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Full Length Research Paper

Seroprevalence of canine leptospirosis, in Urban and Periurban, Morogoro, Tanzania

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A cross-sectional study was carried out in the Morogoro region, Tanzania, to determine the seroprevalence of canine *Leptospira* exposure. A total of 232 sera were collected from apparently healthy dogs in Mvomero, Morogoro Urban and Morogoro Rural districts. The microscopic agglutination test (MAT) was performed following standard procedure using panel of six *Leptospira* serovars. Within the districts, positive reactions against five serovars were detected: Sokoine (4.3%); Pomona (4.3%); Lora (3.0%); Grippotyphosa (2.2%), and Kenya (0.9%). The overall seroprevalence was found to be 9.5%. Male dogs were at significantly greater risk than the female dogs ($p < 0.05$); but no significant difference in prevalence was observed with respect to age and breed ($p>0.05$). The growing urbanization, which allows high interaction between different maintenance hosts, may cause infection spill over and consequently a rising prevalence. The presence of *Leptospira* antibodies suggests that leptospirosis is common in this study area. Therefore, further serological surveys followed by isolation and identification of isolates in this study area and other areas of the country need to be undertaken to report infective serovars in canine population.

Key words: Seroprevalence, leptospirosis, serovars, epidemiology, dogs.

INTRODUCTION

Leptospirosis is one of the most important bacterial diseases in dogs (Ghasemzadeh and Namazi, 2015). Due to the climate change and shift in infective serovars, it is now regarded as re-emerging disease (Chomel, 2014; Knoepfler, 2015). Serosurveillance followed by production and usage of vaccines consisting of circulating serovars in the region are essential preventive tools in endemic regions (Senthil et al., 2013). Human leptospirosis, has received critical attention in Tanzania and animals have been suspected to be the source of the disease (Biggs et al., 2011). Although that clinical evidence of the disease exists, no isolation of the microorganism has been achieved in dogs in the country (Barnabas and Muhairwa, 2015).

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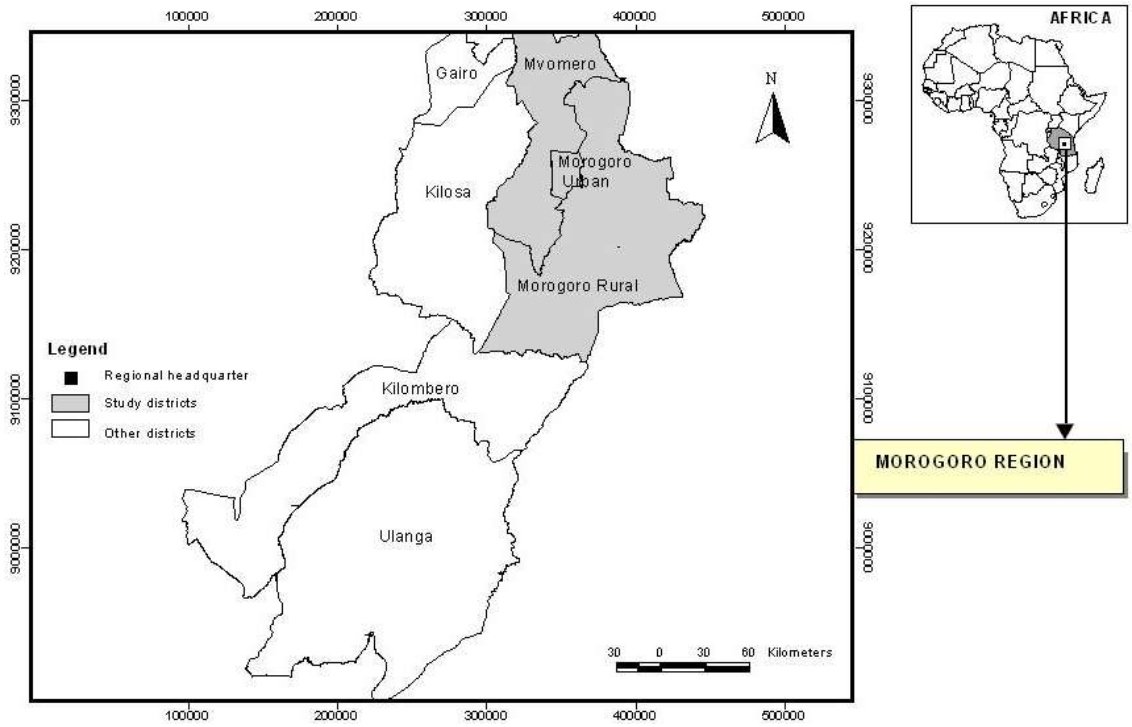

Figure 1. Map of the Morogoro region showing the study areas.

In United States and Canada, leptospirosis has been found to be more prevalent in adult male dogs (Ward et al., 2002a). In a subsequent study, Ward et al. (2002b) showed rainy tropical climate to be particularly favourable for the survival of the pathogen. Furthermore, flooding and growing urbanization are drivers for leptospirosis (Lau et al., 2010). In a study by Meeyam et al. (2006), dogs in contact with sewage-contaminated environments, or that consumed raw meat had a higher risk of contracting leptospirosis.

Canine leptospirosis has been reported in a number of African countries with varying prevalence such as Senegal 83% (Roqueplo et al., 2016), Nigeria 16.7%, Ethiopia 8.3%, South Africa (Coastal regions) 4.7%, South Africa (Gauteng Province) 50%, and Zimbabwe 15.6% (de Vries et al., 2014). In Tanzania, early studies by Machang'u et al. (1997) showed that 37% of dogs were seropositive to serogroup Icterohaemorrhagiae; this was higher than that of cattle, rodents and humans. Subsequent investigations provided further evidence of seroprevalence in African giant rats (Machang'u et al., 2004), pigs (Kessy et al., 2010) and cattle (Assenga et al., 2015). The reported seroprevalence data exhibits widespread exposure to *Leptospira* species in humans and animals in Tanzania, with dogs ranking highest, however, no clear link to the clinical disease in dogs has been established. According to Barnabas and Muhairwa (2015), records from clinic and pathology at the Sokoine University of Agriculture (SUA) Animal Hospital show the existence of seasonal morbidity and mortality suggestive of leptospirosis syndrome in adult dogs, which occur after the rainy season. Similar trends have been observed in different parts of the country such as Dar es Salaam and Dodoma, however, no research data is available to support the observations. Moreover, dogs are fed with raw condemned meats, including, kidneys from pigs and cattle, which expose them to risk of *Leptospira* infection.

There is no recent information about *Leptospira* seroprevalence in canines and no attempt has been made on identification of the circulating serogroups in Tanzania. Also, the studies carried out in dogs in the country did not determine risk factors possibly associated with the disease. The aim of this study was therefore, to determine the seroprevalence and risk factors associated with *Leptospira* infection in healthy dogs in three districts of Morogoro region, Tanzania. Knowing the *Leptospira* seroprevalence in the mentioned study areas will help to advocate a 'One Health' approach to promote multidisciplinary research efforts to improve understanding of the animal to human transmission of *Leptospira* infection in Tanzania and elsewhere.

MATERIALS AND METHODS

Study area

The study was carried out in Morogoro region, in the three districts of Mvomero, Morogoro Urban, and Morogoro Rural (Figure 1) from

November 2016 to July 2017. Morogoro region is situated between 5°58' and 10°0' S and 35°25' and 35° 30' E. The population of dogs in each district was estimated at 5645, 4624 and 10,125 in Mvomero, Morogoro Urban, and Rural districts, respectively (as per communication through District Veterinary Offices). The following wards were selected in each district; Morogoro Rural (Kingolwira and Pangawe); Mvomero (Mangae and Mzumbe); (Kihonda, Misongeni, Magadu and Mazimbu) Morogoro Urban. The study districts and wards were purposively selected based on the population of dogs.

Study animals

The study included only dogs of one year of age and above, to exclude possibility of maternal antibodies known to exist up to that age (Chappuis, 1998), currently not vaccinated against leptospirosis. Exclusion criteria included dog aggressiveness and poor health conditions not necessarily related to leptospirosis, such as emaciation and poor demeanor/obvious ill dogs.

Study design

A cross-sectional study design with random sampling of dogs was used. The sample size was estimated according to Fosgate (2009). A cross-sectional study design with random sampling of dogs was used. The wards in each district were selected purposively based on the number of dogs kept. From a list of wards that had dog population, villages were selected using a simple random technique. The list was obtained from the Village Administrative Office and was used as primary sampling unit. A total of 232 blood samples were collected. This was a convenience sampling based on the availability of both male and female dogs in the study area.

