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A SEROLOGICAL INVESTIGATION OF CAPRINE ARTHRITIS ENCEPHALITIS VIRUS (CAEV) IN GOATS BY COMPETITIVE ELISA AT KAFRELSHEIKH GOVERNORATE, EGYPT

(With One Table and 3 Figures)

By

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دراسة سيرولوجية لمرض التهاب المفاصل والأعصاب باستخدام اختبار الاليزا التنافسية بمحافظة كفر الشيخ

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تربى الماعز فى مصر بنظام تربية تقليدى. وجود الامراض المعديى مثل مرض التهاب المفاصل والاعصاب فى الماعز ربما يكون مسئولا عن انخفاض الانتاجية. فى هذة الدراسة تم فحص 362 عينة سيرم ماعز تم تجميعها من قطعان عشوائية تربى مع الغنم فى قرية متبول بمحافظة كفر الشيخ وتم فحصها باستخدام اختبار الاليزا التنافسية لتحديد وجود ونسبة الاصابة بعدوى فيروس التهاب المفاصل والاعصاب فى الماعز وذلك فى الفترة من شهر اغسطس عام 2010 الى شهر يناير عام 2011 . لم تسجل كل القطعان رد فعل مناعى موجب لاختبار الاليزا حيث من فحص عدد 17 قطيع ماعز (362 ماعز)، 11عدد قطيع بنسبة 64.71% كانت سالبة مناعيا، وعدد 6 قطعان بنسبة 35.29% كانت موجبة مناعيا. كانت نسبة الاصابة الكلية 16.02% (عدد 362/58). بالنسبة الى جنس الماعز، بفحص عدد 146 ذكر وجد عدد 19 بنسبة (13.01%) موجبة مناعيا وبالنسبة لنتيجة فحص عدد 216 انثى وجد عدد 39 بنسبة 18.06% موجبة مناعيا. بالنسبة للعمر، المجموعة العمرية من سن 6 شهور الى عامان، تم فحص عدد 209 ماعز ووجد 34 ماعز بنسبة 16.27% موجبة مناعيا. بالنسبة للمجموعة العمرية اكبر من سن عامان، تم فحص عدد 153 ماعز، كانت النتيجة 24 ماعز بنسبة 15.69% موجبة مناعيا. حدوث الاصابة بهذا المرض المعدى للقطعان المختبرة ربما يمثل عاملا هاما فى نقص الانتاجية فى الحيوانات. هذة النتائج تحتاج دراسات اضافية لكى نتعرف على العلاقة بين النتائج الايجابية مناعيا ونقص الانتاجية. كل الماعز التى تم فحصها كانت سليمة ظاهريا بدون اى اعراض او تاريخ اصابة بهذا المرض فى الماعز. هذا البحث يمثل اول بحث على فيروس مرض التهاب المفاصل والاعصاب فى الماعز بمحافظة كفر الشيخ بجمهورية مصر العربية.

SUMMARY

Goats in Egypt are kept under traditional management system. The occurrence of infectious diseases, such caprine arthritis–encephalitis (CAE) may in part be responsible for sub-optimal production. In the present study, 362 serum samples were collected from randomly distributed goat herds reared with sheep from Matbool village of Kafrelsheikh Governorate and examined using competitive enzyme linked immunosorbent assay (cELISA) to determine the existence and prevalence CAEV infection from the period of August 2010 to January 2011. Not all tested flocks presented seroreactive animals for CAEV, Out of 17 goat/herds investigated (362 goats), 11 herd/flocks (64.71%) were seronegative and 6 herd/flocks (35.29%) were seropositive with overall seroprevalence 16.02% (n=58/362). According to sex, out of 146 males tested 19 (13.01%) were seropositive and out of 216 females tested, 39 (18.06%) were seropositive. According to age, for age group from 6 month-2 years old, 209 goats were tested and 34 (16.27%) were seropositive, while for age group over 2 years old, 153 were tested and 24 (15.69%) were seropositive. The occurrence of this infectious disease in the tested flocks may represent an important factor contributing to the decreased productivity of the animals. These findings require further study to define the relationship between seropositivity and reduced production. All goats tested were apparently normal without showing clinical signs and without history of any specific clinical signs for CAE viral infection. This is the first research on CAEV in goats at Kafrelsheikh governorate, Egypt.

Key words: Caprine arthritis-encephalitis, CAE, Goats, Competitive-ELISA

INTRODUCTION

According to Devendra (1990), approximately 95% of all goats world-wide are located in developing countries. Nearly all caprine and ovine are raised by traditional methods of husbandry in small herd/flocks with or without other species in small settlements with restricted movements except for grazing or water sources.

