



CONCORDANCE BETWEEN MICOLOGICAL CULTURE AND CYTOPATHOLOGY IN THE DIAGNOSIS OF DERMATOPHYTOSIS IN GUINEA PIGS

CONCORDANCIA ENTRE EL CULTIVO MICOLÓGICO Y LA CITOPATOLOGÍA EN EL DIAGNÓSTICO DE DERMATOFITOSIS EN CUYES

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Abstract

Dermatophytosis is a disease that affects the stratum corneum of the skin, hair and nails in guinea pigs, causing bad aspect of the carcass, affecting its commercialization and generating economic losses. For the study 189 samples of guinea pigs with dermatological lesions were collected in intensive breeding farms; the guinea pigs were analyzed by cytopathology and mycological culture in the Laboratory of Microbiology and Microscopy of Universidad Científica del Sur. The frequency of dermatophytosis was $18.5 \pm 5.5\%$ by mycological culture and $43 \pm 7.1\%$ by cytopathology; according to the age stratum, the dermatophytosis frequency was 0% / 0% in breeding, 25.6% / 62% in rearing, and 4.8% / 6% in reproductive guinea pigs by mycological culture and cytopathology, respectively. About the location of the lesions, a frequency of 0% / 0% was found in cages by both techniques, while for animals raised in pools a frequency of 26.5% / 61% was found by culture and cytopathology, respectively. The grade of congruity between these two tests was determined by the value of Kappa (κ) equal to 0.46. The result indicates that there is a moderate degree of association.

Keywords: Guinea pigs, dermatophytoses, mycological culture, cytopathology.

Resumen

La dermatofitosis es una enfermedad que afecta al estrato córneo de la piel, pelo y uñas de los cuyes, causando un mal aspecto en la carcasa, afectando su comercialización, y generando pérdidas económicas. Se colectaron 189 muestras de cuyes con lesiones dermatológicas en granjas de crianza intensiva; las cuales fueron analizadas mediante cultivo micológico y citopatología en el Laboratorio de Microbiología y Microscopía de la Universidad Científica del Sur. Se

halló una frecuencia de dermatofitosis de $18.5 \pm 5.5\%$ por cultivo micológico y $43 \pm 7.1\%$ por citopatología; según el estrato etario la frecuencia de dermatofitosis fue de $0\% / 0\%$ en lactantes, $25.6\% / 62\%$ en recría y $4.8\% / 6\%$ en reproductores, por cultivo micológico y citopatología, respectivamente. Según la ubicación de la lesión la frecuencia de dermatofitosis fue mayor en las regiones frontal y nasal, con $41.7\% / 70\%$ y $28.1\% / 67\%$, por cultivo micológico y citopatología, respectivamente; en cuanto al tipo de instalación, se presentó una frecuencia de $0\% / 0\%$ en animales criados en jaulas, y $26.5\% / 61\%$ en animales de crianza en poza, por la técnica de cultivo micológico y citopatología, respectivamente. Al evaluar el grado de concordancia entre ambas técnicas se halló un valor de Kappa (κ) igual a 0.46, considerada moderada.

Palabras clave: Cuyes, dermatofitosis, cultivo micológico, citopatología.

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1 Introduction

Guinea pig (*Cavia porcellus*) is a rodent mammal from the Andean region. This species is important because it represents a product with a great nutritional value for high Andean rural areas. In addition, it is highly rustic, with competitive advantages over other species, and with commercial and economic viability (Morales, 2013; Solórzano, 2014). Despite its rusticity, there are factors that can predispose guinea pig to various diseases, such as variations in temperature, humidity, air currents, high population density, among others (Morales-Cauti, 2018). Dermatophytosis is one of the diseases that most affect guinea pig; it is a dermatophyte fungal infection that affects the stratum corneal of the skin, hair and nails. It is caused by fungi of the genera *Trichophyton* and *Microsporum*, and is transmitted by contact between sick animals or through contaminated facilities or tools. The most commonly found dermatophyte in guinea pig is *Trichophyton mentagrophytes*, which usually manifests with clinical signs such as non-itchy diffuse flaking and alopecia in the nose, ears, face and/or limbs (White et al., 2016). This infection may be accompanied by a secondary bacterial infection, where the lesion is suppurative known as weeping eczema (Burke, 1994; Indranil, 2015).