Sample collection and handling

Basic descriptive characteristics of the dog were initially recorded (that is, gender, age and breed). Apparently healthy dogs were manually restrained and blood was collected from the cephalic vein using a 25G, 2 ml syringe. Blood (2 ml) samples were then transferred to plain Vacutainer® tubes, allowed to clot overnight in a refrigerator for separation of serum. The sera were subsequently harvested and dispensed into appropriately labeled 1.5 ml cryovials, and stored at -20°C freezer at the Pest Management Centre-Leptospirosis Research Laboratory (SPMC-LRL) until subjected to MAT.

Serological analysis

Live leptospiral antigens representing six commonly found serogroups for MAT in Tanzania were used. Two of the reference serogroups Hebdomadis (*Leptospira santarosai* serovar Hebdomadis) and Pomona (*Leptospira interrogans* serovar Pomona) were initially obtained from the WHO Reference Laboratory at the Royal Tropical Institute (KIT), Amsterdam, Netherlands. The remaining were local serogroups Grippotyphosa (*Leptospira kirshneri* serovar Grippotyphosa), Icterohaemorrhagiae (*L. interrogans* serovar Sokoine), Australis (*L. interrogans* serovar Lora) and Ballum (*Leptospira borgpetersenii* serovar Kenya) provided by the Pest Management Centre, Morogoro, Tanzania. The serovars were grown in fresh Ellinghausen and McCullough medium-modified by Johnson and Harris (EMJH) (Difco-USA) for 5 to 7 days, reaching a density of 3×10^8 leptospires/ml on the MacFarland scale, according to the guidelines of WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis of the Royal Tropical Institute, Amsterdam, Netherlands.

Antibody detection

The MAT was applied to determine *Leptospira* antibodies in dog sera as described elsewhere (Cole et al., 1973). Briefly, 10 µl of the sera were mixed with 90 µl phosphate buffered saline (PBS) in 'U-shaped' microtiter plates to obtain 100 µl (1:10 dilutions). Further doubling dilutions (1:20 to 1:160) were prepared in subsequent wells and then 50 µl of the fully-grown serovars in EMJH medium was added to the sera in the microtiter plate wells and mixed gently and then incubated at 30°C for 2 h. The serum antigen mixture was then visualized by DF microscopy for the presence of agglutination and the titres recorded. A sample was considered positive if 50% or more of the microorganisms in the microtiter well agglutinated at the titre ≥1:160 (Assenga et al., 2015). This was determined by comparing 50% of spirochaetes, which remained free with a control culture diluted 1:2 with PBS (Korver, 1992). Positive samples were further diluted to titres of 1:5120 to appreciate the end point titres. Phosphate buffered saline was used as a negative control, whereby 50 µl volume of PBS was mixed with an equal volume of the different antigens.

Ethical considerations

This research was conducted in adherence with the Sokoine University of Agriculture (SUA) Code of Conduct for Research. The clearance for conducting this research was obtained from the Ethical Clearance Committee of the College of Veterinary Medicine and Biomedical Sciences, SUA, and from the District Executive Director of each of the three districts involved in this study. A verbal consent was sought from the dog.

Data analysis

Data was stored using Microsoft Office Excel® 2013. Descriptive statistics to determine prevalence was computed using EpiInfo™ software version 7.1.4.0 (2014). Statistical significance was determined at 95% CI at critical probability of ($p < 0.05$). Identification of determinant factors for *Leptospira* infection such as gender, breed and age of dogs was analyzed by Logistic Regression Model whereby all variables were entered once at a time using SPSS version 20.

RESULTS

A total of 232 dogs with no history of vaccination against leptospirosis were sampled from the three districts; Morogoro Rural (n=32), Morogoro Urban (n=122) and Mvomero district (n=78) were screened for canine leptospirosis using MAT test. The collected samples per ward are shown in Table 1. The MAT detected 14 (11.5%) positive samples from Morogoro Urban district, Morogoro Rural, 4 (12.5%) and Mvomero, 4 (5.1%). The overall *Leptospira* seroprevalence in this study was 9.5%. The serovars that showed seropositivity were: Sokoine (4.3%); Pomona (4.3%); Lora (3.0%); Grippotyphosa (2.2%); Kenya (0.9%) (Table 2). Table 3 shows that male dogs had a higher seropositivity (15.3%) compared to female dogs (2.0%). Results of the multivariable logistic regression for MAT seropositivity showed that only

Table 1. MAT test results of dogs by individual districts.

District	Ward	No. of samples	Positive samples	Seroprevalence (%)
Mvomero	Mangae	46	2	4.3
	Mzumbe	32	2	6.3
Sub total	-	78	4	5.1
Morogoro Rural	Kingolwira	12	2	16.7
	Pangawe	20	2	10
Sub total	-	32	4	12.5
Morogoro Urban	Kihonda	20	0	0
	Misongeni	46	10	21.7
	Magadu	30	4	13.3
	Mazimbu	26	0	0
Sub total	-	122	14	11.5
Total: 3 districts	8 Wards	232	22	9.5

Table 2. Prevalence of *Leptospira* serovars in canine sera collected in Morogoro Urban, Morogoro Rural and Mvomero districts.

Serovar	Number	%	95% CI	
			Low	High
Hebdomadis	-	-	-	
Kenya	2	0.86	0.24	3.08
Sokoine	10	4.31	2.36	7.75
Grippotyphosa	5	2.16	0.93	4.95
Lora	7	3.02	1.47	6.1
Pomona	10	4.31	2.36	7.75

Table 3. Seroprevalence of *Leptospira* infection in dogs by sex, age and breed, in Morogoro, Tanzania.

Risk factors	Categories	Seropositivity	
		Number	%
Sex	Male	20	15.3
	Female	2	2.0
Age	1 year	16	11.4
	More than 1 year	6	6.5
Breed	Mongrel	17	8.7
	Mixed	5	13.5

gender difference was statistically important. Specifically, male dogs (15.3%) were more likely to have canine leptospirosis antibodies ($p < 0.004$, 95% CI = 2.1 - 44.4) compared to female dogs (Table 4).

Table 5 shows frequency of MAT titres of dog sera collected from Morogoro urban, Morogoro rural and Mvomero districts against six *Leptospira* antigens. A total of 22 sera showed relatively high titers, suggesting an active infection. Fifty-two (22.4%) of the tested animals had lower antibody levels (1:20 to 1:80) that is, below the cut-off point of 1:160 recommended (WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis of the Royal Tropical Institute, Amsterdam, The Netherlands).

Table 4. Multivariable logistic regression analysis of sex as a risk factor for leptospirosis in dogs in Morogoro, Tanzania.

Variable	p-value	OR	95% CI	
			Lower	Upper
Sex (reference female)	0.004	9.590	2.070	44.436
Age (reference more than 1 year)	0.370	1.618	0.565	4.630
Breed (reference mongrel)	0.843	0.875	0.233	3.288

Table 5. MAT titres by serovar of dog sera collected from Morogoro Urban, Morogoro Rural and Mvomero districts.

Serovar	1:20	1:40	1:80	1:160	1:320	1:640	1:2160	1:5120
Hebdomadis	3	1	1	-	-	-	-	-
Kenya	2	2	4	2	-	-	-	-
Sokoine	8	6	6	4	4	1	-	1
Grippotyphosa	4	4	0	3	2	-	-	-
Lora	4	11	5	4	3	-	-	-
Pomona	9	3	6	3	1	4	2	-

DISCUSSION

This study was carried out with the aim of establishing seroprevalence and risk factors associated with *Leptospira* infection in healthy dogs in three districts of Morogoro region, Tanzania. It was found that, 9.5% of dogs were seropositive for *Leptospira*. Age, sex and breed were the risk factors analysed, however, only sex has shown significance. Moreover, seroprevalence was higher in male as compared to female dogs. Seroprevalence among the three districts was reported as follows: Morogoro Urban district (11.5%), Morogoro Rural (12.5%), and Mvomero (5.1%).

