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ASSOCIATION BETWEEN POSITIVE CANINE HEARTWORM (*DIROFILARIA IMMITIS*) ANTIGEN RESULTS AND PRESENCE OF *ACANTHOCHEILONEMA ODENDHALI* MICROFILARIA IN CALIFORNIA SEA LIONS (*ZALOPHUS CALIFORNIANUS*)

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Abstract: This study establishes a relationship between positive canine heartworm (*Dirofilaria immitis*) test results frequently observed in California sea lions (*Zalophus californianus*) and infection with the filarid nematode *Acanthocheilonema odendhali*. Four commercially available canine heartworm antigen tests were evaluated for cross-reaction with *A. odendhali* in California sea lions. Sera were tested from fifteen California sea lions with *A. odendhali*-associated microfilaremia, confirmed by blood smear, and with no evidence of *D. immitis* infection at necropsy. Ninety-five percent of tests were falsely positive for *D. immitis*. This study also determined that the prevalence of *A. odendhali* infection in stranded California sea lions from central California is approximately 23% by comparing the number of findings of mircofilaremia to the total number of California sea lions sampled at The Marine Mammal Center between 2005 and 2011, inclusive. *Acanthocheilonema odenhali* microfilaremia in California sea lions is likely to cross-react with canine heartworm antigen tests, and clinicians should interpret results with caution.

Key words: *Acanthocheilonema odendhali*, California sea lions, canine heartworm, *Dirofilaria immitis*, false positive, *Zalophus californianus*.

INTRODUCTION

California sea lions (*Zalophus californianus*) are routinely tested for infection with canine heartworm (*Dirofilaria immitis*) prior to introduction into a zoological collection due to evidence that captive sea lions are susceptible to *D. immitis*.[9] It has also been common for zoos and aquaria to use heartworm preventive in California sea lions since the practice was first described in the literature in 1977.[1] Literature review reveals only two reports of *D. immitis* infections in captive California sea lions, both of which occurred in areas highly endemic with canine heartworm, Florida and Louisiana.[3,8] No cases of *D. immitis* have been reported in wild sea lions; however, positive *D. immitis* antigen test results are common in sea lions tested at The Marine Mammal Center (TMMC), a research and rehabilitation hospital for marine mammals which receives animals from the central California coast.

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Infection with the nematode *Acanthocheilonema odendhali* is common in California sea lions, and microfilaria are often evident when conducting routine blood smear evaluation.[2] The microfilaria are fairly easily identified by their approximate size.[6,7] This parasite was originally identified in 1965 in California sea lions and has since been described in Steller sea lions (*Eumetopias jubatus*) and Northern fur seals (*Callorhinus ursinus*).[6,7] *Acanthocheilonema odendhali* is thought to be nonpathogenic in otariids, with adult worms living in the muscle fascia of the thorax, but very little additional information is known about the parasite.[5]

The true prevalence of *A. odendhali* in wild California sea lion populations is not known; however, 35 cases of *A. odendhali*-associated microfilaremia were observed at TMMC in 2011. By use of commercially available assays, this study reports the association between positive canine heartworm test results and microfilaremia due to *A. odendhali* infection. It also determines the frequency of cross-reaction for four different commercially available canine heartworm assays and, based on those results, recommends protocols for heartworm testing in California sea lions. Additionally, the study reports the prevalence of *A. odendhali* in stranded sea lions in central California based on routine diagnostic screening conducted on California sea lions from TMMC between the years 2005 and 2011, inclusive.