There are two essential methods for the diagnosis of dermatophytosis: direct examination and mycological culture; however, there are other methods such as Wood lamp or dermatoscopy (Hnilica and Patterson, 2017; Moriello et al., 2017). Skin cytopathology is not commonly used for the diagnosis of dermatophytosis, but it is mentioned as a valid technique (Mendelsohn et al., 2006; Joyce and Vandy, 2007; Scurrel, 2011; Miller et al., 2013; Wiebe, 2015; Albanese, 2017). However, mycological culture is the standard gold test for the diagnose of dermatophytosis and it should be performed whenever the disease is suspected (Patel and Forsythe, 2008). *aburaud dextrose Agar* is a peptone medium supplemented with dextrose to promote fungal growth. While peptone works as a source of nitrogenous growth factors, dextrose provides an energy source for the growth of microorganisms, the medium is not selective for dermatophytes since there is no inhibition of saprophyte fungi, thus to modify it chloramphenicol must be added to inhibit gram-negative and positive bacteria (Sparkes et al., 1993).

Dermatophytes are identified macroscopically based on their growth rate, appearance, texture, surface color and reverse color (Indranil, 2015). For performing the microscopic examination, the colonies are transferred to a slide, using a tape or sterile swab. Lactophenol cotton blue is added since it highlights the appearance of the hyphae and conidia; for identifying it, hyphae, macroconidial and/or microconids should be searched (Helton and Werner, 2018). On the other hand, skin cytology is the second most common technique for diagnosing dermatological diseases. It consists of identifying bacterial or fungal organisms (yeasts) and evaluating the types of inflammatory cells, neoplastic cells or acantholytic keratinocytes found in the skin (Hnilica and Patterson, 2017). Findings that may suggest a dermatophytosis infection are the presence of neutrophils, macrophages, keratinocytes and acantholytic cells. The first two are the most commonly detected cells in skin lesion samples. This type of mixed inflammation is often associated with foreign bodies, fungal infections, mycobacteria infections, granulomas and other chronic injuries (Raskin and Meyer, 2015).

For a definitive diagnosis of dermatophytosis, septate hyphae and/or arthroconidia should be detected on the surface of these corneocytes (Gross et al., 2005; Albanese, 2017). Purple or blue hyphae and spores may be observed with a Diff Quick stain (Neuber and Nuttall, 2017). In Peru, studies on the use of cytopathology in the diagnosis of dermatophytosis have not yet been reported. For this reason, the purpose of this study is to find the concordance between the mycological culture technique and the cytopathological technique in the diagnosis of dermatophytosis in intensively reared guinea pigs, and determine their frequency according to their own characteristics, so that cytology can be established as a rapid diagnostic technique for this disease, establishing appropriate treatment and decreasing its impact on the producer.

2 Materials and Methods

2.1 Date and place of the research

This study was conducted from January to March 2018, with an average environmental temperature of 19,5°C and humidity of 87% (INEI, Instituto Na-

cional de Estadística e Informática, 2018). The skin samples collected for the diagnosis of mycosis were transported for their evaluation to the Microbiology and Microscopy Laboratory of the Faculty of Veterinary and Biological Sciences of Universidad Científica del Sur. Populations of guinea pigs from intensive breeding systems, from both sexes and different productive stages (infant, breeding, reproductive) were studied. The samples were taken from animals with skin lesions such as flaking and alopecia, using surface skin scraping and deep skin scraping techniques.