The serology results indicate that 9.5% of dogs, with no history of vaccination had previous exposure to the *Leptospira*. These findings are consistent with those of Moch et al. (1975), who found seroprevalence of 8.3% in Ethiopia but differ from those of Mgode et al. (2015) who reported 39% prevalence in dogs. A possible explanation to the findings by Mgode et al. (2015) is that, all the reactors at titres 1: 20 were considered positive while in this study, only reactors with a titre ≥1:160 were considered positive (Cole et al., 1973; Assenga et al., 2015). According to Miller et al. (2011), there is no consensus on cut off titre that constitutes seropositivity by MAT in leptospirosis. Titres lower than 1:160 in the present study can be considered indicative of an early phase of leptospirosis or previous exposure to the organism from diverse reservoir hosts.

The current study found that dogs in the households examined have been exposed to at least five serovars, namely, Sokoine (4.3%), Pomona (4.3%), Lora (3.0%), Grippotyphosa (2.2%) and Kenya (0.9%). This study differs from that of Mgode et al. (2015), where the most predominant serovars in dogs were Sokoine (39%), Kenya (26%), Grippotyphosa (10%), Pomona (9%), Hardjo (9%), and Canicola (5%). This study agrees with studies elsewhere that, there is a high level of variation in *Leptospira* serovars in different areas. For example, in Ethiopia and Nigeria the most predominant serovar was Grippotyphosa (Moch et al., 1975; Okewole and Ayoola, 2009), while in Uganda, predominant serovars were Icterohaemorrhagiae (42.8%), Canicola (39.2%), Pyrogenes (21.4%), Tarassovi (10.7%), Grippotyphosa and Australis (7.2%) (Millan et al., 2013). Direct comparisons of seroprevalence are often difficult as the vaccination history of dogs, the MAT panel of serovars and the detection method vary among studies. Limitation of the current study was that, only six reference serovars were used in the MAT panel, living out two more serovars Hardjo and Canicola, thus might have missed the antibodies and misrepresented other unknown infecting serovar. According to Mgode et al. (2015), the requirement for leptospirosis serodiagnosis in Tanzania is to include eight serovars (Sokoine, Kenya, Grippotyphosa, Lora, Pomona, Hardjo, Hebdomadis, and Canicola). However, the panel may not be complete as other serovars could be present in the country.

Leptospirosis is therefore, a potential public health threat in the study area. This study provides information on circulating serovars in canine population and contributes to our overall knowledge to help us in designing possible preventive measures. One such measure is vaccination of dogs using the local circulating serovars to protect dog health in the study districts and elsewhere in Tanzania.

In this study, occurrence of the "pig" serovar Pomona (4.3%) in dogs, suggests that pigs in the study areas could be important maintenance hosts of this serovar. Indeed, close contact of dogs and pigs is common in the study districts due to keeping of pigs in peridomestic areas. This finding is consistent with those of United States and Canada which implicate serovar Pomona as among the main serovars causing canine leptospirosis (Prescott et al., 2002). This study has demonstrated a decline in host serogroup specificity, which according to Goldstein (2010), there are serovars pathogenic to dogs other than Icterohaemorrhagiae and Canicola. The reported seroprevalence to serovar Sokoine, which was first isolated from urine of cattle by Mgode et al (2006) and serovar Pomona which was previously reported by Kessy et al. (2010) in pigs in Morogoro suggests there is a "sharing" of *Leptospira* serovars between dogs, cattle and pigs, in Morogoro. In other countries, the serogroups of *Leptospira* found in cattle and pigs were different from those found in Morogoro. For example, in Brazilian cattle were found serogroups Sejroe, Hardjo, Tarrassovi, Bratislava, and Icterohaemorrhagiae (Guitian et a., 2001), while in pigs in Japan serogroups Australis, Icterohaemorrhagiae, and Pomona were reported (Kazami et al 2002). In Tanzania, rodents are natural carriers of *Leptospira* serovars Lora, Grippotyphosa and Kenya (Assenga, 2003; Mgode et al., 2015), which were found in this study suggesting that, rodents may be a source of *Leptospira* infection in dogs in Tanzania.

The present study observed that sera agglutinate to more than one serogroup, which implies serological cross-reactions. These reactions between serovars belonging to different serogroups can be due to mixed or two different past infections. One of the sera showed cross positivity for serogroup Icterohaemorrhagiae (*L. interrogans* serovar Sokoine) and Pomona (*L. interrogans* serovar Pomona), while two samples were positive for serogroup Grippotyphosa (*L. kirshneri* serovar Grippotyphosa) and Pomona (*L. interrogans* serovar Pomona); and one sample each for serogroup Ballum (*L. borgpetersenii* serovar Kenya), Icterohaemorrhagiae (*L. interrogans* serovar Sokoine), Grippotyphosa (*L. kirshneri* serovar Grippotyphosa), Australis (*L. interrogans* serovar Lora) and Pomona (*L. interrogans* serovar Pomona), and for serogroup Grippotyphosa (*L. kirshneri* serovar Grippotyphosa), Australis (*L. interrogans* serovar Lora) and Pomona (*L. interrogans* serovar Pomona), respectively. According to Felt et al. (2011), cross-reactions are reported in acute or early convalescent sera, whereby the host, infected previously with one serogroup, may successively become infected by another serogroup, and the recently acquired serogroup may cross-react to the previous one, leading to activation of the memory response against the subsequent serogroup.

A significantly higher seroprevalence was recorded amongst male dogs ($p < 0.004$) as compared to female dogs similar to what was previously reported by Ward et al. (2002a). This is possibly due to increased outdoor activities, sniffing habits and the licking of external genitalia by infected males and females (Luna et al., 2008; Cisneros et al., 2002). Similar trend was observed in another study conducted by Meeyam et al. (2006), who reported dogs with increased outdoor activities or that consumed raw meat to be at a higher risk of leptospirosis. However, the later was not the case in this study area, as majority of the dog owners fed their dogs with stiff porridge mixed with sardines. However, there was no significant difference in prevalence with respect to age which contradicts with Ward et al. (2002a) who reported that male dogs' age 4 to 10 years old had significantly increased *Leptospira* antibody levels. The majority of the dog sampled were the mixed breed (n = 195) and a few Mongrel breeds (n = 37). This sample was not adequate to detect differences in prevalence of positive leptospiral titres by breed in the total population of 232 dogs sampled.

This study demonstrated *Leptospira* antibodies in unvaccinated, clinically healthy dogs at titres ≥160. The findings provide further support to the assertion that dogs could serve as maintenance hosts of the spirochete to other animals. Improving hygiene and removal of rodents may also reduce the risk of infection in dogs.

Conclusion

This study has found that leptospirosis is a potential health risk to dogs in Tanzania.The significantly higher seroprevalence amongst dogs from Morogoro rural compared with those from Morogoro urban and periurban districts may be indicative of differences in area-level risk factors. Sex was identified as a risk factor for leptospirosis where male dogs are significantly at higher risk of infection as compared to female dogs. Moreover, sharing environment amongst domestic animals, that is, dogs, pigs and cattle may result into infection spill over, consequently rising prevalence and risk of acquiring leptospirosis by dog keepers.

Further serological surveys using antigen panels with more serovars is required. In addition, isolation and identification of locally circulating serovars are needed. Vaccination of dogs using local leptospiral antigens should be encouraged and done alongside thee control of distemper, hepatitis and other canine diseases. Also, disease prevention programs for pet owners need to be instituted to reduce the public health risk of pet leptospirosis. Furthermore, implementation of efficient management of rodents and infection in livestock must be emphasized.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Assenga JA (2003). Habitat use and social structure of *Cricetomys gambianus* in Morogoro Tanzania, with reference to its potential role as disease reservoir. M.Sc. Dissertation, Sokoine University of Agriculture.

Assenga JA, Matemba LE, Muller SK, Mhamphi GG, Kazwala RR (2015). Predominant leptospiral serogroups circulating among humans, livestock and wildlife in Katavi-Rukwa ecosystem, Tanzania. PLOS Neglected Tropical Diseases, 9(3):1-14.

Barnabas E, Muhairwa AP (2015). Clinicopathological information on suspected cases of Leptospirosis in Dogs submitted for postmortem examination at the College of Veterinary and Medical Science, Sokoine University of agriculture, Tanzania. Paper presented at 33rd Tanzania Veterinary Association Scientific conference on 1st- 3rd December 2015 at AICC, Arusha Tanzania.

Biggs HM, Bui DM, Galloway RL, Stoddard R, Shadomy SV, Morrissey AB, Saganda W (2011). Leptospirosis among hospitalized febrile patients in northern Tanzania. The American Journal of Tropical Medicine and Hygiene, 85(2):275-281.

