INVESTIGATION ON THE EFFECT OF SOME ANTIPARASITIC AND ANTIBIOTICS ON THE IMMUNE RESPONSE OF DOGS TO RABIES VACCINE

Mohamed I. Emam[a], El-Sayed M. Galila[b], Abdelfatah M. Abdelfatah[b], Abdelmonem M. Mustafa, Faysal K. Hamouda[b] and Mohamed Khodeir[c]

[a] police academy, [b] Faculty of Veterinary Medicine, Benha University, veterinary Serum and Vaccine Research Institute، Abbassia, Cairo.

A B S T R A C T

The present study was planned to determine the effect of some familiar drugs used for dog treatment including antibiotic as E-Mox®; antiparasitic drugs as levamisol® and Yomesan® on the immune response of dogs to the locally produced inactivated rabies vaccine, through the application of SNT and indirect solid phase ELISA. SNT revealed that specific rabies neutralizing antibodies stated from the first week post vaccination in vaccinated all puppy groups then increased gradually to reach their peak by the 4th week later then still unchanged till the 12th month. It was visible that the highest levels of serum neutralizing rabies antibodies were determined in puppies received Levamisol® reached 256. Other treated puppy groups did not show significant differences between them. The vaccinated un-treated group and Yomesan® treated group were reached 32 meanwhile the treated group with E-Mox® which showed the lowest antibody titer was 16. It was clear that the results of ELISA were in parallel to those of SNT showing that the highest levels of rabies ELISA antibodies were determined in puppies received Levamisol®. Other treated groups did not show significant differences. The results of the vaccinated un-treated group and Yomesan® treated group were similar. While the treated group with E-Mox® showed the lowest antibody titer. Similarly, the serum proteins showed elevation in the Levamisol® treated group. On the other hand the E-Mox® treated group showed the lowest serum proteins level. There were no significance differences the level of the serum protein among the other groups.

Key Words: Dog, Antiparasitic, Antibiotic, Rabies vaccine, SNT

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1. INTRODUCTION

Rabies virus is the type species of the Lyssavirus genus, in the family Rhabdoviridae, order Mononega-virales, Lyssaviruses have helical symmetry, with a length of about 180 nm and a cross-sectional diameter of about 75 nm [1]. From the point of entry, the virus is neurotropic, traveling quickly along the neural pathways into the central nervous system (CNS), and then further into other organs [2].The salivary glands receive high concentrations of the virus thus allowing further transmission. Any warm-blooded animal (including humans) may become infected with the rabies virus and develop symptoms [3]. Infected bats, [4] monkeys, raccoons, foxes, skunks, cattle, wolves, coyotes, dogs, mongoose (normally yellow mongoose) [5] or cats present the greatest risk to humans. Rabies may also spread through exposure to infected domestic farm animals, groundhogs, weasels, bears and other wild

carnivores. Small rodents such as squirrels, hamsters, guinea pigs, gerbils, chipmunks, rats, and mice and lagomorphs like rabbits and hares are almost never found to be infected with rabies and are not known to transmit rabies to humans [6].

The virus is usually present in the nerves and saliva of a symptomatic rabid animal [7]. The route of infection is usually, but not always, by a bite. In many cases the infected animal is exceptionally aggressive, may attack without provocation, and exhibits otherwise uncharacteristic behavior [8]. There are an estimated 55,000 human deaths annually from rabies worldwide, with about 31,000 in Asia, and 24,000 in Africa [9]. In these countries the virus is primarily transmitted through canines (feral dogs and other wild canine species). Recent reports suggest that wild rabid dogs are roaming the streets.

It was reported that, rabies is enzootic in jackals and common in dogs [10] and it was concluded that dogs and wolves are the primary vector animal for transmission of rabies to cattle in the Middle East [11].

The aim of this work was directed to investigate the effect of some drugs on the immune status of rabies vaccinated dogs.

2. MATERIALS AND METHODS

2.1. *Dogs:*

Twenty five native breed young puppies of 3 months age were used in the present study and all of them were found to be free from rabies antibodies as screened by serum neutralization test.

