

PREVALENCE OF TOXOPLASMA ANTIBODIES
AMONG HORSES IN IRAQ

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SUMMARY

Sera from 525 Arabian horses were tested for the presence of antibodies to *Toxoplasma gondii* by the indirect haemagglutination test. Of these sera, 39(7.43%) were positive. Thirty eight (3.15%) of seropositive animals were horses of two to 13 years of age and only one of the yearlings (1.5 year old) was seropositive. Twenty six of males (6.57%) and 13 of females (10.08%) were seropositive. There was no significant ($P>0.05$) difference in the prevalence of toxoplasmosis between males and females.

INTRODUCTION

Toxoplasmosis is an infectious disease affecting nearly all species of animals. It is of particular importance in human beings, sheep and goats as a cause of abortion, stillbirth or neonatal death (Watson, 1972; Blewett and Watson, 1984; Dubey *et al.*, 1981). In horses, *T. gondii* causes multifocal myelomalacia (Cusick *et al.*, 1974). While infection is common in several species of animals, it does not often develop into clinically recognizable disease. There are few studies on the prevalence of toxoplasmosis in horses (Vanderwagen *et*

al., 1974; Riemann et al., 1975) and non has been reported from Iraq. The present study was conducted to determine the prevalence of *Toxoplasma* antibodies among 525 Arabian horses.

MATERIALS AND METHODS

Blood samples were collected from the jugular vein of 525 apparently normal Arabian horses. Their ages ranged from one month to 13 years. Serum was separated and stored at -20C until tested. All sera were heat-inactivated at 56C for 30 minutes then screened for the presence of antibodies against *T.gondii* by the indirect haemagglutination test using a ToxHA test kit (Wellcome Reagent Ltd, Beckenham, England). Sera were screened at 1:64 dilution in microtitration plates and those gave positive reactions were titrated to the end point.

RESULTS

Of the 525 sera tested, 39(7.43%) were positive. None of the foals (< 1 year) were seropositive for *T. gondii*, but one of the yearlings (1.5 year) and 38 (8.15%) of the adults (2 to 13 years) were seropositive (Table 1). Statistically there was no significant ($P > 0.05$) difference in the prevalence of infection between males and females. Two out of 12 mares were seropositive (titres of 1:64) while all their 12 foals were seronegative.

DISCUSSION

A serological survey of toxoplasmosis in Baghdad revealed that 70.1% of sheep and 72.7% of goats examined were positive to the indirect haemagglutination test (Rasheed, 1984). The high rates of reactors were probably due to the extensive grazing of these animals on pastures

contaminated with cat feces. The prevalence of *Toxoplasma* antibodies among horses examined in the present study was 7.43%. The low incidence of reactors might be attributable to the fact that these horses were kept in stables at one confined area where the exposure to the sources of infection, such as cats, was minimal.

Seropositive horses were mainly in the group of two to 13 years of age. Similarly, Riemann *et al.* (1975) reported that the rate of infection in horses increased with advancing age. There was no significant ($P > 0.05$) difference in the incidence of reactors between males and females. This agreed with the findings of Riemann *et al.*

Table 1. Distribution of antibody titres to *Toxoplasma gondii* among horses according to age and sex.

	No. tested	No. seropositive(%)	Serum antibody titres		
			1:64	1:256	1:1024
Age in years					
< 1	12	0	0	0	0
1-2	47	1(2.13)	1	0	0
2-13	466	38(8.15)	27	8	3
Sex					
Males	396	26(6.57)	18	6	2
Females	129	13(10.08)	10	2	1
Total	525	39(7.43)	28	8	3

(1975) and Aganga and Ortese (1984) who surveyed the serological prevalence of *Toxoplasma gondii* in horses and dogs respectively.

Two mares had titres of 1:64 while their three and four month old foals were seronegative. This was apparently due to the short duration of persistence of maternal antibodies to *T. gondii*.

Further studies towards the epidemiology of toxoplasmosis in the whole country are needed.

