): Specific ELISA test for DNA-vaccine against salivary gland protein of A. persicus.

Concentration (ug/kg)	After 1st injection	After 2nd injection	After 3rd injection	One week after feeding
Control	0.08 ± 0.02	0.192 ± 0.013	0.120 ± 0..026	0.139 ± 0.017
50	0.05 ± 0.016	0.155 ± 0.012	0.106 ± 0.031	0.173 ± 0.02
100	0.07 ± 0.03	0.181 ± 0.049	0.075 ±0.005	0.123 ± 0.007
200	0.08 ± 0.024	0.175 ± 0.025	0.082 ± 0.006	0.190 ± 0.010
400	0.08 ± 0.028	0.156 ± 0.02	0.081 ± 0.013	0.162 ± 0.013
600	0.074 ± 0.016	0.149 ± 0.041	0.095 ± 0.016	0.176 ± 0.005
800	0.055 ± 0.023	0.158 ± 0.013	0.095 ± 0.016	0.306 ± 0.012
1000	0.058 ± 0.031	0.163 ± 0.022	0.071 ± 0.008	0.135 ± 0.012

Table (2): Non-Specific ELISA test for DNA-vaccine against salivary gland protein of H. drommedrii,.

Concentration (ug/kg)	After 1st injection	After 2nd injection	After 3rd injection	One week after feeding
Control	0.052 ± 0.005	0.038 ± 0.01	0.007 ±0.001	0.05 ± 0.006
50	0.029 ± 0.007	0.032 ± 0.003	0.018 ± 0.006	0.07 ± 0.004
100	0.027 ± 0.005	0.013 ± 0.003	0.015 ± 0.003	0.062 ± 0.007
200	0.049 ± 0.01	0.025 ± 0.005	0.014 ± 0.003	0.086 ± 0.01
400	0.034 ± 0.008	0.025 ± 0.011	0.009 ± 0.001	0.064 ± 0.004
600	0.055 ± 0.021	0.055 ± 0.017	0.019 ± 0.005	0.085 ± 0.011
800	0.041 ± 0.01	0.018 ± 0.004	0.011 ± 0.004	0.08 ± 0.011
1000	0.036 ± 0.009	0.019 ± 0.009	0.008 ± 0.001	0.06 ± 0.004

Table (3): Specific ELISA test for DNA-vaccine against gut protein of A. persicus

oncentration (ug/kg)	After 1st injection	After 2nd injection	After 3rd injection	One week after feeding
Control	0.051 ± 0.005	0.128 ± 0.009	0.171 ±0.011	0.077 ± 0.015
50	0.06 ± 0.01	0.17 ± 0.006	0.178 ± 0.008	0.149 ± 0.024
100	0.094 ± 008	0.140 ± 0.021	0.142 ± 0.011	0.120 ± 0.012
200	0.115 ± 0.015	0.375 ± 0.02	0.122 ± 0.009	0.326 ± 0.21
400	0.098 ± 0..014	0.195 ± 0.024	0.234 ± 0.02	0.516 ± 0.014
600	0.112 ± 0.03	0.228 ± 0.011	0.205 ± 0.01	0.187 ± 0.027
800	0.068 ± 0.01	0.118 ± 0.008	0.063 ± 0.010	0.202 ± 0.017
1000	0.087 ± 0.013	0.269 ± 0.037	0.085 ± 0.017	0.112 ± 0.004

Table (4): The relative content of each protein bands fractionated from DNA-vaccinated chicken plasma compared to the control group.