2.2. *Rabies virus:*

Cell culture adapted ERA strain of rabies virus was kindly supplied by the Department of Pet Animal Vaccine Research; Veterinary Serum and Vaccine Research Institute, Abassia, Cairo. It was supplied in a lyophilized form with a titer of 103.5 TCID50 / ml. This virus was used in serum neutralization test and for preparation of rabies antigen for ELISA.

2.3. *Rabies vaccine:*

Inactivated cell culture rabies vaccine was obtained from Veterinary Serum and Vaccine Research Institute, Abassia, Cairo. It was used for vaccination of experimental puppies.

2.4. *Drugs:*

2.4.1. E-Mox®

It is a broad spectrum antibiotic composed of 250 mg amoxicillin sodium injected intramuscularly every 8 hours from first day.

2.4.2. Yomesan®:

It is composed of 0.5g niciosamide /tablet and acts by direct contact with the tapeworm causing necrosis of the head and adjoining segments and thus the worm loses its hold and it's eliminated intact or in pieces with feces. One tablet was grind and giving in liquid after breakfast for 7 days from first day.

2.4.3. Levamisol®:

It was supplied by Bayer, Leverbusen, Germany and used at the dose of 0.7ml/ kg body weight injected subcutaneous as anthelmintic drug from first day.

2.5. *Cell culture:*

Baby hamster kidney cell line (BHK-21) established by (12) was used in serum neutralization test.

2.6. *Anti-dog immunoglobulin conjugated with horse radish peroxidase*

Anti-dog immunoglobulin [IgG whole molecule] conjugated with peroxidase [Horse Radish Peroxidase enzyme (HRP)] was obtained from Sigma chemical company(USA) and treated before use by double dilution to cover the expected range and the suitable dilution was selected by using P.B.S with bovine albumin and tween 20 which gives suitable color where that titer was1:10,000.

2.7. *Kits of serum proteins*

Biocan Laboratory Reagents (Germany) were used to estimate total serum protein and serum albumin in sera of experimental dogs.

2.8. *Experimental vaccination of puppies*
Twenty five puppies were divided into 5 groups (5 puppies/ group) as follow:
Group-1 was treated with E-Mox 250mg/8hours injected intramuscularly for 5 days. Rabies vaccine was inoculated on the first day of E-Mox treatment using 2ml injected subcutaneous.
Group-2 was vaccinated with rabies vaccine on the first day of the puppy's treatment with Yomesan using one tablet daily for 7 days.
Group-3 was treated with levamesol at the dose of 0.7ml/kg body weight injected subcutaneous in each puppy vaccinated with rabies vaccine.
Group-4 was vaccinated with rabies vaccine without any treatment.
Group-5 was kept without vaccination and without treatment.
All puppy groups were housed under hygienic measures in separate kennels receiving balanced diet and adequate water.

2.9. *Sampling*
Blood samples were obtained from the experimental puppies through the jugular vein puncture under complete aseptic conditions according to [13].

2.10. *Serum Neutralization test (SNT)*
SNT was carried out using the micro technique according to [14].

2.11. *Indirect ELISA*
The indirect method of ELISA was for quantitative determination of rabies antibodies according to [15] and [16].

3. RESULTS

Rabies antibodies were followed up in the sera of all experimental puppy groups through the application SNT and indirect solid phase ELISA. SNT revealed that vaccination of puppies with the inactivated cell culture rabies vaccine resulted in induction of neutralizing antibodies from the first week post vaccination in all puppy groups increased gradually to reach their peak by the 4th week later then still unchanged till the 12th month (table 1). It was visible that the highest levels of serum neutralizing rabies antibodies were determined in puppies received Levamisol (256). Other treated puppy groups did not show significant differences between them and the vaccinated un-treated group and Yomesan treated group (32) except the treated group with E-Mox which showed the lowest antibody titer (16).