Chappuis G (1998). Neonatal immunity and immunisation in early age: lessons from veterinary medicine. Vaccine, 16:1468-1472.

Chomel BB (2014). Emerging and re-emerging zoonoses of dogs and cats. animals. Animals (Basel), 4(3), 434-445.

Cisneros P, Moles C, Gavaldón R, Lara A, Bolaños G (2002). Diagnóstico serológico de leptospirosis en perros callejeros capturados en el Área metropolitana. Memorias del XVIII Congreso Panamericano de Veterinaria, La Habana.

Cole JR, Sulzer CR, Pursell AR (1973). Improved microtechnique for the leptospiral microscopic agglutination test 1. Applied Microbiology, 25(6):976-980.

de Vries SG, Visser BJ, Nagel IM, Goris MGA, Hartskeerl RA, Grobusch MP (2014). Leptospirosis in Sub-Saharan Africa: A systematic review. International Journal of Infectious Diseases, 28:47-64.

EpiInfo™ software version 7.1.4.0 (2014). Data collection, management, analysis, visualization, and reporting software for public health professionals, Centers for Disease Control, Atlanta, Georgia.

Felt SA, Wasfy MO, El-Tras WF, Samir A, Rahaman BA, Boshra M, Parker TM, Hatem ME, El-Bassiouny AA, Murray CK, Pimentel G (2011). Cross-Species Surveillance of leptospira in domestic and peri-domestic animals in Mahalla City, Gharbeya Governorate, Egypt. The American Journal of Tropical Medicine and Hygiene, 84(3):420-425.

Fosgate GT (2009). Practical sample size calculations for surveillance and diagnostic investigations. Journal of Veterinary Diagnostic Investigation, 21(1):3-14.

Ghasemzadeh I, Namazi S (2015). Review of bacterial and viral zoonotic infections transmitted by dogs. Journal of Medicine and Life, 8(4):1-5.

Goldstein RE (2010). Canine leptospirosis. Veterinary Clinic of North America: Small Animal Practice, 40(6): 1091-1101.

Guitian F, Garcia-Pena F, Oliveira J, Sanjuan M, Yus E (2001). Serological study of the frequency of Leptospiral infections among dairy cows in farms with suboptimal reproductive efficiency in Galicia, Spain. Veterinary Microbiology, 80:275-284.

Kazami A, Watanabe H, Hayashi T, Kobayashi K, Ogawa Y, Yamamoto K, Adachi Y (2002). Serologic survey of leptospirosis in sows with premature birth and stillbirth in Chiba and Gunma prefectures of Japan. The Journal of Veterinary Medical Science, 64:735-737.

Kessy MJ, Machang'u RS, Swai ES (2010). A microbiological and serological study of leptospirosis among pigs in the Morogoro Municipality, Tanzania. Tropical Animal Health and Production, 42(3):523-530.

Knoepfler SV (2015). Laboratory diagnostic and radiological finding and course in 99 dogs with Leptospirosis (2006 - 2013). Inaugural Dissertation, Free University Berlin.

Korver H (1992). Microscopic agglutination test (MAT) for the diagnosis of leptospirosis and serotyping of leptospires. In: Leptospirosis on the African continent. Proceedings of a CEC/STD 3 Research Meeting. Harare, Zimbabwe. pp. 148-155.

Lau C, Smythe LD, Craig SB, Weistein P (2010). Climate change, flooding, urbanisation and leptospirosis: Fuelling fire? Transactions of the Royal Society of Tropical Medicine and Hygiene, 104:631-638.

Luna A, Moles C, Salazar G, Nava V, Gavaldón R (2008). La Leptospirosis canina ysu problemática en México. Revista de Salud Animal, 30:1-11.

Machang'u R, Mgode G, Mpanduji D (1997). Leptospirosis in animals and humans in selected areas of Tanzania. The Belgian Journal of Zoology, 127:97-104.

Machang'u RS, Mgode GF, Assenga J, Mhamphi G, Weetjens B, Cox C, Verhagen R, Sondij S, Goris MG, Hartskeerl RA (2004). Serological and molecular characterization of leptospira serovar Kenya from captive African giant pouched rats (*Cricetomys gambianus*) from Morogoro Tanzania. FEMS Immunology and Medical Microbiology, 41(2):117-121.

Meeyam T, Tablerk P, Petchanok B, Pichpol D and Padungtod P. Seroprevalence and risk factors associated with leptospirosis in dogs. Southeast Asian J Tropic Med Public Health, 31(1):148.

Mgode GF, Machang 'u RS, Mhamphi GG, Katakweba AS, Mulungu LS, Durnez L, Leirs H, Hartskeerl RA, Belmain, SR (2015). Leptospira Serovars for diagnosis of leptospirosis in humans and animals in Africa: Common leptospira isolates and reservoir hosts. PLoS Neglected Tropical Diseases, 9(12):1-19.

Mgode GF, Machang'u RS, Goris MG, Engelbert M, Sondij S, Hartskeerl RA (2006). New Leptospira serovar Sokoine of serogroup Icterohaemorrhagiae from cattle in Tanzania. International Journal of Systematic and Evolutionary Microbiology, 56: 593-597.

Millan J, Chirife AD, Kalema-Zikusoka G, Cabezon O, Muro J, Marco I, Muro J, Marco I, Cliquet F, León-Vizcaíno L, Wasniewski M, Almería S, Mugisha L (2013). Serosurvey of dogs for human, livestock, and wildlife pathogens, Uganda. Emerging Infectious Diseases, 19:680-682.

Miller MD, Annis KM., Lappin MR, Lunn KF (2011). Variability in the results of the microscopic agglutination test in dogs with clinical leptospirosis and dogs vaccinated against leptospirosis. Journal of Veterinary Internal Medicine, pp. 426-432.

Moch RW, Ebner EE, Barsoum LS, Botros BA (1975). Leptospirosis in Ethiopia: a serological survey in domestic and wild animals. The Journal of Tropical Medicine and Hygiene, 8:38-42.

Okewole EA, Ayoola MO (2009). Sero-epidemiological survey on canine leptospirosis in Nigeria. Veterinarski arhiv, 79(1):87-96.

Prescott JF, McEwen B, Taylor J, Woods JP, Abrams-Ogg A, Wilcock B (2002). Resurgence of leptospirosis in dogs in Ontario: recent findings. The Canadian Veterinary Journal, 43(12): 955-961.

Roqueplo C, Demoncheaux JP, Mediannikovc O, Diarra M, Tine R, Pasqualini C, Mariée JL, Davoustf B, Kodjog A (2016). Serological survey of leptospirosis in equids, dogs, and domestic ruminants from Senegal. International Journal of Infectious Diseases, 53: 128.

Senthil NR, Palanivel KM and Rishikesavan R (2013). Seroprevalence of leptospiral antibodies in canine population in and around Namakkal. Journal of Veterinary Medicine, pp. 1-4.

Ward MP (2002a). Seasonality 2 of canine leptospirosis in the United States and Canada and its association with rainfall. Preventive Veterinary Medicine, 56(3):203-213.

Ward MP, Glickman LT, Guptil LF (2002b). Prevalence and risk factors for leptospirosis among dogs in the United States and Canada: 677 cases (1970-1998). Journal of the American Veterinary Medical Association, 20(1):53-58.

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Full Length Research Paper

Exploratory study of hepatitis D virus infection in HBsAg carriers in Abidjan, Côte D'ivoire in 2016

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In sub-Saharan Africa, the prevalence of co-infection with hepatitis D (HDV) and hepatitis B viruses (HBV) is poorly known. Chronic infection with HBV is currently treated by nucleoside analogs whereas interferon is used to inhibit HDV. Nevertheless, Hepatitis Delta is not routinely diagnosed in Côte d'Ivoire. This study aims to estimate the current prevalence of Hepatitis D infection among HBV-infected patients in Abidjan to determine whether it is necessary to implement its routine practice in the country. A cross-sectional analytical study was conducted from January 2016 to June 2016 at the Pasteur Institute of Côte d'Ivoire (Abidjan). Patients were screened for Hepatitis D Virus infection through detection of anti-HDV antibodies. A total of 87 patients between 17 and 70 years, including 12 anti-HIV positive, were recruited. A subset of 16 (18.4%) have acute hepatitis B while 71 (81.6%) were chronically infected with HBV. Concerning viral loads, 37 patients (42.5%) displayed values > 1.0 E+05 UI/mL; 22 (25.3%) range between 20 and 1.0 E+05 UI/mL and 28 (32.2%) were undetectable for HBV DNA. Out of these, 20 (22.9%) was positive for anti-HDV. Infection with HDV was not associated with any clinico-pathological variables such as age, gender, disease stage or even HBV DNA loads. The current seroprevalence of anti-Delta antibodies among HBV carriers is high in Abidjan. The study provides important information pledging for the introduction of systemic anti-Delta testing in HBV-infected patients in Côte d'Ivoire.