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مدى انتشار داء المقوسات في الخيول في العراق

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الخلاصة

لقد تم فحص 525 عينة مصل خيول للكشف عن وجود الاجسام
المناعية للـ Toxoplasma gondii بواسطة اختبار تلازن الدم الغير
مباشر. وقد اظهرت 39 (7.43%) عينة نتائج موجبة. تراوحت اعمار
38 من الحيوانات الموجبة مصليا بين 2-13 سنة وحيوان اثبتت
اصابته مصليا كان بعمر سنة ونصف. وكانت مصول 26 من الذكور (6.57%)
و 13 من الاناث (10.08%) موجبه بهذا الاختبار. لم يظهر اي فرق
معنوي ($P > 0.05$) في مدى انتشار داء المقوسات بين الذكور والاناث.

INDUCTION OF ESTRUS DURING THE NON BREEDING
SEASON IN AWASSI EWES BY EXOGENOUS
HORMONAL TREATMENT

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SUMMARY

Sixty four randomly selected Awassi ewes were used for estrus induction. Four types of hormonal regimes were tested for this purpose. these ewes were diagnosed clinically anestrus after careful clinical examination and teasing by a detector ram for a period of thirty five days during late spring and summer. The ewes were divided into four groups. The first group received 7.5 mg Luprostiol and the second group received 125 ug Cloprostenol. Both drugs are synthetic analogues of prostaglandin F₂ alpha. The third group was treated with intravaginal sponges impregnated with 60 mg medrox acetate progesterone (MAP) for 14 days. The fourth group was subjected to the same vaginal sponges treatment for only 9 days followed by an injection of 500 IU of pregnant mare serum gonadotrophin (PMSG).

Luprostiol (PGF₂ alpha) and progesterone in the form of (MAP) did not induce estrus in the treated groups. Cloprostioli (Synthetic PGF₂ alpha) induced estrus in only 20% of the ewes. The combination of progesterone and PMSG had the best results. This last hormonal combination induced estrus in 93% of the ewes. The

results indicated that induction of estrus in the clinically anestrus ewes during the non breeding season (late spring and summer) through the use of hormonal treatment may be an effective and feasible method to increase the reproductive life of ewes.

INTRODUCTION

Production of sheep depends on their reproductive efficiency and on the application of a good breeding program (Ott and Memon, 1980). Sheep reproduction, however, is affected by the duration of light and to some extent by temperature (Hulet *et al.*, 1976; Mears *et al.*, 1979). Good reproduction in this species is peculiar since it requires manipulation of its breeding pattern by induction of estrus and the utilization of estrus synchronization techniques. These techniques not only shortens anestrus intervals but affect breeding management and improves it (Alifakiotis *et al.*, 1982). Induction of estrus is a major component of breeding program in a seasonally breeding ewes and in clinically anestrus ewes (Hulet and Stormshake, 1972). It can maximize breeding efficiency and conception during breeding season in seasonal breeding ewes like those in the Middle East. Induction of estrus can also be used as a tool of estrus synchronization (Lemon, 1970). Many authors tried to induce fertile estrus by manipulating hypothalamo-pituitary-gonadal axis through the use of exogenous hormones (Mallampati, Pope and Casida, 1971. Laster and Glimp, 1974; Alifakiotis *et al.*, 1982). Estrus and ovulation can be induced in anestrus ewes by administering progestagen and pregnant mare serum gonadotropin (PMSG). Good lambing rates were achieved in the anestrus ewes received two injections of PMSG at 14 to 16 day intervals, the first PMSG injection was

preceded by a long term (16 days) progestagen treatment (Laster and Glimp, 1974) or by short term (3 to 7 days) treatment (Hulet and Foote, 1967; Christenson, 1976). The second PMSG injection was also preceded by short term progestagen treatment (7days) Bruner *et al.*, 1964). Long term progestagen treatment, however, was associated with a depression of fertility of the induced estrus (Laster and Glimp, 1974). Altered uterine and tubal contractions after long term treatment was claimed to affect sperm transport in the female genital tract (Hawk and Conley, 1972). It is therefore, advisable to use shorter progesterone regime. Treatment of anestrus ewes with progestagens for 12 days followed by PMSG injection induced growth of ovarian follicles in 82% of treated ewes (Lemon, 1970). Attempts had also been made to induce estrus in the ewe by combining the use of exogenous hormones and environmental control factors (Mears *et al.*, 1979). Hormonal regime that consists of daily injection of progesterone (10 mg) for 14 days followed by a single injection of PMSG (500 IU) and Estradiol-17B (30 ug) along with controlled temperature and lighting visualized in 80% conception rate of treated ewes. Induction of estrus in noncycling lactating ewes was achieved by using implants contains 1.3 mg Norgesteromet. This might be a useful procedure in improving reproductive efficiency (Alifakitis, 1982).