In addition, it was clear the results of ELISA were parallel to those of SNT showing that the highest levels of rabies ELISA antibodies were determined in puppies received Levamisol (2.91), Other treated puppy groups did not show significant differences between them and the vaccinated un-treated (2.5) group and Yomesan treated group (2.3) except the treated group with E-Mox which showed the lowest antibody titer (1.5) as tabulated in table (2).

It was noticed that the highest levels of serum proteins were determined in puppies received Levamisol (8.87±0.25; 1.9±0.07 & 6.7±0.22 g/dl for total protein; albumin and globulin respectively).Other treated puppy groups did not show significant differences between them and the vaccinated un-treated group and Yomesan treated group (8.3±0.55; 1.98±0.07&6.32±0.33g/dl and 7.78±0.14; 1.3±1.1& 5.9±0.9g/dl in untreated and puppies received Yomesan for total protein; albumin and globulin respectively) except the treated group with E-Mox which showed the lowest levels (6.9±1.1; 2.5±0.04& 4.4±0.30 for total protein; albumin and globulin respectively) as tabulated in table (3).

4. DISCUSSION

Dogs may come suffering from a disease caused by different infections needed rapid treatment using different drugs of different

Table 1. Rabies serum neutralizing antibody titers in vaccinated puppies

Periods post vaccination	Mean rabies SNT titer*				
	E-Mox®	Yomesan®	Levamisol®	Rabies vaccine	Control
1W**	2	2	16	4	0
2W	4	8	32	8	0
3W	16	8	64	16	0
4W	16	16	256	32	0
2M***	16	32	256	32	0
4MP	16	32	256	32	0
6M	16	32	256	32	0
8M	16	32	256	32	0
10M	16	32	256	32	0
12M	16	32	256	32	0

*Rabies antibody titer: the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100 $TCID_{50}$ of rabies virus.**WPV: week post vaccination.***MPV= month post vaccination.

Table 2: Rabies ELISA antibody titers in vaccinated puppies

Periods post vaccination	Mean ELISA antibody titer (log 10/ml)				
	E-Mox®	Yomesan®	Levamisol®	Rabies vaccine	Control
1W*	0.80	1.50	2.37	1.69	0.24
2W	0.94	2.00	2.84	2.10	0.38
3W	0.96	2.10	2.86	2.30	0.70
4W	1.50	2.30	2.91	2.50	0.30
2M**	1.49	2.30	2.90	2.52	0.25
4M	1.50	2.31	2.92	2.50	0.44
6M	1.52	2.30	2.92	2.50	0.32
8M	1.50	2.28	2.93	2.51	0.44
10M	1.49	2.29	2.91	2.53	0.36
12M	1.49	2.30	2.89	2.54	0.23

*WPV= week post vaccination. **MPV= month post vaccination

Table 3: Serum protein levels in treated vaccinated puppies

Drug	Serum proteins (gm/dl) on weeks post treatment								
	Total serum protein			Serum albumin			Serum globulin		
	1W*	2W	3W	1W	2W	3W	1W	2W	3W
E-Mox®	6.4 ±1.1	6.5 ±0.4	6.9 ±0.3	2.9 ±0.15	2.89 ±0.09	2.5 ±0.09	3.5 ±0.90	3.75 ±0.45	4.4 ±0.30
Yomesan®	7.2 ±0.23	7.5 ±0.22	7.98 ±0.14	1.9 ±0.8	1.6 ±0.11	1.3 ±1.1	5.3 ±0.22	5.6 ±0.33	5.9 ±.09
Levamisol®	7.9 ±0.1	8.1 ±0.12	8.87 ±0.25	2.1 ±0.7	1.9 ±0.15	1.5 ±0.45	5.0 ±0.0	5.99 ±1.1	6.7 ±0.22
Rabies vaccine	7.45 ±0.33	7.8 ±0.45	8.30 ±0.55	2.5 ±0.15	2.01 ±0.02	1.98 ±0.07	4.95 ±0.49	5.79 ±0.59	6.32 ±0.33
Control	6.1±0.15			3.1±0.32			2.5±0.07		

*W: week post treatment.

actions and accordingly the present work was planned to investigate the effect of some antibiotics as E-Mox®; antiparasitic drugs as levamisol® and Yomesan® on the immune response of dogs to the locally produced inactivated rabies vaccine in addition to investigate the effect of such drugs on the levels of serum proteins.