Key words: Hepatitis D virus, frequency, hepatitis B carriers, Abidjan-Côte d'Ivoire.

INTRODUCTION

Hepatitis D virus (HDV) is a defective virus which requires helper functions of hepatitis B virus (HBV) for its propagation. Similar to many RNA viruses, HDV exhibits high genetic heterogeneity. RNA recombination may reflect the template-switching activities of host RNA polymerases (Lin et al., 2017). This can be a source of therapeutic failure. It is known that co-infection of HDV/HBV tends to accelerate the progression of infection towards cirrhosis and hepatocellular carcinoma. It is also estimated that up to 80% of all chronic patients

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with hepatitis D will develop cirrhosis (Amanullah et al., 2014; Wedemeyer, 2010). According to the World Health-Organization (Wedemeyer, 2010), among 240 million chronic HBV carriers reported worldwide, approximately 15 to 20 million individuals are also infected with HDV. In Africa, approximately one fourth of the 65 million of HBsAg-positive individuals is suspected to be dually infected with HDV. In western Africa, prevalence of the HDV infection is estimated to range between 1.3 and 50% (Andernach et al., 2014). Nevertheless, the anti-HDV antibody prevalence in HBsAg carriers was reported only in few countries including Cameroun (17.6%), Gabon (15.6 to 70.6%) (Makuwa et al., 2009), Ghana (11.3 %) (Asmah et al., 2014).

The aim of this study was to determine the prevalence of HDV in HBsAg carriers in Abidjan.

METHODS

The study was conducted in HBsAg carriers coming for biological investigations to the Institute Pasteur within the framework of their follow-up from January 2016 to June 2016. All participants gave written informed consent. Ethical approval was obtained from the National Committee of Ethics and Research (NCER).

A case report form was used to collect the socio-demographic data (sex, age), clinical data (clinical information) and biological data (viral load and HBsAg) of the patients. Viral load, serological HIV and HBsAg were collected from the patient registry of the Pasteur Institute of Côte d'Ivoire.

Serum samples were collected for subsequent anti-HDV antibodies detection. The presence of anti-Delta IgG was qualitatively determined using a commercially available ELISA kit (ETI-AB-DELTAK-2, DiaSorin, Italy) based on a competitive assay. All samples were tested and confirmed following the manufacturer's instructions.

Data analysis was performed using Epi-info version 3.5.4 software (July 30, 2012). Anti-HDV prevalence was described with a 95% confidence interval.

RESULTS

Epidemiological data

A total of 87 patients positive for HBsAg were included during a six months period (January to June 2016) of this study. The median age of the patients was 42 years (range 17-70). Gender proportions were balanced (51.7% males and 48.3% females). Acute hepatitis B (IgM anti-HBc-positive) was found in 16 patients (18.4%) while 81.6% were affected from a persistent infection.

Biological data

The viral load and HIV results were obtained from Institut Pasteur registry of patients. Concerning viral loads, 37 patients (42.5 %) displayed values above 1.0 E+05 UI/mL; 22 (25.3%) range between 20 and 1.0 E+05 UI/mL and 28 (32.2%) were undetectable for HBV DNA. HBV DNA loads do not depend on age ($p = 0.22$); sex ($p = 0.27$) and clinical status ($p = 0.64$, Table 1).

Among the patients, 12 (13.8%) were anti-HIV positive. HIV status was not related to age ($p = 0.07$), sex ($p = 0.54$) nor the clinical status ($p = 0.61$) of the patients. It was detected that among these we noted the presence of anti-HDV Ab in 2 patients, thus the existence of triple infection. The latter have a chronic clinical stage, evidence of an acceleration of complications of associated liver diseases.

The samples were screened for IgG directed against HDV and 23.0% of them were positive. Anti-HDV was more prevalent in females than in males (31% vs 15%) but this difference is not statistically significant ($p = 0.14$). Regarding age of the patients, the prevalence of HDV antibody was higher between 31 and 45 years with 29.7%. However, the difference with anti-HDV negative subjects is not statistically significant ($p = 0.43$).

Prevalence of anti-HDV was different between patients with acute hepatitis B (12.5%) and chronic carriers (33.9%) of HBV albeit without reaching the level of significance ($p = 0.40$). In patients with HIV, 16.7% were carriers of anti-HDV antibodies but this figure was not statistically different in anti-HIV negative patients ($p = 0.72$). The distribution of anti-HDV according to the different variables is shown in Table 1.

DISCUSSION

The prevalence of HDV infection in this study was 23.0%, a figure considerably higher than the estimated worldwide prevalence of 5% (Rizzetto and Ciancio, 2012). This result is lower than the results of other studies conducted in Bangladesh (24.4%) and those conducted by Seetlani et al. (2009). It is higher than the results of Nwokediuko and Ijeoma (2009) and Opaleye et al. (2016) with 12.5% in Nigeria and similar to Darwish et al. (1992), with 23.53%. This high prevalence rate was found again; the HDV virus being an RNA virus, with its great capacity of heterogeneity during these replications, as shown by the works of Lin et al. (2017) in Taiwan could explain the therapeutic failures observed in patients on ARVs. But in a lot of countries such as Italy, this high frequency of HDV infection has declined where the prevalence has dropped from 22% in 1987 to 8.2% in 1997 (Gaeta et al., 2000). There are several factors such as improvement in socioeconomic status of the population, proper vaccination against HBV (Lin et al. 2015) in Taiwan, better guidance and education regarding safety measures and systematic screening of the general population (Gaeta et al. (2001). This is not the case of Côte d'Ivoire that is still in the exploratory phase concerning HDV.

The prevalence of HDV virus infection is higher in patients with chronic hepatitis B in Côte d'Ivoire while it is higher in patients with acute hepatitis B in Pakistan (Amanullah et al., 2014). This situation might be

Table 1. Relationship of HDV antibody with different variables.

Variable	Total patients (n = 87)	Anti-HDV Ab positive (n = 20)	Anti-HDV Ab negative (n = 67)	P-Value
Age (years)				
< 15	1	0	1	0.43
16-30	25	6	19	
31-45	37	11	26	
> 45	24	3	21	
Gender				
Female	42	13	29	0.14
Male	45	7	38	
Clinical stage				
Acute hepatitis	16	2	14	0.40
Patient on treatment	4	0	4	
Patient with liver disease	6	2	4	
Chronic hepatitis	61	16	45	
Viral load (UI/ML)				
Undetectable	28	6	22	0.71
20 to 100000	22	4	18	
>100000	37	10	27	
Anti-HIV g				
Positive	12	2	10	0.72
Negative	75	18	57	

explained by the high frequency of co-infection HDV/HBV in both populations. Patients between 30 and 40 years were most concerned about HDV infection in Nigeria but differ with the findings observed in Pakistan (Khan et al., 2011). This difference could be explained by the characteristics of the populations in different study particularly differences in proportions of the various categories of HBV and/or different study population profiles.

Analysis of the sex revealed that females (31.0%) were more often infected than males (15.6%). In a similar study, Roshandel et al. (2008) found female predominance (9.9%) in Northeast Iran. However, Nwokediuko in Nigeria found males were most infected than females by 12.8 and 7.1% respectively (Nwokediuko and Ijeoma, 2009). This situation could be explained by the different proportion of male and female in different study.

According to HIV status, this study has shown the existence of triple infection, a source of accelerated liver complications, such as the work of Lee et al. in Taiwan in 2013 (Lee et al., 2015). Also, future works require more investigations.

According to the viral load, the study showed that HDV antibodies were frequently presented in patients with high viral load (> 100000 UI/ML). This result is different from the outcome of Iris and colleague, 2014 (Lorenc et al., 2017). The study also revealed the presence of HDV antibodies in 21.4% of HBV undetectable viral load. Hence, it is important to look for HVD even at a low viral load, as highlighted by the work of Beata Lorenc and colleagues in Poland in 2016 (Mumtaz et al., 2005).

