

Original Research Article

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Study on Ringworm in Donkeys: Risk Factors, Clinical Pathology, and Treatment in Alfola City, West Kordofan State

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ABSTRACT

The current study was carried out to identify and to evaluate some risk factors, clinical picture, pathological changes and treatment in donkey naturally infected by ringworm. A questionnaire was designed to 100 owners of suspected donkey with ringworm and included age, sex, breed, season, source of drug, drug use, lesion, housing, bedding, disposal of manure and animal purpose. A total of 36 donkeys naturally infected by the disease were examined visually (lesion shape, size, distribution, configuration and depth lesion). Health parameters were examined (respiratory and pulse rates and rectal temperature). Blood for serum was taken to determine total protein, albumin, creatinine, aspartate aminotransferase, alanine aminotransferase, zinc and copper. Skin scraping was used for mycological examination and skin biopsy was taken for histopathological changes. The treatment was applied using clotrimazole cream (1%) and *Azadirachta indica* examination. The results of the respondents revealed that old animals were infected by the disease (43 owners), and males (60 owners) were infected more than females (40 owners). Most of the lesions appeared in winter (37 owners) and treated by antifungal (41 owners). The majority of these animals were tethered (48 owners) and their bedding was sand (63 owners). Health parameters of infected donkeys showed no statistically significant differences ($P < 0.05$). But blood biochemical parameters revealed statistically significant difference at P-value 0.05 in infected animals. Mycological examination identified two genera and these were *Trichophyton spp* and *Microsporm spp*. histopathological changes were production of keratinized-epithelial cells, presence of inflammatory cells and mycelia around the hair follicles and desquamation of epithelial cells. All animals were treated by clotrimazole (1%) and 32 (88.8%) were recovered and also 27 (75%) were response to the herbal therapy. In conclusion, ringworm is spreading in donkeys in the study area and these animals are important for human purposes. The diagnosis of the disease can clinically, mycologically, histopathologically and estimation of the blood chemistry. The herbal therapy can be developed as the drug for treatment of the disease.

Keywords

Ringworm,
Donkeys, Alfola
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Introduction

Dermatophytosis or ringworm or Tinea is superficial skin infection caused by closely related keratinolytic fungi (Rossed *et al.*, 2008; Moriarty *et al.*, 2012). The disease is contagious among animals and great economic loss resulting from cost of treatment in public health. The importance of ringworm is zoonotic disease and all animal-associated dermatophytes are transmitted to humans (Chermette *et al.*, 2008; Bond, 2010). Soil where the animals rolling and lying down and this is depended on the fungal species, age and health condition of the skin surface and grooming. Also, the spores remain viable in suitable environments for up to 12-20 months, but some spores persist for at least nine years in salty water (Acha and Szylfres, 2003).

The pathogenic fungi causing ringworm in donkeys as zoonotic agents are *Trichophyton mentagrophytes* and *Microsporum racemosum* (Rodostits *et al.*, 2007). Recently, *Microsporum racemosum* has been isolated from naturally infected donkeys (Nardoni *et al.*, 2016). Dermatophytes as keratinophilic fungi are able to infect keratinous tissues skin (the stratum corneum layer), hair and nail in human via keratinase enzymes and in animals they are degraded cloths, leathers, hooves, horns, wools (Moriarty *et al.*, 2012).

The lesions mainly involve the head and neck. In the head especially around the eyes, muzzle and at the base of the ears. Also, these lesions may extent to the neck, back and perineum (Cam *et al.*, 2007; Ozanlar *et al.*, 2009; Jameel *et al.*, 2016; Simone *et al.*, 2016).

For confirmation, evaluation of the condition in animals by physical examination (Temperature, pulse rate, respiratory, appetite and morbidity rate (Kelly, 1984). Laboratory examination including wood's lamp, culture of dermatophytes and histopathology (Moretti *et al.*, 1998; Hainer, 2003; Behzadi and Behzadi *et al.*, 2003; Chaya and Ponda, 2007; Arbatzis *et al.*, 2007; Chermette *et al.*, 2008). Also, advanced molecular diagnostic tools are

provided successfully approaches for rapid diagnosis of pathogenic dermatophytes high accuracy, sensitivity and specificity (Behzati and Behzati, 2012).

In Sudan, donkeys are important animals by giving the new socio-economic situation that being used in labor and transportation (Ali *et al.*, 1981). The population of these animals was estimated about 7.5 million (MARF, 2009). The disease is known as Dalag in animal and Goub in human. A severe outbreak was occurred in this country to 69 about domestic donkeys caused by *T. mentagrophytes* (Ali *et al.*, 1981). Today *T. verrucosus* been isolated from infected donkeys (Wisal *et al.*, 2005).

Systemic treats are recommended for infected animals by injection of 10% sodium iodide for oral administration of griseofulvin. Also, topical application of lesions by Clotrimazole miconazole. Preparation is effective (Radostits *et al.*, 2007). Most of vaccines prepared from *T. verrucosus* are a live monovalent vaccines (Gokee *et al.*, 1999). The aim of the paper was to identify the dermatophytes causing ringworm in donkeys with clinical and pathological changes and examination of the treatment of the disease by Clotrimazole cream (1%) and *Azadirachta indica* extract (Neem leaves).