SNT revealed that vaccination of puppies with the inactivated cell culture rabies vaccine resulted in induction of specific rabies neutralizing antibodies from the first week post vaccination in all puppy groups increased gradually to reach their peak by the 4th week later then still unchanged till the 12th month (table 1). It was visible that the highest levels of serum neutralizing rabies antibodies were determined in puppies received Levamisol® (256). Other treated puppy groups did not show significant differences between them and the vaccinated un-treated group (32) except the treated group with E-Mox® which showed the lowest antibody titer (15).

In addition, it was clear the results of ELISA were parallel to those of SNT showing that the highest levels of rabies ELISA antibodies were determined in puppies received Levamisol® (2.91). Other treated puppy groups did not show significant differences between them and the vaccinated un-treated (2.5) group and Yomesan® treated group (2.3) except the treated group with E-Mox® which showed the lowest antibody titer (1.5) as tabulated in table (2).

These results came to be parallel to and confirmed by the findings of [17];[18];[19];[20];[21] and [22] who stated that the cell culture inactivated rabies vaccine is safe for all animal species and clarified that the protective neutralizing antibody titer should not be less than 1:5.

Regarding the effect of the used drugs on the immune response of vaccinated puppies, it was stated that Levamisol® has an immune stimulant effect on treated vaccinated puppies where it was showed that Levamisol® fast the formation of protective levels of specific antibodies (23). It was found that Levamisol® enhances the immune response to influenza vaccines [24] and it was mentioned that Levamisol® enhances specific and nonspecific immunity to Corynebacterium [25]. Also Levamisol® increased the antibody levels to many viral vaccines as demonstrated by [26];[27];[28];[29];[30];[31];[32]; and [33].

On the other side the low titer of rabies antibodies in puppies treated with E-Mox® could be attributed to its content of amoxicillin which has an immune suppressive effect in vaccinated animals where it was established that amoxicillin administration durably altered the gut microbiota of these animals [34]. They observed that the induction of the specific humeral response to ovalbumin was impaired when it occurred during antibiotic administration to the rat pups. Globally, these data suggest that antibiotic administration early in life negatively affects the specific immune response to a luminal antigen when it is first introduced during antibiotic administration.

Estimation of serum proteins showed that the highest levels of serum proteins were determined in puppies received Levamisol® (8.87±0.25; 1.9±0.07 & 6.7±0.22 g/dl for total protein; albumin and globulin, respectively). Other treated puppy groups did not show significant differences between them and the vaccinated un-treated group and Yomesan® treated group (8.3±0.55; 1.98±0.07&6.32±0.33g/dl and 7.78±0.14; 1.3±1.1& 5.9±0.9g/dl in untreated and puppies received Yomesan® for total protein; albumin and globulin, respectively) except the treated group with E-Mox® which showed the lowest levels (6.9±1.1; 2.5±0.04& 4.4±0.30 for total protein; albumin and globulin, respectively) while un-vaccinated un-treated puppies had 6.1±0.15; 3.1±0.32& 2.5±0.07g/dl for total protein; albumin and globulin, respectively as tabulated in table (3). These findings were obtained by the 3rd week post vaccination. In general; the increased levels of total serum protein is attributed to the induced antibodies which