R_f value	Molecular Weight (kDa)	Injected DNA-vaccine in µg/kg body weight.							
		Control	50	100	200	400	600	800	1000
0.037	276	100	73.81	50.95	92.78	119.19	53.04	134.04	70.32
0.055	260	100	142.33	196.25	248.84	267.23	364.55	792.12	521.74
0.062	251	100	133.35	158.78	101.16	210.98	167.02	230.00	285.12
0.089	225						00.00	00.00	00.00
0.098	220		00.00	00.00	00.00	00.00	00.00	00.00	00.00
0.105	212	100	80.46	95.87	68.32	75.14	78.81	93.36	98.55
0.139	186	100	125.31	117.87	66.69	174.23	93.75	106.82	157.99
0.163	170			00.00	00.00	00.00	00.00	00.00	00.00
0.175	163	100	70.83						
0.178	155	100	80.69	98.56	83.10	104.87	34.53	38.22	37.09
0.203	146	100	150.60	125.09	121.28	134.22	93.43	218.93	237.14
0.219	138	100	66.36	73.36	77.12	72.90	69.85	257.84	262.00
0.257	120	100	86.31	102.40	114.25	95.91	99.28	101.97	98.13
0.298	104	100	9.02	10.21	151.83	176.69	106.04	183.22	145.70
0.343	90	100			172.23	75.83	105.01	75.74	99.85
0.369	83		00.00	00.00	00.00	00.00	00.00	00.00	00.00
0.380	80	100							
0.410	73	100	95.74	115.47	123.94	115.13	112.37	143.78	150.57
0.613	43	100	75.42	45.80	106.01	5.24	58.57	66.72	64.77
0.661	39	100	97.35	101.61	144.82	71.27	89.99	132.50	75.35
0.695	37	100	125.64	129.26	129.49	83.63	71.96	118.23	111.76
0.909	28	100	195.52	215.29	194.62	142.76	91.80	155.52	124.16

00.00= Band not detected in the control

الملخص العـربـى

دراســـة تمهـيديـــة على التـطعـيم بالحمض النـووى لوقايــة الدجــاج ضـد القراد *Argus persicus* (Oken, 1818)

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تراوحت النسـبة المئوية للقراد الذى رفض التغذية على الدجــاج المطعـم بالـ حمض النووى DNA مابين ٧٤,٦٤ ±٦,٣٣ و ٩,٣٩ ±٣,١٥ %. وأظهر التفاعل بين سيرم الدجاج الذى تم تطعيمه والبروتينات المستخلصة من الغدد اللعابية للقراد بواسطة اختبار الـ ELISA تفاعلا موجبا والذى تراوح بين ٠,١٩٠ ±٠,٠١ و ٠,٣٠٦ ±٠,٠١٢ وحدات امتصاص وذلك للجرعتين ٢٠٠ و ٨٠٠ ميكروجرام DNA/كيلوجرام من وزن الدجاجة. وكانت وحدات الامتصاص فى حالة الكنترول ٠,١٣٩ ±٠,٠١٧ . وأعطى اختبار الـ ELISA نتائج سـلبية مع نفس السيرم و عند اسـتخدام بروتين الغدد اللعابية من قراد الجمــال *Hyalomma dromedarii* (أنتيجين غير متخصص)

وقد ظـهرت تفاعلات إيجابية بين سيرم الدجاج المطعم وبروتينات القناة الهضمية لقراد الدجاج وذلك بعد الحقن الأول. بعد الحقن الثاني أظهرت التركيزات ٢٠٠ ، ١٠٠٠ و ٦٠٠ ميكروجرام/كيلوجرام من وزن الجسم تأثيرا معنويا حيث كان قيمة الامتصاص ٠,٣٧٥ ±٠,٠٢ ، ٠,٢٦٩ ±٠,٠٣٧ و ٠,٢٢٨ ±٠,٠١١ على التوالى. كما أظهر التركـيز ٤٠٠ ميكروجرام / كيلوجرام زيادة فى الاستجابة المناعية للدجاج ضد بروتينات القناة الهضمية للقراد حيث كلن قيمة الامتصاص فى اختبار الـ ELISA ٠,٥١٦ ±٠,٠١٤ بالمقارنة بقيمة الكنترول ٠,٠٧٧ ±٠,٠١٥ أن عدم ظهور أى اسـتجابة إيجابية فى الاختبارات المناعة على الانتيجين غير الـمتخـصص والمستخلص من النوع *H. dromedarii* يدل على أن الأجسام المضادة فى سيرم الدجاج المعامل متخصص لبروتينات من نوع القراد *A. persicus* .

أظهر التفريد الكهربائى لبروتينات السيرم الخاص بالدجاج المطعم ثلاثة بروتينات جـديدة ذات نقطة سريان (R_f) ٠,٠٨٩ ، ٠,٠١٦٣ و ٠,٣٦٩ وذات أوزان جزيئيه ٢٢٥ ، ١٧٠ ، و ٨٣ كيلودالتون، على التوالى.وهذه البروتينات توضح نشاط الجهاز المنــاعى للدجاج ضد القراد.