No reports as far as we know are available on the prevalence of CAEV infection among goat populations at Kafrelsheikh Governorate (Egypt) to determine the distribution of the disease.

Caprine arthritis-encephalitis (CAE) virus is one of only two lentiviruses belongs to the Retroviridae family, that currently are known to infect sheep and goats (Pugh, 2002); Rowe and East (1997). CAEV is genetically and antigenically related to visna-maedi virus (MVV) of sheep (Ravazzolo *et al*., 2001). CAE can affect goats of both sexes, of various different races, and at all ages. However, encephalitis occurs more frequently in young animals, while arthritis in more mature animals (Callado *et al*., 2001).

Countries with the highest prevalence of CAEV infection include the United States, France, Norway, Switzerland, and Canada (Smith, and Sherman, 1994). It has been diagnosed in North America, Europe, Kenya, Peru, Australia, and New Zealand (Knight and Jokinen, 1982; Pugh, 2002; Smith and Sherman, 1994). The results from Kenya and Mexico showed that 4-5% of the goats were infected (Adams *et al*., 1984**),** higher seroprevalence rates were recorded in the USA (Cutlip *et al*., 1992) and Australia (Grewal *et al*., 1986), where intensive husbandry is generally practiced.

The development of clinical disease takes a few months to a few years, however, most infected animals remained subclinically infected (Cheevers *et al*., 1988). Most goats infected with CAE virus are asymptomatic, but there are five major clinical presentations associated with viral infection including arthritis, encephalitis, interstitial pneumonia, mastitis, and progressive weight loss (Matthews, 1999; Smith and Sherman 1994; Van Maanen *et al.* 2010).

The virus is transmitted to kids primarily via colostrum, although transmission via aerosol, animal-to-animal contact, and sexual activity can also occur (Phelps and Smith, 1993; Rowe and East, 1997**;** Peterhans *et al.*, 2004). Other clinical presentations can include a hard udder or mastitis which greatly diminishes the milk production (Narayan and Clements, 1989; Callado *et al*., 2001; Leitner *et al.,* 2010). Horizontal transmission is possible by prolonged contact with infected goats via shedding of the virus in the saliva, the urogenital secretions, and the feces (Rowe and East, 1997). Contact with the blood of an infected animal can also transmit the disease (Matthews, 1999). Other potential sources of viral transmission include viral contamination of milking equipment, needles, tattooing equipment; and breeding an infected animal with a non-infected animal (Adams *et al*., 1983).

Despite humoral and cellular immunity, CAEV causes a lifetime infection and infected goats serve as a reservoir of the virus for the entire lives (Adams *et al*., 1980; Dawson, 1989). Infected goats are carriers

without showing clinical signs and delayed seroconversion are impediment in control and eradication programs (Rowe *et al*., 1992; Rimstad *et al*., 1993). Some reports confirmed that the serological methods are not sufficient enough to detect all infected animals which could be attributed to longtime seroconversion after infection (Rimstad *et al*., 1993) or to the fact that some already infected goats may become temporary seronegative (Harkiss and Watt, 1990). It is also important to note that the maternal antibodies passed in the colostrum are not protective for kids ingesting the colostrum (Adams *et al*., 1983).

Therefore, the presence of antibodies indicates that the animal is infected with CAE virus (Knowles, 1997). The gold standard in testing for CAE antibodies is the immunoprecipitation assay (IP), but it is too expensive to use as a common diagnostic tool. There is a competitive ELISA (cELISA) that has just been developed to detect serum antibodies to CAE. The cELISA is more sensitive than the IP test in detecting CAE antibodies. The cELISA can evaluate undiluted serum, thus detecting lower titers of anti-CAE antibody than the indirect ELISAs. This feature may possibly allow for the earlier detection of CAE positive animals. Validation of this CAEV cELISA for use in sheep was reported previously (Herrmann *et al.*, 2003). Recommendation of several authors, who stated that ELISA is a more reliable test due to its higher sensitivity (Rosati *et al*., 1994; Castro *et al*., 1999).

The aim of this work is to detect the possible occurrence of CAEV in goats and to analyse the epidemiological status with the presence of specific antibodies using cELISA.

MATERIALS and METHODS

1- Area

The present study was conducted during the period from August 2010 to January 2011 at Matbool village of Kafrelsheikh Governorate, Egypt.

2- Animals

Data from sporadic herds comprising 362 goats of both sexes of different age groups (6 month – 6 years) were collected and analysed for CAEV. Flocks were chosen randomly and samples were collected from each herd (All herds goats reared with sheep). At the time of sample collection, no information on the history of each goat was provided to avoid sampling bias. The number of goat heads per herd/flock range between 12-33. Goats sera were collected as 146 males and 216 females.