mainly composed of globulin coming in agreement with it was stated that serum proteins could be increased in animals after immunization [35]. It was reported that serum total protein; albumin and globulin were found to be 6.1-7.8gm/dl; 3.1-4.0gm/dl and 1.3-3.3gm/dl respectively in healthy dogs [36]. The albumin was found to be decreased due to inhibition of synthesis of albumin or an increase in globulin concentration. Alternation in globulin especially Gama globulin was usually a response of reticulo-endothelial system to antigenic stimulation (viral; bacterial or parasitic); it was found that low levels of serum albumin were accompanied with high levels of beta and Gama globulins which might be due to reduction in albumin synthesis in the liver[37]. It was stated that in dogs, the total serum protein, albumin and globulin were 5.7-7.7gm/dl; 2.5-4.0gm/dl and 3.2-3.7gm/dl respectively [38]. Total serum protein level was found to be increased with age increased with antigenic stimulation; it was showed that there was an increase in the levels of total serum protein due to the increased globulin in dogs vaccinated with rabies; canine distemper and canine parvovirus vaccines under normal conditions[39]. It was clearly noticed that high levels of serum proteins were determined in a parallel manner with high levels of induced rabies antibodies in vaccinated puppies treated with Levamesol®, with lower levels of serum proteins and antibodies in puppies treated with E-Mox® which suppressed puppy immune response.

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استبيان تأثير بعض مضادات الطفيليات والمضادات الحيوية على الاستجابة المناعية للقاح السعار

محمد ابراهيم امام[1]، السيد مصطفى ابراهيم[2] ، عبد الفتاح منجد عبد الفتاح[2] ، عبد المنعم محمد مصطفى[2] فيصل خليل حمودة[2] ومحمد حسن خضير[3]

[1]اكاديمية الشرطة، [2]كلية الطب البيطري –جامعة بنها، [3]معهد بحوث الامصال واللقاحات البيطرية بالعباسية–القاهرة

الملخص العربي

لقد تم تصميم الدراسة الحالية لبحث تأثير بعض العقارات الشائع استخدامها في علاج الكلاب وتشمل المضادات الحيوية: مثل الايموكس ومضادات الطفيليات: مثل الليفاميزول واليوميزان على الاستجابة المناعية للكلاب للقاح السعار المصنع محليا بالإضافة لبحث تأثير تلك العقارات على مستوي البروتين في مصل الدم. وقد تم تتبع مستوى الاجسام المناعية للسعار في مصل الدم لكل الاجراو المقام عليها التجربة باستخدام اختبار المصل المتعادل واختبار الانزيم المناعي المرتبط الغير مباشر

اوضح اختبار المصل المتعادل ان تحصين الاجراو بلقاح السعار قد ادي الي ظهور اجسام مناعيه من الاسبوع الاول للتحصين والتي تزايدت حتى وصلت لأعلي مستوي لها في الاسبوع الرابع وظلت كما هي حتى الشهر الثاني عشر. وقد لوحظ ان اعلي مستوي لتلك الاجسام قد ظهر في الاجراو التي تم علاجها بعقار الليفاميزول(265) بينما لم يلاحظ اختلاف بين تلك التي لم يتم علاجها (32) وتلك التي عولجت باليوميزان (32)،اما ما تم علاجه بعقار الايموكس فقد لوحظ فيه انخفاض لمستوي الاجسام المناعية في الجسم (16)عن تلك التي لم يتم علاجها باي عقار. وقد كانت النتائج الناتجة عن اختبار الانزيم المناعي المرتبط الغير مباشر موازيه لتلك التي تمت بطريقه اختبار المصل المتعادل واوضحت ان اعلى مستوى للاجسام المناعيه قد ظهر فى تلك المجموعة التي تم علاجها بعقار الليفاميزول(2.91)، بينما التي لم يتم علاجها (2.5) لم تظهر اختلاف عن المجموعة التي تم علاجها بعقار اليوميزان(2.3) ،فيما اظهرت النتائج انخفاض نسبه الاجسام المناعية فى المجموعة التي تم علاجها بالايموكس (1.5). بقياس مستوى البروتينات فى مصل الدم لوحظ ارتفاع مستواه فى المجاميع التي تم علاجها بالليفاميزول، بينما لم ترتفع فى المجموعة التي تم علاجها بعقاراليوميزان وانخفض فى المجموعة التي تم علاجها بعقار اليموكس.

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