2.2 Sample size

In determining the sample size of the study, reference was taken from the research that determined dermatophytosis in guinea pigs at the headquarters of INIA (Celis, 1998), in which 93% of prevalence was obtained; and the infinite population formula was used for this purpose. 189 samples were processed by opportunity and resource availability, and the distribution of which was as follows: Farm 1 (n=86), farm 2 (n=40), farm 3 (n=5) and farm 4 (n=73).

2.3 Obtaining of samples

The samples were collected using two techniques: surface skin scraping and deep skin scraping, mentioned by Bexfield et al. (2014). Surface scraping was performed for the mycological culture, while deep skin scraping for the cytology technique as bleeding helps to generate more contrast and facilitate the visualization of fungal structures alongside inflammatory cells of the lesion. The surface skin scraping was performed with a scalpel throughout the peripheral area of the lesion, taking scales and scabs. These were transported in sterile containers with a threaded lid for their processing in the laboratory. For the deep skin scraping, the surface of the skin continued to be scraped with the scalpel until capillary bleeding occurred. The sample was then transferred to a slide, and the sample was extended by passing one slide against another, using the squash technique (Valenciano and Cowell, 2014).

2.4 Citology processing

The slides with the samples were stained with the Diff Quick staining protocol. First they were immersed in the alcoholic fixer 3 times from 2 to 3 se-

conds at a time; then these were drained on filter paper; later, the samples were immersed in the basic red staining; and finally, in the purple acid staining during 8 seconds. They were then immersed in water to remove the excess of dye and were allowed to dry for 5 minutes (Albanese, 2017). The dyed and dried slides were observed under the microscope with a lens of 100X, in order to identify the types of existing cells and possible fungal organisms (Hnilica and Patterson, 2017). The samples considered positive were those that indicated the presence of hyphae and/or spores. Spores are small, rounded or oval structures with a clear pericellular halo. Hyphae were recognized for their peculiar bamboo branch shape, such as segmented linear filaments (Mendelsohn et al., 2006; Raskin and Meyer, 2015; Albanese, 2017).

2.5 Micological culture processing

Sabouraud Dextrose Agar medium with a 5.6 pH was used for the cultivation of the samples, to which chloramphenicol was added in a concentration of 50mg/dL. The samples were sown with the help of Falcon tubes containing the culture medium, in an inclined position or flute beak to avoid drying. Cultures were kept at a temperature of 22°C for 21 days, monitoring daily to observe the colony growth (Cuétara, 2007; Kraemer et al., 2012). Samples that on day 10 day had flat white, cottony or woolly colonies, golden edges with or without depressed center, and yellow orange or orange brown on the back (compatible with *M. canis*) were considered positive; as well as flat colonies with powdery texture, and cream color with white edges, white myceliums and pale yellow or brown on the back (compatible with *M. gypseum*); or flat colonies with a white or creamy powdery surface, and copper brown or dark red on the back (compatible with *T. mentagrophytes*) (Moriello, 2001; Miller et al., 2013). Colonies with other aspects were compared with an atlas of mycology to be considered positive or not to the diagnosis of dermatophytosis.

2.6 Microscopic identification

From the cultivated plates, the colonies were transferred to slides. Lactophenol cotton blue was used on the slides, and were then covered with tape. They were then observed under the microscope (Helton and Werner, 2018). Findings of fusiform

macroconidias of 6 or more segments with thick spiny walls (*M. canis*) were considered positive as well as findings of elliptical-shaped macroconidia with up to 6 segments and thin walls (*M. gypseum*); and findings of globose microconids with occasional presence of cigar-shaped macrochondicates with thin, smooth walls and sporadic spiral hyphae (*T. mentagrophytes*) (Miller et al., 2013). Kappa test was used to determine the concordance between the two techniques used, for which a 2×2 contingency table was prepared and is detailed below.

