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Coccidioidomycosis in Alpacas in the Southwestern United States

RUNNING HEAD: Coccidioidomycosis in Alpacas

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Summary

An anonymous web-based survey of alpaca owners was used to learn more about the clinical presentation, diagnosis, and treatment of coccidioidomycosis in alpacas in the United States. Thirty-seven owners, with 1,117 alpacas, completed the survey. Over 4% of alpacas included in the study were diagnosed with coccidioidomycosis between 2005 and 2016 (5 post mortem, 46 clinically). Immunodiffusion titers ranged from 1:4 to ≥ 1:256 in sick animals. Alpacas residing in Arizona counties with a high incidence of human disease were 5.8 times more likely to contract coccidioidomycosis than animals residing in other areas of the state. Treatment was reported in 23 alpacas, and 78% of those animals died or were euthanized. Necropsy records from a veterinary diagnostic laboratory in Tucson, AZ were reviewed to estimate the severity of disease in this species. Nine cases identified for review died of disseminated coccidioidomycosis; the disease was extensive in most animals, with lungs, lymph nodes and liver most frequently affected. Alpacas appear to be highly susceptible to severe illness as a result of infection by *Coccidioides* spp., frequently resulting in death. More research is needed to better understand the epidemiology, clinical signs, and treatment protocols for coccidioidomycosis in alpacas.

Keywords: Camelids, New World; *Coccidioides*; Coccidioidomycosis; Communicable Diseases; Veterinary Medicine

Introduction

Coccidioidomycosis, or Valley Fever, is an infectious fungal disease caused by *Coccidioides immitis* or *C. posadasii*. The *Coccidioides* spp. are endemic to parts of the Western Hemisphere, including the southwestern United States and northern Mexico and scattered regions in Central and South America. Areas in the United States considered endemic include regions of Arizona, California, New Mexico, southern Nevada and Utah, and Texas (Laniado-Laborin, 2007). Recently, the fungus was found in the soil of an area in eastern Washington state after three human cases were identified (A. P. Litvintseva et al., 2015). Domestic livestock such as cattle and sheep are susceptible to infection, although clinical illness is rare. South American camelids (SAC), on the other hand, appear to be prone to widespread disseminated illness (Fernandez, Hidalgo, Hodzic, Diab, & Uzal, 2018; Shubitz, 2007).

The life cycle of *Coccidioides* occurs in two distinct phases. In the soil, the hyphal form grows during the rainy season. As the soil dries out, the hyphae form into arthroconidia, which are dispersed through wind or other soil disruption. Infection is typically through inhalation, and this initial respiratory infection ranges from asymptomatic to severe. In the body, arthroconidia transform into spherules which develop internal endospores. When the spherules rupture, endospores may disseminate to nearly any location in the body. The presentation of the disseminated disease is variable as clinical signs will relate to the organ affected (Nguyen et al., 2013).

There are estimated to be more than 76,000 llamas (*Llama glama*) and about 140,000 alpacas (*Vicugna pacos*) in the United States (Vilsack & Clark, 2014). An estimated nearly 20,000 alpacas live in the region endemic for *Coccidioides* spp. South American camelids appear to be prone to development of widespread, often fatal, disease due to *Coccidioides* spp. based on

reports in llamas plus conversations with pathologists, veterinarians, and animal owners in southern Arizona (Coster, Ramos-Vara, Vemulapalli, Stiles, & Krohne, 2010; Fowler, Pappagianis, & Ingram, 1992; Maddy, 1959; Muir & Pappagianis, 1982). A review of necropsies performed on camelids in California between 1992 and 2013 found that 4% of the animals died from coccidioidomycosis; the fungus was found in extrapulmonary tissue in most of these animals (Fernandez et al., 2018). There is one case report of coccidioidomycosis causing spontaneous abortion in an alpaca with infection of the placenta and fetus. The dam also tested positive for coccidioidomycosis with a titer of 1:256 and was euthanized. Upon necropsy, she was found to have disseminated disease in multiple organs, including the uterus (Diab et al., 2013). However, information about the infection and mortality rate is scant for both alpacas and llamas. The purpose of this study was to obtain more information about the frequency, clinical signs, treatment, and outcome of coccidioidomycosis in alpacas. Secondarily, we reviewed necropsy reports from Arizona Veterinary Diagnostic Laboratory (Tucson, AZ) and cataloged the extent of disease in alpacas that died of coccidioidomycosis.