3- Clinical Examination

Goats were examined for encephalitis, mastitis and arthritis, which could suggest the infection. All tested animals did not show any apparent clinical signs of CAE.

4- Sample collecting

ten ml blood samples were collected from each goat from jugular vein using vacutainer tubes with a separate needle for each sample, allowed to clot, and transferred onto ice as quickly as possible to the Central Laboratory of Faculty of Veterinary Medicine, Kafrelsheikh University. The sera were separated by centrifugation at 2000 rpm for 10 min: and aspirated in eppendorf tubes using Pasteur Pipettes, identified and stored at -20°C until testing

5- Serological test (Laboratory assay procedures)

Serum samples were examined for specific antibodies using a commercially available Competitive ELISA (cELISA). VMRD, Inc. PO Box 502. Pullman, WA 99163 USA Phone: 1-509-334-5815. VMRD 's cELISA detect antibodies to caprine arthritis encephalitis virus (CAEV) in goat sera. The test is, however, only qualitative and so antibody titres were not measured.

Test Procedure

All reagents were stored at 2-7°C (35-45°F)**.** Warm up reagents by bringing the serum samples, reagents and plate(s) to room temperature at least 30 min prior to starting the test. Remove the plate(s) from the foil pouch(es). Place strips to be used in the frame and number the top of each strip to maintain orientation with the Setup Record. Run both the Positive Control (B) and Negative Control (C) in duplicate. Load controls and serum samples using a pipettor set at 50 µl, transfer controls and serum samples to the Antigen-Coated Plate. Serum samples are tested undiluted. Tap the side of the loaded assay plate several times to make sure the samples coat the bottom of the wells. Incubate the plate 1 hour at room temperature (21-25°C), (70-77°F). Prepare 1X Wash Solution by diluting one part of the 10X Wash Solution Concentrate (F) with 9 parts of deionized or distilled water. Approximately 1.5 ml are needed per well. After the 1-hour incubation, wash the plate three times and dump contents of the wells into a sink and remove the remaining sera and controls by sharply striking the inverted plate four times on a clean paper towel, striking a clean area each time. Immediately fill each well with 1X Wash Solution using repeating syringe with a manifold, wash bottle or multichannel pipettor. Dump out the Wash Solution and strike the inverted plate sharply on a clean paper towel as above. Repeat

the washing procedure two more times (three washes total). Prepare 1X Antibody-Peroxidase Conjugate by diluting one part of the 100X Antibody-Peroxidase Conjugate (D) with 99 parts of Conjugate Diluting buffer (E). For 96 wells, mix 60 µl of Antibody-Peroxidase Conjugate (D) with 5.940 ml of Conjugate Diluting Buffer (E) to yield 6 ml of ready-to-use Antibody-Peroxidase Conjugate. Fifty microliters (50µl) are needed per well. Tap the side of the loaded assay plate several times to make sure the conjugate coats the bottom of the wells. Incubate for an additional 30 minutes at room temperature (21-25°C, 70-77°F). After the 30-minute incubation, repeat the washing procedure described before (3 washes total). Add 50 µl of Substrate Solution (G) to each well. Tap the side of the loaded assay plate several times to make sure the substrate coats the bottom of the wells. Incubate 20 minutes at room temperature (21-25°C; 70-77°F). Do not empty wells. Add 50 µl of Stop Solution (H) to each well. Gently mix the well contents by tapping the side of the plate several times. Do not empty wells. Immediately after adding the Stop Solution, the plate should be read on a plate reader. Set the optical density (O.D.) reading wavelength to 620, 630 or 650 nm. Test Validation: The mean of the Negative Controls must produce an optical density ≥0.300. The mean of the Positive Controls must produce ≥35% inhibition. Calculation of percent inhibition (% I): % I = 100 - [(Sample O.D. x 100) ÷ (Mean Negative Control O.D.)]. Interpreting the Results: If a test sample produces ≥35% inhibition, it is positive. If a test sample produces <35% inhibition, it is negative.