Table 1. Serum canine heartworm test results from 15 sea lions confirmed to be infected with *A. odendhali.*

Field no.	IDEXX–ELISA Antigen	Antech–ELISA Antigen	Witness Heartworm	DiroCheck
8247	Positive	Positive	Positive	Positive
8571	Positive	Positive	Positive	Weak positive
8673	Weak positive	Borderline positive	Positive	Negative
8680	Positive	Positive	Positive	Weak positive
9765	Positive	Borderline positive	Positive	Weak positive
9821	Positive	Positive	Positive	Weak positive
9831	Positive	Positive	Positive	Positive
9925	Weak positive	Positive	Positive	Weak positive
9931	Positive	Positive	Positive	Weak positive
9975	Positive	Borderline positive	Positive	Weak positive
10222	Positive	Positive	Positive	Weak positive
10343	Positive	Borderline positive	Positive	Weak positive
10376	Weak positive	Negative	Positive	Positive
10452	Positive	Borderline positive	Positive	Negative
10495	Positive	Positive	Positive	Weak positive

MATERIALS AND METHODS

Individual sea lions were identified as infected with *A. odendhali* during routine clinical blood smear evaluation by the lab technician at TMMC based on approximate size of microfilaria. Samples identified as microfilaremic were then assayed with four commercially available canine heartworm antigen tests. Canine heartworm antigen testing was conducted using Witness® Heartworm in-clinic antigen tests (Zoetis Inc., Florham Park, New Jersey 07932, USA, serial #1300449), DiroCHEK® Lab Pack test kit (Zoetis, serial #1300278), IDEXX Heartworm Antigen by ELISA-Canine in lab (Idexx Laboratories Inc., Westbrook, Maine 04092, USA), and the Antech Heartworm Antigen in lab test (Antech Diagnostics Inc., Irvine, California 92614, USA).

Serum samples from 15 California sea lions, which were positive for *A. odendhali* on blood smear and negative for *D. immitis* at postmortem examination, were tested for *D. immitis* infection using the above four commercially available antigen tests. These serum samples had been frozen and stored at –80°C for up to 4 yr. Each sample was thawed and refrozen once before use during the course of this study. Both of the in-clinic tests, Witness and DiroCHEK, were run by the author (DRK) according to the manufacturer's specifications. The serum samples were submitted to IDEXX and Antech on ice. IDEXX staff ran the serum samples using proprietary equipment and testing protocols utilizing enzyme-linked immunosorbent assay (ELISA) technology. Antech processed the samples using the Quadraspec Bio-CD® (Quadraspec, Inc.–Antech Diagnostics, West Lafayette, Indiana 47906, USA).

There were three possible results for the Antech, IDEXX, and DiroCHEK tests: positive, weak-borderline positive, or negative. The Witness test could only be interpreted as positive or negative based on manufacturer's instructions. Weak or borderline results approximate the cut-off value of a positive result, as defined by the manufacturers.

Control serum samples from a group of five presumably naïve sea lion neonates and fetuses were submitted to Antech Diagnostics for ELISA antigen testing for *D. immitis.* Three samples had been stored for 3 yr, one sample for 4 yr, and the remaining sample for 11 yr at –80°C.

In order to establish the prevalence of *A. odendhali* in the stranded sea lion population, the number of animals infected with *A. odendhali* microfilaria was compared to the total number of individual sea lion whole blood samples that TMMC had screened from 2005 throughout 2011.

RESULTS

The results of all of the antigen screening tests were interpreted by either the lab providing the test or according to the manufacturer's instructions. All five of the serum samples from naïve sea lion neonates and fetuses returned negative results. All of the samples run on the Witness and IDEXX tests produced positive results. Fourteen out of 15 samples run by Antech produced positive results as did 13 out of 15 samples run on DiroCHEK. Table 1 details each result obtained from every test run. In all

Table 2. The total number of positive results for each test out of the 15 sea lions confirmed to be infected with *A. odendhali*.

Test kit	No. of "weak" or "borderline" positives	No. of positives	Total no. of positive results
Witness	N/A	15	15
DiroCHEK	10	3	13
IDEXX in lab	3	12	15
Antech in lab	5	9	14

borderline cases, the manufacturers recommend additional diagnostic testing to verify heartworm infection. Table 2 summarizes the numbers of each result by test.