Materials and Methods

Survey data were collected and managed using REDCap electronic data capture tools hosted at The University of Arizona (Harris et al., 2009). A public, anonymous, web-based survey collected data about the number, location, and housing of alpacas. Respondents were asked to report whether one or more of their animals had been definitively diagnosed with coccidioidomycosis by a veterinarian between 2005 and 2016. Those with diagnosed animals were then asked for additional information about the clinical signs, diagnostic tests, treatment, and outcome.

The Alpaca Breeders of Arizona association dispersed the link to the survey to their members and all were encouraged to share the link with other alpaca owners wherever they lived. Some respondents informed the investigators that they also used social media to disperse the survey link. Additionally, a link to the survey was provided on the Valley Fever Center for Excellence's webpage and Facebook page.

The database of Arizona Veterinary Diagnostic Laboratory was searched for coccidioidomycosis in alpacas. Cases that were serological submissions only were not utilized. Records were reviewed for cases with cytological or tissue specimens or complete necropsies.

Statistical analysis

Data were exported to IBM SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, N.Y., USA) for analysis. Frequencies and means were calculated. An odds ratio was calculated to determine the risk of clinical illness by location. Due to the small sample size, a Fisher's exact test was used to analyze the risk of housing animals on a dry lot. All assumptions for the Fisher's exact test were met. Significance was set at 0.05.

Results

Geographic distribution of alpacas with coccidioidomycosis – The survey was open to all alpaca owners across the United States; in 2012 there were 9,353 alpaca farms in the country (Vilsack & Clark, 2014). As we distributed the survey through online resources, including a breeder association in Arizona, we anticipated that most responses would come from the highly endemic region of the country. Thirty-eight owners submitted at least partial responses to the survey. Only two surveys were received from areas outside the known endemic region for *Coccidioides* spp. In one case, while the alpacas were diagnosed in New Hampshire, the veterinarian contacted investigators to inform them that the alpacas had been imported from

Crane, Texas (Kitt Hollister, DVM, personal communication, 10/28/16). For the analysis, these two animals were considered to be Texas cases. A second survey received from an owner in New Jersey reported no cases of coccidioidomycosis and the herd was removed from the analysis.

Most respondents were from Arizona (n=23, 62%), followed by California (n=7, 19%), New Mexico (n=5, 14%), and Texas (n=2, 5%). Reported herd size ranged from 2 to 100 alpacas (mean = 30.9), with a total of 1,117 alpacas. Twenty-two of the 37 owners reported having had at least one alpaca diagnosed with coccidioidomycosis by a veterinarian between 2005 and 2016. (Table 1) Owners reported a total of 51 ill animals (4.6% of all alpacas included in the survey), with respondents reporting between 1% and 78% of their herd having been affected with coccidioidomycosis (mean = 11%, SD = 17.4). The largest number of animals diagnosed with coccidioidomycosis in one herd originated from a respondent in Crane, Texas, reporting 18 ill animals with an approximate herd size of 23 alpacas. A veterinarian in that area of Texas was contacted and she confirmed that this attack rate was consistent with her experience with camelids in the region (Jessica Todia, DVM, personal communication, 11/14/16).

Because human coccidioidomycosis is reportable in Arizona, there is relatively complete case rate data by county for this state, where most of the alpacas in the survey resided. The Arizona respondents represented about 10% of the farms in the state, which was greater than the other states (California: 1.5%, New Mexico: 7%, Texas: 0.05%). Therefore, odds of diagnosis by location was only performed on the Arizona herds. Arizona counties with a high incidence of human disease (greater than 44 cases/100,000 people on average between 2010 and 2014) for which we had alpaca data are Maricopa, Pinal, Mohave, and Cochise ("Valley Fever: 2015 Annual Report," 2015). Alpacas in the high incidence counties were found to have a 5.8 times

greater chance of contracting coccidioidomycosis compared with alpacas in other Arizona counties (p=0.0049).

Thirty-one owners (84%) reported housing their alpacas exclusively on dry lots, which are fenced areas with no or minimal vegetative cover of the dirt. There was no significant difference found in the number of animals diagnosed with coccidioidomycosis when comparing animals on a dry lot versus other housing conditions, which included both irrigated and native grass pastures (p=0.0854).