RESULTS

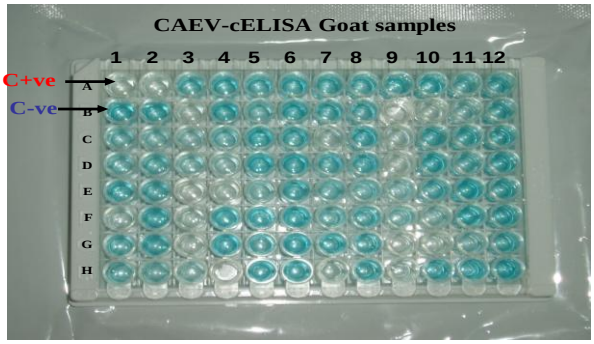
Overall results

Out of 17 goat/herds investigated (362 goats), 11 herd/flocks (64.71%) were seronegative and 6 herd/flocks (35.29%) were seropositive with overall seroprevalence 16.02% (n=58/362). According to sex, out of 146 males tested 19 (13.01%) were seropositive and out of 216 females tested, 39 (18.06%) were seropositive. According to age, for age group from 1-2 years old, 209 goats were tested and 34 (16.27%) were seropositive while for age group over 2 years old, 153 were tested and 24 (15.69%) were seropositive.All goats were apparently normal without showing clinical signs and without history of any specific clinical signs for CAE vial infection (Table 1).

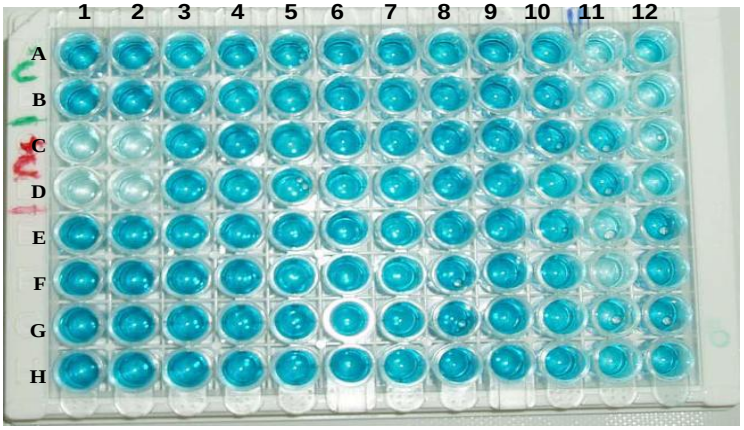
Herd	Herd	Total	Sero	Male	Female	Contact

				6 month-2 y.		Over 2 y.		6 month-2 y.		Over 2 y.		
		No.	%	ELISA +	%	ELISA +	%	ELISA +	%	ELISA +	%	
1	12	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	Sheep
2	19	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	sheep
3	27	9	33.33	2	7.41	1	3.70	4	14.82	2	7.41	Sheep
4	16	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	Sheep
5	19	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	Sheep
6	22	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	Sheep
7	28	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	Sheep
8	18	6	33.33	1	5.56	1	5.56	3	16.67	1	5.56	Sheep
9	33	14	42.42	4	12.12	2	6.06	5	15.15	3	9.09	Sheep
10	17	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	Sheep
11	25	12	48.00	3	12.00	1	4.00	4	16.00	4	16.00	Sheep
12	17	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	Sheep
13	22	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	Sheep
14	23	8	34.78	1	4.35	1	4.35	3	13.04	3	13.04	Sheep
15	24	9	37.5	1	4.17	1	4.17	3	12.5	4	16.67	Sheep
16	19	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	Sheep
17	21	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	Sheep
Overall	362	58	16.02	12	20.69	7	12.07	22	37.93	17	29.31	

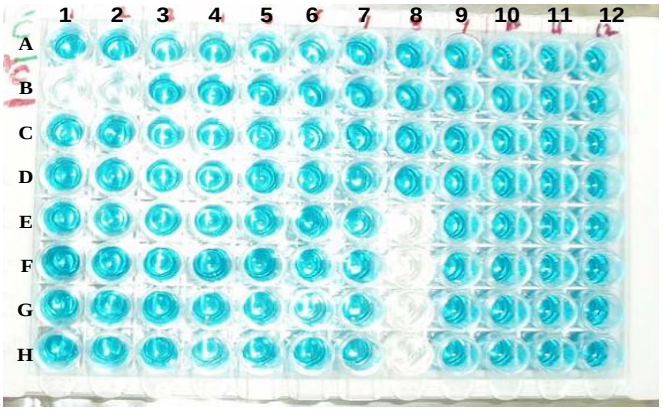
Table 1: Seroprevalence results of CAE ELISA positive goats from 17 herds at Matbool village of Kafrelsheikh governorate, Egypt.


CAEV-cELISA Goat samples
1 2 3 4 5 6 7 8 9 10 11 12
C+ve
C-ve
A
B
C
D
E
F
G
H

CAEV-cELISA Goat samples



CAEV-cELISA Goat samples



Competitive ELISA plates showing deep blue color negative wells and faint color seronegative wells.