Limitation of the study

The main limitation of this study is the small size of the population studied. This situation enabled the proposal of only reasonable speculations regarding age, sex, acute or chronic HBV infection and viral loads of the patients.

Conclusion

The prevalence of HDV antibodies among HBsAg-positive patients in this study was high with 23% despite the decreasing trend affecting HDV infection prevalence worldwide. This study is crucial both for public health, that is, to improve the future control of HDV and for clinical practice as it means that antiviral nucleosides are not relevant around one fourth of the HBsAg-positive patients in Côte d'Ivoire.

Future studies of HDV infection to determine genotypes are necessary to improve surveillance. A prospective study supported by Ivorian authorities should be urgently implemented to define more accurately the actual prevalence of HDV infection in HBV carriers throughout Côte d'Ivoire.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Amanullah Abbasi, Abdul Rabb Bhutto, Nazish Butt, Khalid Mahmood. (2014). HDV seroprevalence in HBsAg positive patients. Journal of the College of Physicians and Surgeons Pakistan 24(9):624-627.

Andernach IE, Leiss LV, Tamagda ZS, Tahita MC, Otegbayo JA, Forbi JC, Omilabu S, Gouandjika-Vasilache I, Komas NP, Mbah OP, Muller CP (2014). Characterization of hepatitis delta virus in sub-saharan Africa. Journal of Clinical Microbiology 52:1629-1636.

Darwish MA, Shaker M, Raslan OS, Abdel-Raouf T (1992). Delta virus infection in Egypt. Journal of Egyptian Public Health Association 67:147-61.

Gaeta GB, Stornaiuolo G, Precone DF (2001). Type B and D viral hepatitis: epidemiological changes in southern Europe. Forum (Genova) 11:126-33.

Gaeta GB, Stroffolini T, Chiaramonte M, Ascione T, Stornaiuolo G, Lobello S, Sagnelli E, Brunetto MR, Rizzetto M (2000). Chronic hepatitis D: a vanishing disease? An Italian multicenter study. Hepatology 32:824-827.

Khan AU, Waqar M, Akram M, Zaib M, Wasim M, Ahmad S, Niaz Z, Ali S, Ali H, Idrees M, Bajwa MA (2011). True prevalence of twin HDV-HBV infection in Pakistan: a molecular approach. Virology Journal 8:420.

Lee CY, Tsai HC, Lee SS, Wu KS, Sy CL2, Chen JK, Chen YS (2015). Higher rate of hepatitis events in patients with human immunodeficiency virus, hepatitis B, and hepatitis D genotype II infection : A cohort study in a medical center in southern Taiwan Journal of Microbiology, Immunology and Infection 48:20-27.

Lin CC, Lee CC, Lin SH, Huang PJ, Li HP, Chang YS, Tang P, Chao M (2017). RNA recombination in Hepatitis delta virus: Identification of a novel naturally occurring recombinant Journal of Microbiology, Immunology and Infection 50(6):771-780.

Lin HH, Lee SS, Yu ML, Chang TT, Su CW, Hu BS, Chen YS, Huang CK, Lai CH, Lin JN, Wu JC (2015). Changing hepatitis D virus epidemiology in a hepatitis B virus endemic area with a national vaccination program. Hepatology 61(6):1870-1879.

Lorenc B, Sikorska K, Stalke P, Bielawski K, Ziętkowski D (2017) Hepatitis D, B and C virus (HDV/HBV/HCV) coinfection as a diagnostic problem and therapeutic challenge. Journal of Clinical and Experimental Hepatology 3(1):23-27

Makuwa M, Mintsa-Ndong A, Souquiere S, Nkoghe D, Leroy BM, Kazanji M. (2009). Prevalence and Molecular diversity of hepatitis B virus and hepatitis delta virus in urban and rural populations in northen Gabon in central Africa. Journal of Clinical Microbiology 47:2265-2268.

Mumtaz K, Hamid SS, Adil S (2005). Epidemiology and clinical pattern of hepatitis delta virus infection in Pakistan. Journal of Gastroenterology and Hepatology 20:1503-1507.

Nwokediuko SC, Ijeoma U (2009). Seroprevalence of antibody to HDV in Nigerians with hepatitis B virus related liver diseases. Nigerian Journal of Clinical Practice 12:439-4342.

Opaleye OO, Japhet OM, Adewumi OM, Omoruyi EC, Akanbi OA, Oluremi AS, Wang B, van Tong, H, Velavan TP, Bock CT (2016). Molecular epidemiology of hepatitis D virus circulating in Southwestern Nigeria. Virology Journal 13:61.

Asmah RH, Boamah I, Afodzinu M, Brown CA, Brandful J, Adjei DN, Adiku T, Gyasi R, Wiredu EK (2014). Prevalence of Hepatitis D infection in patients with Hepatitis B virus-related Liver Diseases in Accra Ghana. West African Journal of Medicine 33(1):332-36.

Rizzetto M, Ciancio A (2012). Epidemiology of hepatitis D. Semin. Liver Dis. 32:211-219.

Roshandel G, Semnani S, Abdolahi N, Besharat S, Keshtkar AA, Joshaqani H, Moradi A, Kalavi K, Jabbari A, Kabir MJ, Hosseini SA, Sedaqat SM, Danesh A, Roshandel D, Hedayat-Mofidi SM (2008). Prevalence of hepatitis D virus infection in hepatitis B surface antigen-positive subjects in Golestan province, northeast Iran. Journal of Microbiology, Immunology and Infection 41:227-230.

Seetlani NK, Abbas Z, Raza S, Yakoob J, Jafris W. (2009). Prevalence of hepatitis D in HBsAg positive patients visiting liver clinics. Journal of the Pakistan Medical Association 59:434-437.

Wedemeyer H (2010). Re-emerging interest in hepatitis delta: new insights into the dynamic interplay between HBV and HDV. Journal of Hepatology 52:627-629.

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African Journal of Microbiology Research

Full Length Research Paper

Exploration of sulfate reducing bacteria from polluted waters

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Sulfate reducing bacteria (SRB) was successfully isolated from Estuary Dam in Suwung Denpasar, Indonesia. This estuary catches highly polluted water from Badung River which runs across and hence carries pollution due to waste disposal from Denpasar City. SRB was studied in detail for their ability to reduce sulfate to sulfide with organic material as an oxidizing agent. SRB exploration of the estuary ecosystem of the contaminated dam was accomplished through isolation, selection and characterization of the isolates obtained. The result of this study found superior SRB named DPS 1711, DPS 1705 and DPS 1703. The bacteria have the ability to grow at pH 3, room temperature and uses compost as organic substrate. This ability is an important factor for the application of isolates in the treatment of acid mine waste. Isolates have optimum optical density under the pH range of 4 to 7 and the best at pH 5 have a growth rate profile at a temperature range of 25 to 40°C. The isolates observed were Gram-negative stem, motile bacteria which only grow in anaerobic condition. Physiological-biochemical characterization showed the three isolates, namely DPS 1703, DPS 1705 and DPS 1711 were SRB groups identified as *Desulfotomaculum orientis*.

Key words: Sulfate reducing bacteria, polluted waters, estuary dam ecosystem.

INTRODUCTION

Estuary Dam Suwung is a lagoon formed at the estuary of the Badung River flowing through the city of Denpasar. Badung River has long experienced pollution due to waste disposal from various activities along its bank. The high pollutants load entering the estuary dam of Suwung Denpasar has led to the decreasing quality of the waters and the formation of oxygen deficit zones at the bottom. Pollution containing high concentration of organic materials tends to cause the acidic and anaerobic conditions of the aquatic sediments. These conditions affect the composition of biological species with biogeochemical cycles. The acidic environment can trigger the formation of reactive metals in the form of their ions, thereby causing metal contamination in the aquatic environment. Meanwhile, sludge from wastewater treatment becomes its own problem with heavy metal content and low pH. This high level contamination of heavy metal requires development of a sludge

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management system to treat the acidity and heavy metal content. Biotechnology has been utilized in waste water treatment. Using this technology, the contaminant is removed through the precipitation of the contaminant in the reduced form by the microbial activity. Furthermore, on a certain scale, the precipitated contaminants can be recovered.

Anaerobic biological processes encourage sulfide formation by sulfur reducing bacteria. SRB is heterotrophic bacteria that use simple organic compounds as carbon sources. With metabolic respiration, the bacteria utilize sulfate, thiosulfate, sulfites and other reducible sulfur compounds as electron acceptor (Müller et al., 2014).