Clinical signs and diagnostic testing – Though 51 alpacas were reported with coccidioidomycosis that had been diagnosed by a veterinarian according to the owners, respondents only supplied additional information on diagnosis method, clinical signs, and treatment for 23 animals. Eighteen animals were diagnosed by agar gel immunodiffusion, the standard blood test used in animals for *Coccidioides* species (Sykes, 2014). Six owners supplied additional anticoccidioidal antibody titer details. Two animals had a titer of 1:128, and there was one animal each with titers of 1:4, 1:32, 1:64, and ≥1:256. The other five animals were diagnosed through a necropsy. One of those animals had no clinical signs prior to death.

A list of anticipated clinical signs was developed from reports in the literature (Fowler et al., 1992; Muir & Pappagianis, 1982). Owners were asked to select from a pick list the clinical signs they noted in their ill animals in the categories of respiratory disease (cough), skin disease (swelling or masses under the skin, non-healing sores), bone disease (lameness), polyarthritis (joint swelling), lymphadenopathy, CNS disease (seizures, inability to stand), and general malaise (fever, weight loss, decreased appetite, and decreased energy). The most frequently reported change in the animals was weight loss (n=15), followed by decreased energy (n=9) and decreased appetite (n=8). Two owners observed the loss of fiber as in their sick animals, which is

a previously unreported clinical finding for coccidioidomycosis in South American camelids (Table 2)

Treatment – Out of 51 animals reported as having a diagnosis of coccidioidomycosis, there was at least partial information regarding treatment for the same 23 animals for which diagnostic and clinical information were provided. Twelve alpacas were not treated, and all but one (92%) of them died. Five of these were the animals previously reported as having been diagnosed by necropsy. Six alpacas received fluconazole. Three animals (receiving either 250 or 500 mg BID) recovered, while three on unknown dosages died. Two alpacas were put on ketoconazole. One of those, at an unknown dose, died, while the other, which received 1200 mg of ketoconazole daily, improved. For two alpacas the medication was not reported and another was given flunixin meglumine and antibiotics; all three of those animals died. One owner reported not treating the sick alpaca and the animal was stable. Of these 23 animals, 18 (78%) died or were euthanized, while the remaining 5 (22%) were recorded as recovered, improved, or stable.

Necropsy Findings – Records of Arizona Veterinary Diagnostic Laboratory were reviewed from 2007-2016, the years available in their database, for alpacas with a histological diagnosis of coccidioidomycosis. Up until 2016, this laboratory was the only location in Arizona that performed necropsies on this species. Ten animals, all submitted for necropsy, were identified. Of the 10, one animal died from widespread bacterial infection but had incidental *Coccidioides* spherules observed in one thoracic lymph node. Coccidioidomycosis was the stated cause of death of the other nine, which had abundant to florid spherules present and pyogranulomatous inflammation in diseased sites. Table 3 summarizes the signalment, gross, and histopathological findings in the cases that died from coccidioidomycosis. The median age was

three years and gender appeared not to be a factor in these cases. All nine animals had extensive lung disease with severe involvement of thoracic (8/9) and cervical lymph nodes in the majority of animals; nodes were frequently described as abscessed and measured up to 15 cm diameter. Five of nine alpacas (56%) had granulomas of the heart. One of these animals also had spherules in the pericardium and died from right sided heart failure considered secondary to the infection. Finally, 5/9 animals were noted to have a thin or wasted body condition; body condition was not stated for three animals and only one was reported to be in good condition with adequate fat reserves.

Discussion

There is very limited published information on coccidioidomycosis in South American camelids in general and on alpacas in particular, with only two publications detailing cases in this species, and only one animal for which there is antemortem information published (Coster et al., 2010; Fernandez et al., 2018). Both series reported widespread disease in most animals. This manuscript details findings from a web-based survey of alpaca owners, with a goal to improve our understanding of the scope and impact of the disease on living alpacas. Though the most significant limitations of the survey data are reporting by owners, lack of complete information for their diagnosed animals, and low response rate to the survey, the 80% reported deaths among 23 complete cases underscores the fatality of the disease and the need for robust prospective research to improve diagnosis and treatment.