Materials and Methods

Study area

The study was conducted in Alfola city, Kordofan State, Sudan. The city located between latitude 11° - 20 north and longitude 32° - 22 - 22 - 30.27" east (<https://wku.edu.sd>, 2017).

Questionnaire

A questionnaire was carried out for 100 owners of suspected animals with ringworm and including the characters of animals and these were age, sex, breed, lesions and animal purpose. Also, it was composed of environmental factors and these were housing, bedding, disposal of manure, season. Medical source

was also included in the questionnaire.

Clinical examination

A total number of 36 draught donkeys of the age between 5 – 10 years old were selected according to skin lesions (shape, size, distribution and the time of appearance). All animals were examined clinically for estimation of respiratory and pulse rate and temperature (Kelly, 1984).

Mycological examination

Deep skin scrapping was taken from suspected donkeys (Cheesbrough, 1992) from active margin of the lesion using sterile disinfection (70% ethyl alcohol). The samples (36 samples) were submitted to microbiological laboratory in the Faculty of Veterinary Medicine, West Kordofan University for direct examination of the fungi using (10%) potassium hydroxide and gently heated (Hainer, 2003). Also, these samples were for identification of time of the growth and colony morphology using lactophenol cotton blue.

Biochemical analysis

Whole blood for serum were collected from suspected donkey (36 serum sample) for evaluation of total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), Copper (Cu) and zinc (Zn).

All biochemical parameters were analyzed by test skin (Biosystem, S. A. Spain)

Histopathology

Skin biopsy was taken from skin lesion (8 animals) of the suspected donkeys in 10% formal saline. The formaline fixed tissues were processed according to the method of Culling (1974). The sections were stained with the Hematoxylin and Eosin (Luna, 1968) for microscopic examination.

Drug Candid cream

Drug used for treatment of infected donkeys were 1% Clotrimazole (Candid cream) and Neem leaves extract (*Azadirachta indica*). One drug used by each site of the animal for comparison.

Statistical Analysis

The data analyzed by Social Package for Science (SPSS) version 16.0. The statistical method was used was descriptive and expressed as frequencies. One-way analysis of variance used to generate the analysis for variance table (ANOVA).

Results and Discussion

The questioned people said that animals were infected by the disease (43 owners) and males (60 owners) were infected more than the females (40 owners). Also, Diplawi breed was lowered infected o disease than Hur and mixed bread (Table 1). The lesions appeared mostly in winter season (37 owners) and it was decreased in summer (30 owners). The respondents revealed that these animals their purpose in urban, there was variation of disposing of manure, the majority of these animals were tethered ad their bedding was sand. They were taking their drugs from pharmacy and antifungal drugs were most common used (Table 1).

In visual examination the lesions were scaly, alopecia in face and neck, grey-white areas of skin with an ash-like surface in naturally infected donkeys. Also, some of the lesions were circular in outline and there was enlarged and raised.

There was no statistically significant difference in health parameters ($P < 0.05$), but there were slightly changes in these parameters during the three weeks of treatment.

The identification of fungal scraping from infected donkeys revealed that red, pink, and grayish colonies and after 14 days the color of these colonies change to brownish, white cottony and yellowish color. The colonies become fully after three weeks.

When these colonies were staining, two genera were identified and these were *Trichophyton spp* and *T. microspore spp*.

The results revealed a statistically significant difference at P-value 0.05 in all blood biochemical parameters used (Table 3).

Histopathological changes in diseased animals were present of keratinized epithelial cells, also the inflammatory cells and mycelia around the hair follicles and desquamation of epithelial cells.

The treatment of infected animals was used by application of candid cream at one side and Neem leaves at other side in each infected animal for duration and efficacy of each drug. In the first week candid treated 33.3% of the animals, while Neem leaves treated 25% of these animals. In second week 55.5% of the animals were treated by the cream and 50% of the animals by herbal drug and in the third week. The results showed that 88.8% of these animals were treated by the cream and 75% animals were treated by herbal drug.

In this study, ringworm is causing highly infection in donkeys (Table 1). These results are supported by observation of Nooruddin and Singh (1987) and Radostits *et al.*, (2007).

From this result the diseased was decreased in free animals with hay bedding, disposal of manure in specific time and rural animals. These findings in agreement with recorded by Birthal *et al.*, (2002). Also, the present study showed that naturally infected donkeys in old ages were susceptible than adult and young animals and this can be attributed to decrease of the immunity in these animals. This finding disagreed with the finding of Pascoe (1976). Diplawi breed was lowered infection by dermatophytes may be due to immunological response of the treat and skin sensitivity to the disease. These results revealed that males are more susceptible than females, similar to the results of Elham *et al.*, (2017). Also, the study showed that the disease increased in winter than other seasons

(Nooruddin and Sigh, 1987; Radostits *et al.*, 2007).