The sulfur in the oxidized state will be reduced to sulfide in the anaerobic environment (Barton and Faugue, 2009). SRB is a true anaerobic microorganism with a primitive respirative pathway capable of living in extreme environments. The group of bacteria is generally isolated from aquatic sediments that have extreme conditions, as temperature, pH, alkalinity, sulfate, iron, manganese, ammonium and phosphate contents (Rückert, 2016). This research aims to isolate and characterize SRB from water sediments contaminated with domestic waste.

MATERIALS AND METHODS

Isolation

The soil samples were taken from Estuary Dam in Suwung, South Denpasar area of Bali. There are about 12 sampling points in one area. For SRB exploration, soil samples taken from sediments were immediately inserted into polyethylene bottles and stored in a cooler box for transporting and then stored in a freezer in the laboratory. The number of samples taken at each sampling point ranged from 100 to 200 g of soil. SRB group isolation was based on Postgate medium B liquid media composition (Atlas, 1993). The pH 4 setting was performed with 10% sulfuric acid and 10% sodium hydroxide prior to sterilization. Samples suspended in 0.85% NaCl and diluted to 10^3 were then cultured in an anaerobic tube. The cultures were then incubated at 35°C. Observations were made from the time when color started to change until all turned black. Blacken isolates were then purified in liquid medium (Rückert, 2016). The isolates were suspended and diluted further in the same manner until they reached dilution level of 10^{12}. Growth time of the bacteria was observed from the time of black color appearance until the entire tube turned black. Isolates grown at the final dilution rate were indicated as cultures with one type of SRB cell.

Characterization of SRB cells

Isolates indicated as SRB cells were tested for their ability to reduce sulfate and increase the pH of the media. Isolates were cultured in a liquid medium containing 500 ppm sulfate. Trials were carried out in media with pH variations of 7, 5, 4 and 3. One mL of SRB cells isolate was transferred aseptically into a threaded tube filled with 1/3 liquid medium (500 ppm sulfate with 7, 5, 4, or 3), and filled up with medium, then incubated at 35°C. On the 15th day, the residual sulfate and pH of the solution were measured. The isolates capable of growing at low pH and reducing sulfate (and increasing pH of the media were selected.

Table 1. Isolates growth time.

Sample code	Growth time (days)	Colour intensity
DPS 1701	5	+++
DPS 1702	5	+
DPS 1703	5	++++
DPS 1704	8	++++
DPS 1705	8	+ +++
DPS 1706	9	++++
DPS 1707	5	++
DPS 1708	5	++
DPS 1709	5	+
DPS 1710	7	++
DPS 1711	7	++
DPS 1712	8	+

+: Thin black on the bottom; ++: black evenly distributed almost all parts of the tube; +++: black evenly on all parts of the tube; ++++: solid black evenly across the tube.

RESULTS AND DISCUSSION

Isolation and characterization

The estuary dam from where the samples were collected has undergone siltation, indicated by its blackish brown sediment, low pH (5, 6 and 3) and high pollutant contents. All 12 soil samples isolated with Postgate B liquid media, change from clear to black under anaerobic conditions, which indicate the presence of SRB (Dennis and Julia, 2014). The black color shows the presence of sulfide (reduced sulfur) which is the result of sulfate reduction by SRB (Xu et al., 2013). The growth rate observed by the time required to turn the colour of the suspension black varies widely. The growth time of SRB from the isolated samples is shown in Table 1. The capability of the SRB to reduce sulfate, and therefore increase pH, as measured after 15 days culture, is shown in Table 2. The observation found nine isolates capable of growing at all pH variations, and only three isolates did not grow at pH 3 (Table 2). The pH changes and sulfate reduction efficiency is shown in Table 3. The sulfate reduction efficiency was determined by calculating the percentage of the reduction of the sulfate concentrations. Table 3 shows the sequence of ability to increase pH and sulfate reduction efficiency of nine isolates. High efficiency sulfate reduction is an important factor for sulfide formation associated with pH increase.

Three isolates appear to have a prominent activity compared to the other six isolates. The three isolates namely DPS 1711, DPS 1703 and DPS 1705, are also the ones with highest growth rates (Table 1). Bacterial cells grow rapidly by splitting in the supporting environment. When food is abundant then the term survival of the fittest which implies that, the winning type is the one who can divide the most rapidly. Faster splitting ability allows bacterial populations to adjust immediately

Table 2. pH and sulfate concentrations of the isolates grown at various initial pH and [SO^{2-}] of 500 ppm.

Isolate code	Initial pH 7		Initial pH 5		Initial pH 4		Initial pH 3	
	pH	$[SO_4^{2-}]$(ppm)	pH	$[SO_4^{2-}]$(ppm)	pH	$[SO_4^{2-}]$(ppm)	pH	$[SO_4^{2-}]$(ppm)
DPS 1711	8.1	39.11	7.6	68.32	6.3	78.16	6.1	83.57
DPS 1703	7.9	44.06	7.6	66.35	5.9	83.24	5.8	94. 31
DPS 1705	7.9	48.72	7.4	81.23	5.8	87.05	5.8	91.56
DPS 1701	7.6	88.92	6.5	127.55	5.8	158.54	4.4	188.62
DPS 1710	7.6	93.24	6.3	153.25	5.8	162.37	4.4	192.67
DPS 1706	7.6	91.79	6.3	158.81	5.7	171.28	4.2	197.25
DPS 1704	7.6	101.97	6.2	167.89	5.5	166.37	4.2	267.17
DPS 1707	7.5	124.31	6.0	193.96	4.4	204.36	3.8	285.01
DPS 1708	7.5	122.46	6.0	193.83	4.2	216.54	3.3	326.67
DPS 1702	7.5	129.65	6.0	197.55	4.1	221.42	NA	NA
DPS 1712	7.3	135.28	6.0	200.49	4.0	223.12	NA	NA
DPS 1709	7.3	131.18	5.8	214.03	4.0	226.89	NA	NA
Control	NA	NA	NA	NA	NA	NA	NA	NA

NA: Unmeasured due to unobservable growth.

Table 3. Changes in pH and sulfate reduction efficiency.

Isolate code	pH 7		pH 5		PH 4		pH 3	
	ΔpH	E (%)	ΔpH	E (%)	ΔpH	E (%)	ΔpH	E (%)
DPS 1711	1.2	92.27	2.5	86.45	3.2	84.36	3.7	83.28
DPS 1703	1.0	91.29	2.5	86.84	2.9	83.35	3.3	81.13
DPS 1705	1.0	90.37	2.3	83.89	2.8	82.78	3.3	81.69
DPS 1701	0.8	82.40	2.2	82.64	2.8	68.65	1.9	66.79
DPS 1710	0.7	81.58	2.0	83.49	2.8	66.13	1.9	66.07
DPS 1706	0.7	81.87	2.0	82.39	2.7	66.13	1.3	62.92
DPS 1704	0.7	79.86	1.1	66.71	2.5	67.10	0.8	52.96
Control	NA	NA	NA	NA	NA	NA	NA	NA

NA: Not measured due to unobservable growth.

to changes in the environment (Shiqiang et al., 2014). Microorganisms interact with their environment in a variety of ways. The ability to utilize certain nutrients, producing metabolites that affect other microorganisms and interacting with the physical and chemical environment are the factors that determine the growth of a microorganism. These conditions define the activity and growth character of a species, which is different from the other species (Kato, 2016). The ability to grow at room temperature is a very important factor for the efficient application of SRB isolates in bioreactors. The ten isolates selected in the first selection, were further selected for the purpose of the determination of the reduction efficiency. The tested isolates were cultured on a Postgate B liquid medium containing 1000 ppm sulfate at pH 3 and incubated at room temperature (27 to 30°C).

Measurements and observations of growth time, optical density, pH increase and sulfate reduction different efficiency are shown in Table 4. Measurements of optical density, pH increase and sulfate reduction efficiency at room temperature are performed after a 21 day incubation period with estimated exponential phases being exceeded. Isolates capable of growing rapidly at room temperature, raising the pH of the medium and reducing the sulfate with high efficiency (that is >80%) are selected isolates. The selected isolates are DPS 1703, DPS 1705 and DPS 1711 (Figure 1). Growth at room temperature and activity on acidic medium (low pH) are very important properties in the applications for treating acidic mine waste and are important factors for the efficiency of the bioreactor performance.