This paper compare the results of four hormonal regimes that were based and suggested upon the peculiar reproductive pattern of the local ewes to induce fertile estrus in the non-breeding season.

MATERIALS & METHODS.

Randomly selected ewes, 2-3 years old, of local Awassi breed were used in this experiment. The ewes found

to be free of diseases and were in good general health, after careful inspection and clinical examination. The ewes were subjected to a preventive health program against common parasites and kept in separate pens at the Department of Theriogenology. All received adequate amount of green food and a daily concentrate of half kilogram, which contained energy supplement, protein, minerals and salts. They had access to water adlib. They were kept under observation for two weeks before the actual experimentation, during which they were exposed to a teaser rams daily without actual interaction. After this period, apronized rams were used for estrus detection and suspected ewes were checked for clinical signs of estrus. Estrus detection was conducted three times daily and for a period of two hours each time. Ewes were considered in estrus if they show an interaction with ram, standing to be mounted, appearance of cloudy vaginal discharge and vulva hyperemia.

Clinically anestrus ewes were selected after thirty five days of teasing and regular detection without showing signs of estrus. These ewes were considered for hormonal treatment to induce estrus. The estrus induction regime consisted of four treatments. These treatments, included the use of two types of synthetic prostaglandin analogues, synthetic progesterone and progesterone treatment with pregnant mare serum gonadotropin (PMSG).

Eighteen ewes were selected as not pregnant, not cycling and considered clinically anestrus according to the observation and teasing by detector rams. These ewes received a luteolytic agent which was a synthetic prostaglandin analogue Luprostiole*, It was given in a double dose scheme injection of 7.5 mg according to the

* Prosolvin, Intervet International B.V., Boxmeer-Holland.

manufacturer instruction. These injections were given at nine days interval and after each injection the ewes were checked and observed continuously for five days for signs of estrus.

Another 16 anestrus ewes were given a double injection of another synthetic prostaglandin analogue (Cloprostenol**). Cloprostesnol was given as intramuscular injection of 125 Ug (Manufacturer dose) similar to the first regime. Following the injections, the ewes were observed and detected for estrus as in the first group.

In a third experiment, 16 anestrus ewes were treated with progesterone for purpose of estrus induction. The progesterone was given as intravaginal sponges that contained 60 mg of medroxy acetate progesterone (MAP***). The sponges were inserted by a special applicator into the anterior vagina. The sponges were left in the vagina for 14 days. Following the removal of the sponges all ewes were checked for estrus.

Using the same technique a fourth regime of estrus induction was tested on 14 clinically anestrus ewes. Under similar conditions, the ewes like in the third experiment were kept under progesterone treatment. Intravaginal sponges containing 60mg of MAP were inserted into the vagina of each ewe and kept for nine days. The sponges were removed at the 9th day of treatment, following the removal of the sponges, each ewe was received 500 IU of PMSG****. The ewes were observed for estrus as in the other experiment.

** Estrumate, ICI. Ltd.,

*** Veramix, Upjohn Ltd., Essex, England.

****Folligon, Intervet International, Boxmeer, Holland.

RESULTS

In the pre-experimental period, all ewes that were involved in the experiment did not come to estrus. The vaginal inspection did not reveal any change in the pattern of secretion or consistency of the vaginal mucus. The mucus was scanty, thick and on the wall of the vagina, characteristic of anestrus. Cervix was closed and with no secretion. The vaginal and cervical characteristics did not change for a period equal to two anestrus cycles. These ewes then diagnosed as clinically anestrus and considered for the experimental treatment of estrus induction.

In the first experiment Luproستيol failed to induce estrus in the clinically anestrus ewes after the first and second injections. Observation and ram detection of the treated ewes did not reveal any signs of estrus in all treated ewes. Vaginal changes were similar to those before treatment.