In recent review of necropsy reports from the California Animal Health and Food Safety laboratories, coccidioidomycosis was the cause of death in 4% of camelids (alpacas and llamas) submitted, and 86% had extrapulmonary dissemination at the time of death (Fernandez et al., 2018). The necropsy report does not address the number of alpacas living in the regions from

which the cases were submitted, nor does it address diagnosis rates among animals. We calculated a veterinarian-diagnosed rate of coccidioidomycosis of 4.6% from just short of 1200 animals whose owners responded to the survey, with percentages of afflicted animals per herd varying from 1% to 78%. Information that was not captured in this survey but would be useful to understand is the serological attack rate in herds from endemic areas compared to diagnosed illness rates. Asymptomatic disease is common in other species (Shubitz, 2007; Shubitz, Butkiewicz, Dial, & Lindan, 2005), and recovery from primary respiratory illness without antifungal intervention is also typical in human populations (Galgiani et al., 2005). Neither this survey nor necropsy-based case series capture either of these infection outcomes. Thus the rate of coccidioidal infection in alpacas remains to be determined, preferably through systematic prospective studies in more than one endemic area.

Though our report contains only a small number of animals, the high death rate of nearly 80% of animals for which an outcome was reported (18/23) clearly demonstrates that effective treatment is needed. Among survey respondents, eight people reported treating animals with oral antifungal medication, either fluconazole or ketoconazole, but half of them died. There are no data on oral antifungal medication doses for alpacas, or even whether they are absorbed. In monogastric species afflicted by coccidioidomycosis, such as dogs, cats, and horses, orally administered azole antifungal medications are the backbone of treatment, and limited literature exists regarding pharmacokinetics of various antifungal drugs in SAC. In a study of single-dose oral voriconazole administration, the bioavailability of voriconazole was ~22% and the authors recommended doses of 20-30 mg/kg/day to achieve probable therapeutic antifungal blood levels (H. M. Chan, Duran, Walz, & Ravis, 2009). Similarly, other work has shown that oral medication bioavailability is lower in New World camelids compared to monogastric omnivores

(Busch et al., 1998; Kreuder et al., 2012). Phamacokinetic studies in alpacas to help determine appropriate therapeutic doses of oral antifungal medications would greatly aid veterinarians and likely improve treatment outcomes for coccidioidomycosis.

The frequency of cardiac involvement seen in the necropsy reports was not anticipated. Cardiac lesions in disseminated cases of coccidioidomycosis are reported in both people and dogs, although with less frequency than these alpacas (O. Chan et al., 2016; Shubitz, Matz, Noon, Reggiardo, & Bradley, 2001), or in camelids in California (Fernandez et al., 2018). A similar review of canine coccidioidomycosis reports, also from Arizona Veterinary Diagnostic Laboratory, found that 28% had evidence of the disease in the heart and/or pericardium (Shubitz et al., 2001), while this population of alpacas had a rate of 56%. In the canine cases, the cardiac lesions were considered the likely cause of death in 11/13 dogs, while there was sufficient information for only 1 alpaca to determine that heart failure from cardiac coccidioidomycosis was at least a contributing cause of death. For the other 4 animals, there were so many sites of fulminant disseminated disease that the clinical significance of the cardiac lesions could not be determined.

Although we were able to identify far fewer necropsy reports for alpacas with coccidioidomycosis compared to another review (Fernandez et al., 2018), we did identify similar trends. Our review identified a similar rate of dissemination to the liver (67% compared to 78%), and spleen and kidney (33% compared to 47% each). Our reports showed a higher rate of dissemination to the lymph nodes (78% compared to 54%) and cardiac structures (56% to 34%). We do not believe the low number of identified necropsy reports suggests that this disease is less common in this region compared to California; we suspect that fewer animals are submitted for necropsy, possibly related to the availability of veterinary diagnostic laboratories in the area.

