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 μg (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 compared 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 25°C 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 5°C for 15 min. The plasma were collected and kept refrigerated under -40°C.

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 dromedari*.

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).

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 kDa 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*.

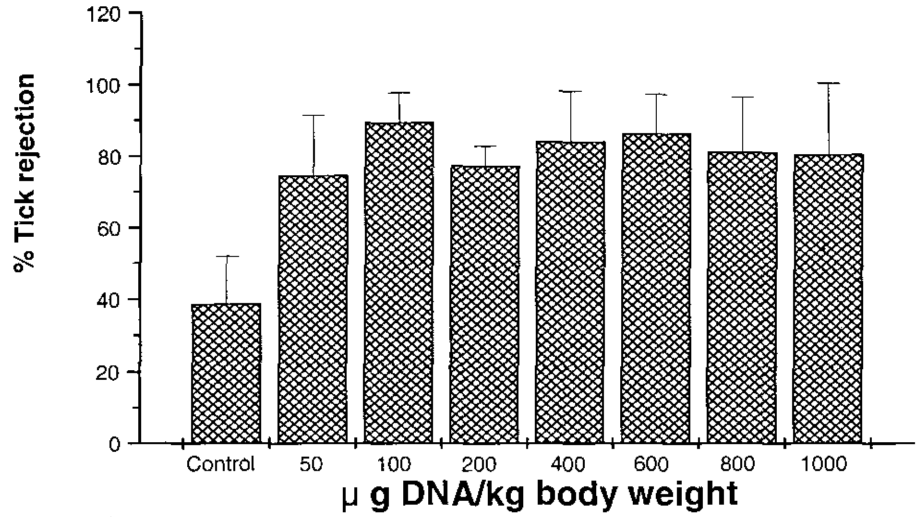

Fig (1): PercentArgas persicusticks rejection after chicken immunization with three weekly doses ofA. persicusegg DNA intramascular injections.

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

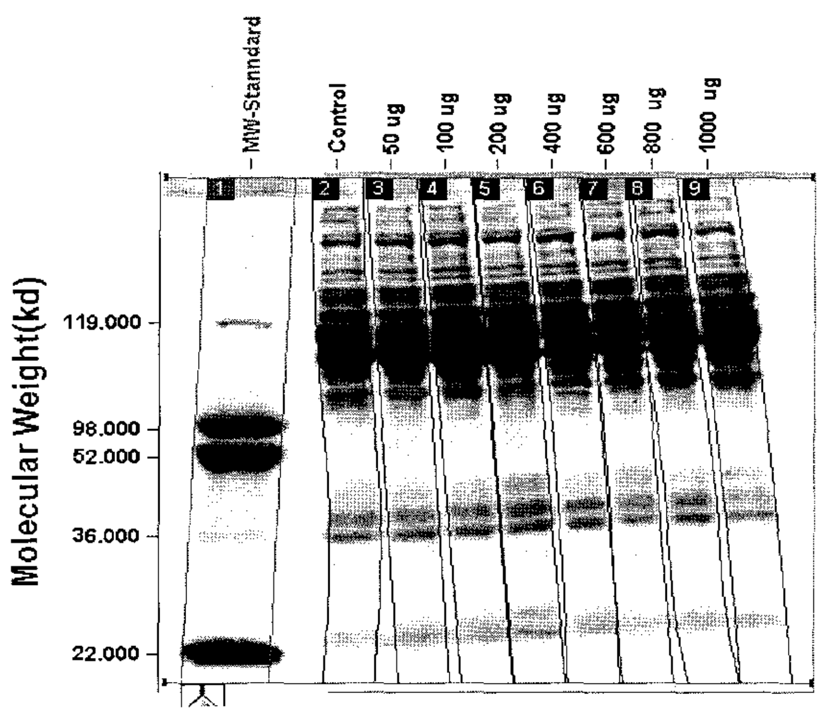

Table (1): Specific ELISA test for DNA-vaccine against salivary gland protein of A. persicus.

Concentration (μg/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 tests for DNA-vaccine against salivary gland protein of H. drommedrii.

Concentration (μg/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 (μg/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

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 *AgMucl*- 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).

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الملخص العربي

دراســـة تمهـيديـــة على التـطـعـيم بالحمض النـووى لوقايـــة الدجـاج ضد القراد
Argus persicus (Oken, 1818)

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تراوحت النســــبة المئوية للقراد الذى رفض التغذية على الدجـــــاج المطعــــم بالـــ حمض النـــووى DNA مـــابين 74.64 ± 6.33 و 89.39 ± 3.15 %. وأظهر التفاعل بين سيرم الدجاج الذى تم تطعيمه والبروتينات المستخلصة من الغـــدد اللعابية للقراد بواسطة اختبار الـــ ELISA تفاعلا موجبا والذى تراوح بـــين 0.190 ± 0.01 و 0.306 ± 0.012 وحـــدات امتصاص وذلك للجرعتين 200 و 800 ميكروجرام DNA/كيلوجرام من وزن الدجاجة. وكانت وحدات الامتصاص فى حالة الكنترول 0.139 ± 0.017 . وأعطى اختبار الـــ ELISA نتائج ســـــلبية مع نفس السيرم و عند اســـــتخدام بروتين الغـــدد اللعابية من قراد الجمــــــال Hyalomma dromedarii (أنتيجين غير متخصص) .

وقد ظـــــهرت تفاعلات إيجابية بين ســـيرم الدجاج المطعم وبروتينات القناة الهضمية لقراد الدجاج وذلك بعـــد الحقـــن الأول. بعد الحقن الثاني أظهرت التركيزات 200 ، 1000 و 600 ميكروجرام/كيلوجرام من وزن الجسم تأثيرا معنويا حيـــث كان قيمة الامتصاص 0.375 ± 0.02 ، 0.269 ± 0.037 و 0.228 ± 0.011 على التوالى. كما أظهـــر التركـــــــيز 400 ميكروجرام / كيلوجرام زيادة فى الاستجابة المناعية للدجاج ضد بروتينات القناة الهضمية للقراد حيث كلن قيمة الامتصاص فى اختبار الـــ ELISA 0.516 ± 0.014 بالمقارنة بقيمة الكنترول 0.077 ± 0.015 أن عدم ظهور أى اســــتجابة إيجابية فى الاختبارات المناعة على الانتيجين غير الـــمتخـــصص والمستخلص من النـــوع *H. dromedarii* يـــدل علـــى أن الأجســـام المضادة فى سيرم الدجاج المعامل متخصص لبروتينات من نوع القراد *A. persicus* .

أظهر التفريد الكهربائى لبروتينات السيرم الخاص بالدجاج المطعم ثلاثة بروتينات جـــــديدة ذات نقطـــة سـريان (R_f) 0.089 ، 0.0163 و 0.369 وذات أوزان جزيئيه 225 ، 170 ، و 83 كيلودالتون، على التوالى.وهذه البروتينـــات توضـــح نشاط الجهاز المنـــــاعى للدجاج ضد القراد.