DISCUSSION

Most indirect ELISA systems presently in use lack specificity to varying degrees. VMRD's competitive ELISA assay for CAEV antibody detection eliminates most of these non-specific reactions. The cELISA is a well documented method for the detection of CAEV infection (Herrmann *et al.,* 2003). Diagnosis of CAEV infection is based on the specific antibody detection (Castro *et al.,* 1983; Zanoni *et al.,* 1994; Keen *et al.,* 1995; Knowles, 1997; Resende and Gouveia, 1999). Sensitivity and specificity of the cELISA against IP were 100% and 96.4%, respectively. Sensitivity and specifity of AGIDT have been shown to be 92.6 % and 98.6 %, respectively, cELISA detects CAEV antibodies and it is based on the serum inhibition of the monoclonal antibody binding to the CAEV gp135 SU glycoprotein. The ability to detect positive sera with low anti-CAEV gp135 SU antibody titres is a major advantage of the cELISA over indirect ELISA, which requires the dilution of the tested sera (Herrmann *et al.,* 2003).

Overall seroprevalence of CAEV infection in this study was 16.02%. The detection of seropositive serum samples with cELISA could be explained by the higher sensitivity of cELISA. Although seroreactivity to an organism does not translate into verification that the animal was clinically affected by that organism, the infectious diseases CAEV seem to be existed amongst goat flocks from Kafrelsheikh governorate and probably represents an important factor that may contributes to the decreased productivity of goats. The seroreactivity rates to CAEV were extremely variable among the studied 17 herds which ranged from 0.0% - 48.0%. Nevertheless, the overall reactivity of 16.02% is significant. We believe that those infections need more attention and a more intensive diagnostic programme is desirable. We believe that the seroprevalence noted in this study may be similar to that observed in many other regions and possibly elsewhere in the developing world. In this study, the seroprevalence of CAEV infection was detected with cELISA is higher than those found in UK 4.3 % (Dawson and Wilesmith, 1985), Switzerland 2% (Krieg and Peterhans, 1990) and lower than serosurveys conducted in Australia (Grewal *et al.,* 1986), USA (Cutlip *et al.,* 1992), Norway (Nord *et al.,* 1998) and Brazil (Garcia *et al.,* 1992), have revealed prevalence rates of 82 %, 73 %, 49.5 %, and 36.5 %, respectively.

Disease control worldwide were reported by many authors as prevention of CAE viral infection is important in goat herd management because there is no treatment that eliminates CAE virus or vaccine to prevent this disease. Seropositive animals should be segregated and culled and goats should only be purchased from CAE virus-free herds (Knight and Jokinen, 1982; Rowe *et al*., 1992; Linklater and Smith, 1993; Rowe and East, 1997; Matthews, 1999; Pugh, 2002). Correlation between CAEV infection, and breed, age and breeding methods has been reported (East *et al.,* 1987; Cutlip *et al.,* 1992; Randall *et al.,* 1992; Nord *et al.,* 1998).

However, in this study, the numbers of seropositive animals were insufficient to achieve any meaningful statistical conclusions. More herds must be investigated over a longer time, and additional managemental, environmental risk factors should be investigated using different methods such as cELISA may be required to assess the true distribution of CAEV in Kafrelsheikh governorate and allover the country. Furthermore, continuous monitoring should be carried out for local goat populations in Kafrelsheikh governorate and trials for virus isolation and molecular characterization should be carried out for future work.

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Herd No.	Herd size	Total	Sero +ve	Male				Female				Contact animals
				6 month-2 y.		Over 2 y.		6 month-2 y.		Over 2 y.		
		No.	%	ELISA +	%	ELISA +	%	ELISA +	%	ELISA +	%	
1	12	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	Sheep
2	19	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	sheep
3	27	9	33.33	2	7.41	1	3.70	4	14.82	2	7.41	Sheep
4	16	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	Sheep
5	19	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	Sheep
6	22	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	Sheep
7	28	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	Sheep
8	18	6	33.33	1	5.56	1	5.56	3	16.67	1	5.56	Sheep
9	33	14	42.42	4	12.12	2	6.06	5	15.15	3	9.09	Sheep
10	17	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	Sheep
11	25	12	48.00	3	12.00	1	4.00	4	16.00	4	16.00	Sheep
12	17	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	Sheep
13	22	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	Sheep
14	23	8	34.78	1	4.35	1	4.35	3	13.04	3	13.04	Sheep
15	24	9	37.5	1	4.17	1	4.17	3	12.5	4	16.67	Sheep
16	19	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	Sheep
17	21	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	sheep