2.7 Interpretation of the results

The determination of Kappa's index, the STATA15.0 statistical package and qualitative interpretation based on the concordance force described by Altman (1990), were used and were qualified as: poor or weak for values lower than 0.40; moderate for values between 0.41 and 0.60; good between 0.61 and 0.80; and very good for values up to 1.13. The determination of the frequency of dermatophytes for each diagnostic technique was obtained from the concordance between the number of positive diagnoses versus the total number of animals evaluated.

3 Results and discussion

The overall frequency of dermatophytosis was $18.5 \pm 5.5\%$ (35/189) by using the culture technique, while in the cytopathology technique, the overall frequency was $43 \pm 7.1\%$ (81/189) (Table 1). The frequency for dermatophytosis in animals with lesions by the mycological culture method was $18.5 \pm 5.5\%$, contrasting with the values found by other authors using the same method in the country. Other authors report higher frequencies, between 50 and 95% of dermatophytosis (Celis, 1998; Jara et al., 2003; Pineda et al., 2009). These high occurrences are due to the environmental factor, since dermatophytes, although ubiquitous, have a higher frequency in warm places and high relative humidity (Helton and Werner, 2018). On the other hand, the immunity of the host also has an influence which depends on the age, feeding and management of animals (Morales, 2013); so this can be circumstantial, and it might have multifactorial influence.

Various factors influence the degree of kappa index (κ) consistency for this study where diagnos-

tic techniques such as mycological and cytological culture are used; these factors are age, gender, type of facilities, degree of training of the operating technicians responsible for obtaining sampling and the execution of diagnostic techniques (Table 2).

As for the frequencies of dermatophytosis per age group in this study using the mycological culture method, these were found to vary from 0% in lactating animals to 25.6% in breeding; these results were similar to those found by Jara et al. (2003), who reported that the breed had the highest percentage of positive animals. This is caused by the incomplete development of the immune system and the low concentration of fungistatic fatty acids present in the sebum (Richardson 2000, Patel 2008). In addition, after puberty the aggressions between males begin, increasing stress and causing injuries that serve as an entry route to the fungus (Jara et al., 2003).

In relation to the location of the lesion, injuries in the nasal and frontal region were much more common, while lesions found on the back of the caudal back and limbs did not show dermatophytosis. This also agrees with Jara et al. (2003) where lesions in the periocular and nasal region were the most reported, while the extremities and back show the lowest frequency of dermatophytosis. In addition, Miller et al. (2013) describe that the most affected areas by dermatophytosis are the nasal, periocular, frontal and atrial area, and can only rarely spread to the lumbosacral area without affecting the limbs. Therefore, this location of injuries could be related to the behavior of the species, easing the contact with contaminated areas of the environment, favored by the moisture of these areas due to the feeding behavior of the species.

As for the type of facility, there is a higher frequency of dermatophytosis in animals raised in pools. Jara et al. (2003) in determining the humidity of intensively reared guinea pigs reported that the pools that stay wet for longer present more animals with dermatological lesions. Therefore, it is confirmed that the type of installation influences the presence or absence of moisture, ventilation and lighting, and potentially the presentation of dermatophytosis.

Table 1. Frequency of dermatophytosis in guinea pig intensively reared according to the sex, age, location of the injury, and type of facilities; by mycological culture and cytology (n=189).