Generally, the high relative humidity has a significant influence on maturation and motility of infective zoospores and this play a role as predisposing factor in spreading of the disease. Also, the dermatophytes are growing best in worm and humid environments and these fungi common in tropical and subtropical areas (Fadlelmula *et al.*, 1994), but their variation according to geographic distribution. The treatment of the disease by antifungal drugs are available (Adams, 2001; Kahn and Line, 2005).

The skin lesions in this study (scaling, alopecia, nummular areas on the face and the neck, grey-white areas with an ash-like surface, circular lesions in outline and raised slightly) are similar to recorded by Chermette *et al.*, (2008) and Simona *et al.*, (2016). The disease can be started as alopecia, crusts, scaling on the flanks, backs, face, ears, legs ad gluteal areas (Suleiman *et al.*, 2017; Boyen *et al.*, 2018). Also, the health parameters (Table 2) showed slightly increased in their measurements may be due to chronicity of pathogenic agent not like other infectious agents (bacteria and viruses). In these results two genera were identified and these were *Trichophyton spp* and *Microsporum spp* in diseased donkey (Ali *et al.*, 1981; Abu-Samra and Ibrahim, 1988; Wisal *et al.*, 2005; OIE, 2005; Radostits *et al.*, 2007; Shathele, 2014; Nardoni *et al.*, 2016; Suleiman *et al.*, 2017; Boyen *et al.*, 2018).

The blood biochemical parameters were estimated in this study revealed that statistically significant difference due to pathogenicity of mycotic agents in the blood constituents. These findings in contrast with findings of Radostitis *et al.*, (2007) who stated that there are no changes in the blood constituents. The treatment by candid cream in infected donkeys showing high efficacy and this result in accordance of Kahn and Line (2005). Also, the treatment by Neem leaves extract giving good responses as medicinal herbal drug (Khan and Wassilew, 1987; Govindach *et al.*, 1998).

Table.1 Summary of questions to owners (n=100) of suspected donkeys by ringworm in Alfoa City, West Kordofan State

Information	Frequency	Percent %
Age/year		
5-6	30	0.3
7-8	27	0.27
9-10	43	0.43
Sex		
Male	60	0.6
Female	40	0.4
Bread		
Hur	36	0.36
Diplawi	30	0.3
Mixed	34	0.34
Season		
Autumn	33	0.33
Summer	30	0.30
Winter	37	0.37
Source of drug		
Clinic	25	0.25
Pharmacy	75	0.75
Drug use		
Antifungal	41	0.41
Anthelmintic	29	0.29
Antibiotic	21	0.21
Insecticide	9	0.09
Lesions		
Distribution	86	0.86
Configuration	96	0.96
Description	100	1
Depth	90	0.9
Quality	94	0.94
Housing		
Zariba	33	0.33
Tethered	48	0.48
Free	19	0.19
Bedding		
Hay	45	0.45
Sand	63	0.63
Disposal of manure		
Weekly	51	0.51
Specific time	49	0.49
Animal purpose		
Rural	42	0.42
Urban	58	0.58

Table.2 Some physical parameters of donkeys (n=36) infected by ringworm – three weeks period in Alfola city, West Kordofan State

Parameters	Mean	Min	Max	mean ±Sd	Sig
Respiration (cycle/min)	23	11	36	19.3±7.23	N
	1				
	11	12	19		
	24	10	30		
Pulse rate (min)	56	40	64	40.36±13.57	N
	33	35	45		
		22	59		
	52				
Temperature (°)	37.8	36	38.8	37.36±0.45	N
	36.9	36.9	39.2		
		37.4	38.5		
	37.4				

N = Non significant P<0.05

Table.3 Blood Biochemical Parameters of Donkeys (n=36) Infected by Ringworm Three Weeks Period in Alfola City, West Kordofan State

Parameters	Neem	Min	Max	Mean±Sd	Sig.
Total protein mg/dll	58	26	71	60±2.66	*
	62	27	78		
	60	25	73		
Albumin mg/dll	19	10	27	20±1.52	*
	20	17	27		
	22	15	29		
Creatinine mg/dll	0.7	0.4	1.1	0.7±0.001	*
	0.7	0.5	1.2		
	0.7	0.4	1.2		
Aspartate Aminotransferase µ/L	4.7	2.2	8.3	40±0.70	*
	4.0	2.1	5.6		
	5.4	2.4	5.8		
Alanine Aminotransferase µ/L	6.8	2.1	11	6.8±0.50	*
	6.5	3.2	10		
	5.8	3.5	11		
Zinc Mg/L	0.17	0.13	0.21	0.18±0.12	*
	0.19	0.15	0.23		
	0.19	0.14	0.20		
Copper Mg/L	0.17	0.13	0.19	0.16±0.01	*
	0.14	0.14	0.18		
	0.18	0.13	0.21		

* ≡ Significance P<0.05

The results of this study showed ringworm is spreading among donkeys in the study area and the diagnosis can be clinically description of the lesions, demonstration of the fungi in the laboratory,

estimation of the constituent of the blood and for confirmation histopathological technique can be used.

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