In the second experiment, Cloprostenoł treatment of the clinically anestrus ewes produced slight different results. After the first injection, one ewe came to estrus. This ewe showed estrus after 48 hours of the treatment. She stood to be mounted by the detector ram and had cloudy vaginal secretion. All other ewes did not come to estrus within five days after this injection. The second cloprostenoł injection, however, induced estrus in two ewes. The first ewe came to estrus after 48 hours of the injections and the second came to estrus after 60 hours. These ewes had similar signs of estrus as previous ewe. The remaining ewes did not show any signs of estrus during five days post injection.

The results of the third experiment, revealed that the treated ewes neither reacted with teaser ram nor

had any vaginal changes for five days after sponge withdrawal.

The fourth treatment regime resulted in estrus induction of nearly all ewes. These ewes came to estrus within four days of progesterone withdrawal and PMSG administration. Eleven ewes came to estrus the second day after PMSG injection and one ewe at the fourth day. All the responded ewes accepted the mounting by the teaser ram and had cloudy vaginal secretion. See Table.1

Table 1: Results of the estrus induction in experiment anestrus ewes.

Experimental groups	No. of ewes used	No. of ewes responded	%response
I	18	0	0%
II	16	3	18.75%
III	16	0	0%
IV	14	12	85.7%

DISCUSSION

Induction of estrus in ewes is an important tool in reproductive manipulation that can ultimately increase lambing potential of the flock. The results of this study revealed that the anestrus period of ewes is a complicated phenomenon that requires better knowledge of the reproductive status of the animal. The ewes of this experiment were diagnosed as clinically anestrus based on clinical examination and estrus detection for approximately 2 anestrus cycles. Exposure to ram believed to have some influence on estrus induction due to

pheromones (Ott and Memon, 1980). It seems that clinically anestrus ewes were not affected by the presence of rams since this exposure did not induce estrus in the experimental ewes.

Prostaglandin treatment failed to induce estrus in the majority of the ewes. This hormone is known to be luteolytic in ewes (Acritopoulou and Haresign, 1980) and also has a central effect on the gonadotropin release (Nett *et al.*, 1979). Luprostiol completely failed inducing estrus in the treated ewes. This may be due to the absence of functional corpus luteum, lack of luteal function or to the lack of the central effect of this prostaglandin analogue. On the other hand, cloprostenol, the other synthetic prostaglandin analogue induced estrus in a small number of ewes. This success might be due to a central effect of this drug but not on a functional corpus luteum, especially, the ewes of this group were treated as ewes received luprostiol.

Intravaginal sponges containing progesterone, were used to mimic a luteal phase in ewes, it also failed to induce estrus in the treated group. It seems that progesterone deepened the anestrus condition and suppressed the synthesis of the gonadotropic hormones. Exogenous gonadotropin successfully induced estrus in clinically anestrus ewes and our results indicated that success rate might reach 100%.

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اربعة طرق لاستحداث الشبق في النعاج العواسية

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الخلاصة

اختيرت ٦٤ نعجة عواسية اختياراً عشوائياً لاستحداث الشبق
(الحني) فيها باربعة طرق علاجية. شخمت انعدام الشبق السريري في
النعاج بعد اختبارها بكبش مخير لمدة ٣٥ يوماً خلال اواخر الربيع
تسمت النعاج الى اربعة مجاميع. اعطيت المجموعة الاولى ٧ ملغم
لويروستيول والمجموعة الثانية ١٢٥ ملغم من الكلوروستينول وكلا
العقارين هما مشتقات البروستاكلاندين ف ٢ الفا المصنع وضعت
المجموعة الثالثة تحاميل مهبلية تحوي ٦٥ ملغم من ميدروستي
اسيتيت بروجستيرون لمدة ١٤ يوماً والمجموعة الرابعة عولجت مثل
الثالثة ولكن الإسفنجات المهبلية تركت لمدة ٩ ايام بالإضافة الى
حظنها بـ ٥٠٠ وحدة عالمية من محرض التند المستخلص من دم الإفراس
الحوامل. لم يلاحظ علامات شبق في المجموعة الاولى والثالثة ولكن
لوحظ شبقاً في ٢٠٪ من نعاج المجموعة الثانية. في المجموعة
الرابعة امكن احداث الشبق في ٩٣٪ من النعاج. نستنتج من هذا ان
النعاج العواسية تكون في حالة انعدام شبق ظلال اواخر الربيع
والصيف ولكن ممكن ان يستحدث فيها الشبق بعد معالجتها بهرموني
البروجسترون ومحرض القند.