	Total of animals	Positive animals (culture)*			Positive animals (cytology)*		
		n	%	± IC 95 %	n	%	+ IC 95 %
Sex							
Female	103	8	7.80 %	± 5.20 %	24	23.00 %	± 8.10 %
Male	86	27	31.40 %	± 9.80 %	57	66.00 %	± 10.00 %
Age							
Lactating	1	0	0.00 %	± 0.00 %	0	0.00 %	± 0.00 %
Breeding	125	32	25.60 %	± 7.70 %	77	62.00 %	± 8.50 %
Reproductive	63	3	4.80 %	± 5.30 %	4	6.00 %	± 5.90 %
Location of the injury							
Nasal	64	18	28.10 %	± 11.00 %	45	70.00 %	± 11.20 %
Frente	12	5	41.70 %	± 27.90 %	8	67.00 %	± 26.60 %
Atrial	5	1	20.00 %	± 35.00 %	3	60.00 %	± 42.90 %
Face	3	0	0.00 %	± 0.00 %	3	100.00 %	± 0.00 %
Medial	96	10	10.40 %	± 6.10 %	17	18.00 %	± 7.70 %
Caudal dorsum	4	0	0.00 %	± 0.00 %	1	25.00 %	± 42.40 %
Limbs	2	0	0.00 %	± 0.00 %	1	50.00 %	± 69.30 %
Periocular	3	1	33.30 %	± 53.30 %	3	100.00 %	± 0.00 %
Facilities							
Cage	57	0	0.00 %	± 0.00 %	0	0.00 %	± 0.00 %
Pool	132	35	26.50 %	± 7.50 %	81	61.00 %	± 8.30 %
TOTAL	189	35	18.50 %	± 5.50 %	81	43.00 %	± 7.10 %

* The concordance determination between both diagnosis techniques was 0.46 (moderate).

On the other hand, the frequency for dermatophytosis in animals with dermatological lesions diagnosed by the cytopathology method was higher compared to the mycological culture, with indices of $43 \pm 7.1\%$ of the animals evaluated. In relation to the frequencies of dermatophytosis per age group in this study, it is reported that these vary from 0% in infants to 62% in breeding. At the level of affected

areas, the highest frequency occurs in the nasal, frontal and atrial region with frequencies between 28.1% and 40.7% (Table 1). As for the type of facility, there is a dermatophytosis frequency of 62% in animals raised in pools, and 0% in animals raised in cages. Showing more sensitivity of this technique for the diagnosis.

Table 2. Proportion of concordance between mycological culture technique and cytology as a diagnosis of dermatophytosis in guinea pigs.

	Cytological technique			
	Negative	Positive	Total	
Mycological culture technique	Negative	108	46	154
	Positive	0	35	35
	Total	108	81	189
Kappa index = 0.46				

The cytopathology technique is often used to determine the presence of etiological agents in dermatological lesions, because it is easy to perform, fast and it is minimally invasive; it is also not too

expensive (Neuber and Nuttall, 2017); however, it is not widely used for this purpose. Within this technique, the best sampling method is that of adhesive tape. However, the amount of fungi found will de-

pend on the intensity of the infection (Albanese, 2017). On the other hand, although the staining of the slides with Diff Quick is very effective to visualize spores and/or hyphae, stains such as Schiff or Gomori allow to distinguish much better these fungal structures in histopathological slides (Albanese, 2017); however, the costs are higher. On the other hand, with regard to the sensitivity of the diagnosis of dermatophytosis, the frequency reported by the cytopathology technique was higher than that reported by the mycological culture, because the latter not only identifies dermatophytes but also tested positive for other fungal species, subsequently defined by mycological culture.

In the study of concordance between the two diagnostic techniques (Table 2), the mycological culture technique and cytopathology determined that a moderate concordance of 0.46 was found using the Kappa test (Altman, 1990), due to the difference between the frequencies reported with both techniques. Despite the false positives of the cytopathology technique, these other fungal species not only cannot be considered as contamination but could potentially be causing the dermatological lesions. In addition, this result represents the first concordance study between the two diagnostic techniques.

Finally, cytopathology could be used as a first-intentioned technique for the diagnosis of dermatomycosis in guinea pigs, and if positive, a definitive diagnosis by mycological culture is necessary.

4 Conclusions

The degree of concordance found between the mycological culture and the cytopathology techniques for detecting dermatophytosis in extensive reared guinea pigs is moderate (Kappa = 0.46).

The presence estimation of dermatophytosis in extensive reared guinea pigs was $18.5 \pm 5.5\%$ using the mycological culture method and $43 \pm 7.1\%$ using the cytopathology method.

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