There were several limitations to our study design. While we created an online survey for ease of access and ideally to reach as many alpaca owners as possible, finding a good method to disseminate the survey proved difficult. As the precise location of alpaca herds in endemic regions is unknown, we relied heavily upon social media and word of mouth to distribute the survey link. Additionally, this was a retrospective study that relied upon the memory of the owners for up to the previous ten years. The survey stated that the animals reported in the study must be diagnosed by a veterinarian. However, veterinary records were not required to be submitted with the survey and anonymity meant we were unable to pursue further inquiry of the owner or the veterinarian that cared for the animals. This reporting mechanism may have resulted in over- or under-reporting of cases due to lack of understanding of owners, or lack of pursuing a diagnosis for sick or dead animals. Owner-reporting of therapies and therapeutic outcomes was both minimal and could lead to significant erroneous conclusions regarding the appropriateness or efficacy of agents. Rather, this information underscored our conclusion that research is needed to provide evidence-based treatment recommendations, preferably with outcome monitoring performed by veterinarians

We also noted that participation in the survey was not consistent even within the highly endemic regions of Arizona. The average human cases per 100,000 people between 2010 and 2014 were highest in Maricopa (211.9), Pinal (156.1), and Pima (132.0) counties ("Valley Fever: 2015 Annual Report," 2015). Unfortunately, we received no survey responses from Pima County; however, six of the alpacas diagnosed by necropsy were from Pima County, along with two from Pinal and one from Santa Cruz counties. While it is not clear why we received no survey respondents from Pima County, it is likely Pima, Pinal, and Santa Cruz were represented

in the AZVDL cases due to proximity of the laboratory, located in Pima County, to the dead animals. Pinal and Santa Cruz counties are adjacent to Pima.

Coccidioidomycosis is not a reportable disease in any veterinary species which inhibits the ability to monitor disease burden in animals, including SAC. In recent years, the human incidence of coccidioidomycosis has been increasing (Ampel, 2010); additionally, *Coccidioides* spp. have been identified in a region not previously known to be endemic for the fungi (Anastasia P. Litvintseva et al., 2015). These factors lead to an increasing concern for the potential effect of this disease in SAC. It would be worthwhile to pursue prospective studies of incidence, prevalence, and clinical presentation of coccidioidomycosis to educate veterinarians and owners about early detection, and to assess the pharmacokinetics of antifungal drugs to advise treatment of sick alpacas.

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Conflicts of interest

The authors declare that there were no conflicts of interest.

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Table 1: Number of animals diagnosed by county

State	County	No Sick/Total (%)
AZ	Cochise	2/20 (10%)
	Coconino	0/2 (0%)
	Maricopa	9/82 (11%)
	Mohave	4/133 (3%)
	Navajo	1/40 (3%)
	Pinal	4/36 (11%)
	Santa Cruz	3/57 (5%)
	Yavapai	0/151 (0%)
CA	El Dorado	0/60 (0%)
	Los Angeles	2/14 (14%)
	Merced	1/75 (1%)
	Riverside	2/8 (25%)
	Tehama	1/45 (2%)
	Ventura	1/42 (2%)
NM	Bernalillo	0/25 (0%)
	Curry	1/70 (1%)
	Sandoval	0/30 (0%)
	Santa Fe	0/60 (0%)
	Socorro	0/44 (0%)
TX	Crane	20/123 16%)

Table 2: Frequency of reported clinical signs noted by owners of alpacas with coccidioidomycosis.

Clinical Sign	**Frequency**
Weight loss	15
Decreased energy	9
Decreased appetite	8
Coughing	6
Lameness	5
Inability to stand/walk	5
Non-healing sores	3
Fever	2
Joint swelling	2
Enlarged lymph nodes	2
Fiber loss	2
Nose bleed	1

Table 3: Alpaca necropsy report findings

Animal Number	**Year**	**Age**	**Sex**	**County**	**Condition**	**Lungs**	**Lymph Nodes**	**Liver**	**Spleen**	**Kidney**	**Heart/Pericardium**	**Other organs affected**
1	2016	3 yr	M	Pima	poor	+	-	+	+	-	+	Stifle periarticular tissue
2	2009	17 mos	M	Pinal	thin	+	+	+	+	-	+	Pancreas
3	2010	3 yr	F	Pinal	unknown	+	+	-	-	-	-	Mediastinal masses
4	2010	8 mo	M	Pima	unknown	+	+	+	-	-	-	
5	2013	adult	F	Pima	poor	+	+	-	-	-	+	
6	2013	3 yr	F	Pima	thin	+	+	+	+	-	+	
7	2009	10 yr	M	Santa Cruz	good	+	+	+	-	+	+	Brain granulomas
8	2008	6 yr	F	Pima	unknown	+	+	-	-	+	-	
9	2008	3 yr	F	Pima	poor	+	+	+	-	+	-	
					Total:	**9**	**7**	**6**	**3**	**3**	**5**	