A total of 550 individuals had *A. odendhali* microfiliaria confirmed via blood smear from 2005 throughout 2011. A total of 2,430 individuals were screened during the same time period, indicating a 22.6% prevalence of *A. odenhali* microfilaremia observed at TMMC.

DISCUSSION

There are few documented cases of *D. immitis* infection in California sea lions.[3,8] Conversely, the prevalence of *A. odenhali* infection, not recognized to cause clinical disease in California sea lions, exceeds 20% in the study population. The data in this study indicate that a positive result on a canine heartworm antigen test in a California sea lion is not specific to infection with *D. immitis* and may be caused by infection with *A. odendhali*. Therefore, the use of the canine heartworm test in wild California sea lions appears likely to produce false-positive results and should be interpreted with caution.

Although the life cycle of *A. odenhali* is unknown,[2] blood samples from naïve neonates and fetuses were used as negative controls in an attempt to eliminate the possibility of the individuals carrying *A. odendhali* or any other parasite. The negative test results from these presumably naïve animals suggest that the positive tests associated with the microfilaremic sea lions are not caused by a previously undescribed unique characteristic of California sea lion serum that is reacting with the tests. No positive control samples from California sea lions infected with *D. immitis* were available for testing. Only two reports of *D. immitis* in California sea lions, totaling four cases, have ever been published, none of which occurred in the last 40 yr.[3,8]

DiroCHEK gave the lowest frequency of cross-reaction, though still very similar, compared to all of the other tests used in this study. While all the other tests produced a majority of positive results, the majority of DiroCHEK's results were weak positives. Most of those weak positives were very difficult to interpret as positives, and they required that the wells be held against a white background, per manufacturer's recommendations, for interpreting unclear results. (Zoetis Corporation, pers. comm.) It is important to note that the DiroCHEK results are highly subjective, and results could easily have been misinterpreted as negative.

The results from this study and interpretation apply only to wild California sea lions. Clinical application and significance of canine heartworm testing are different for sea lions maintained in a collection setting and would be clinically relevant for collections in highly endemic areas of *D. immitis*. If clinical signs indicate a possible canine heartworm infection in a California sea lion housed in a highly endemic area, the authors recommend confirming the diagnosis through additional diagnostics such as radiographs, echocardiograms, and microscopic identification of the microfilaria from blood sample. It is also important to note that phocids are host to the closely related heartworm *Acanthocheilonema spirocauda*, which can cause clinical disease.[2] The conclusions of this paper cannot be extended to *A. spirocauda* without additional investigation.

The prevalence of microfiloremia associated with *A. odendhali* in California sea lions stranded along the central California coast determined by this study, 22.6%, is consistent with the recently published prevalence of *A. odendhali* in the Northern fur seals of the Pribilof Archipelago, Alaska.[4] The prevalence found in the stranded population of sea lions in this study may be a good approximation of the prevalence in the wild population. But this approximation must be made with caution, as all of the stranded individuals tested were malnourished, sick, or injured, and potentially immunocompromised, findings which may lead to an artificially raised, elevated prevalence in the tested population. Due to limitations of this study, and despite the consistency with other published data, the prevalence of *A. odendhali* in wild California sea lion should be verified with additional comprehensive field surveys.

The canine heartworm antigen tests investigated in this study could be used to establish a more-accurate prevalence of *A. odendhali* in wild sea lion populations. The 100% cross-reaction with *A. odendhali* indicated by these data for both the Witness test and IDEXX in lab test suggests

either of these tests could be used to determine a prevalence of *A. odendhali* in wild populations of California sea lions. Using one of these tests would provide a cheaper alternative than developing a unique test for *A. odendhali* and would provide a more-feasible methodology than using postmortem examinations to establish an accurate prevalence. The Witness test is particularly well suited for this use because whole blood may be used and refrigeration is not required for the test kit's long-term storage, making it suited for the field. Additional study is required to determine the positive predictive value for large sample sizes, to verify consistency when using whole blood, and to investigate if other nematodes, including *D. immitis*, produce positive results.

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