Cell morphology and physiology

Observations under microscope of simple staining, Gram staining, and spores to determine cell morphology were performed on all three selected isolates. Physiological

Table 4. Growth time, pH increase, OD, and sulfate reduction of 7 Isolates at pH 3 and room temperature.

Isolate code	Growth time (days)	Optical density	ΔpH	Sulfate reduction ppm	Efficiency (%)
DPS1711	8[a]	0.671[a]	4.3[a]	831.28[a]	83.13[a]
DPS 1703	7[a]	0.650[b]	4.2[a]	829.32[a]	82.92[a]
DPS 1705	8[a]	0.602[c]	3.9[b]	805.24[b]	80.52[b]
DPS1706	11[b]	0.421[d]	2.5[c]	613.68[c]	61.37[c]
DPS 1701	12[b]	0.387[e]	2.3[d]	556.83[d]	55.68[d]
DPS 1710	15[c]	0.382[e]	2.1[e]	547.73[e]	54.77[e]
DPS 1704	15[c]	0.316[f]	1.7[f]	497.34[f]	49.73[f]
Control	NA	NA	NA	NA	NA

NA: Not measured due to unobservable growth.

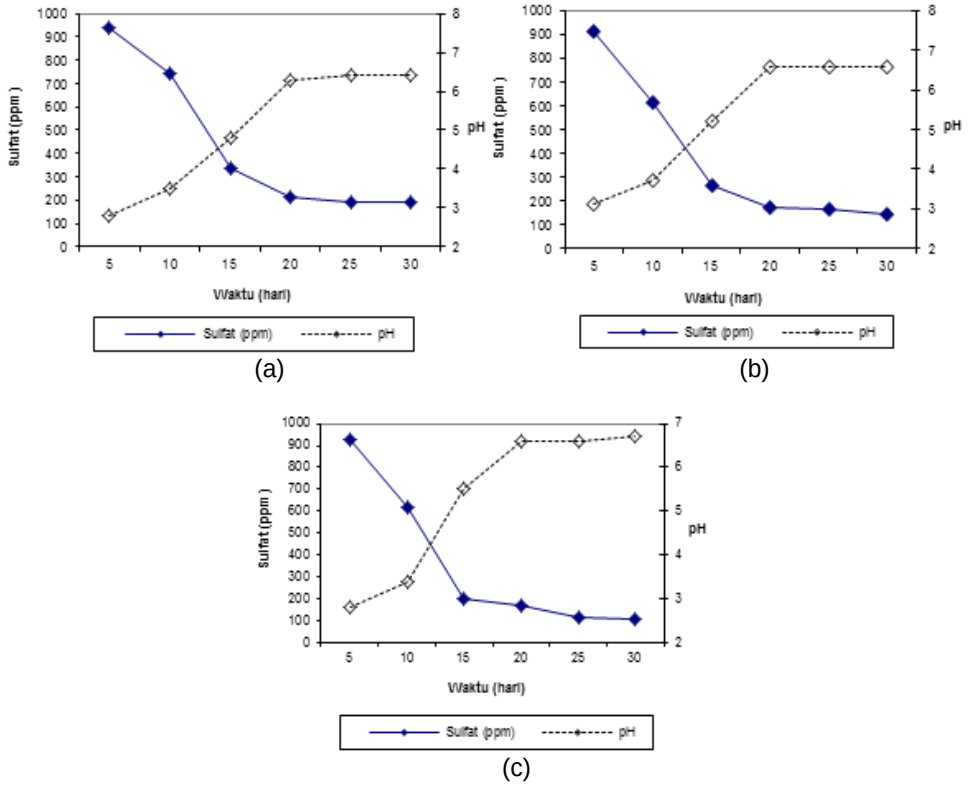

Figure 1. Graph showing the reduction of sulfate and increasing of pH by (a) DPS 1703, (b) DPS 1705 and (c) DPS 1711 isolates.

and biochemical characterization were also performed. The results are shown in Table 5 and the photograph of the isolates in Figure 2. Endospores have very high resistance to heat and are not easily damaged by the effects of chemicals, dryness, radiation, acidity, and dormant for a very long time (Kato, 2016). In Figure 2c, the spherical endospores are in contrast to their rod-shaped vegetative cells. The color difference is caused by the absorption of green color of kit by endospora. Based on the observed characteristics shown by DPS 1703, DPS 1705 and DPS 1711 isolates.

The observed SRB-shaped group of SRB (Figure 2a), with a single arrangement is paired and aggregates. In Figure 2b, the aggregate bacterial cell appears from the DPS 1711 SRB isolate. Characteristics of the cells are Gram-negative, stem, motile and show only growth in anaerobic conditions.

In Figure 2, it is also clear that the morphological type of isolate observed are the form of a stem (bacillus) with a single or group arrangement. The pink bacterial cells showed Gram-negative cells that lose the complex of a purple primer in purple crystals when rinsing with alcohol

Table 5. Determination of morphological and physiological cell of Isolates.

Characteristic	Isolate		
	DPS 1703*	**DPS 1705***	**DPS 1711***
Gram test	Negative	Negative	Negative
Cell form	Rod	Rod	Rod
Colony form	Spherical-irregular	Spherical- irregular	Spherical-irregular
Colony colour	White gray	White gray	White gray
Motility	+	+	+
Oxsidase	+	+	+
Catalase	-	-	-
Anaerob	+	+	+
Aerob	-	-	-
Endospora	+	+	+
Carbon sources			
Lactate	+	+	+
Asetate	-	-	-
Phenol	-	-	-
Butirate	-	-	-
Formate	-	-	-
Growth temperature			
50 - 60°C	-	-	-
25 - 40°C	+	+	+
60 - 65°C	-	-	-
Other characteristics			
$H_2 + CO_2$	+	+	+
3-12% NaCl media	-	-	-
Gas formation during sporulation	-	-	-
Sulfide production	+	+	+

*Desulfotomaculum orientis.

(a) (b) (c)

Figure 2. (a) Rod form, (b) Aggregate cell, (c) spore spots are colored in contrast to their vegetative cells.

and stained with a counter-dye namely safranin. According to Dennis and Julia (2014), the differences in Gram-positive and Gram-negative cell wall structures cause different reactions in dyestuff permeability and the addition of the pale solution.

Most Gram-positive cell wall cells consist of peptidoglycan, while Gram-negative cell wall bacteria have high lipid content compared to Gram-positive cell wall cells. The lipids will dissolve in the pale solution, enlarge the pores of the cell wall and increase the solubility of the violet-iodine crystalline complex. On the other hand, Gram-positive bacteria produce compounds

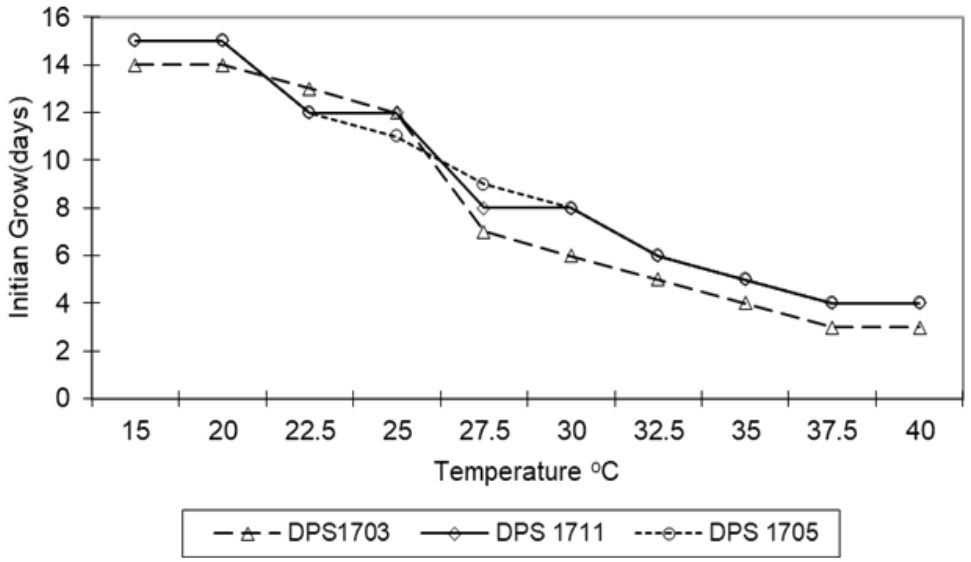

Figure 3. Comparison of temperature and growing time of SRB (Postgate B, pH 5).

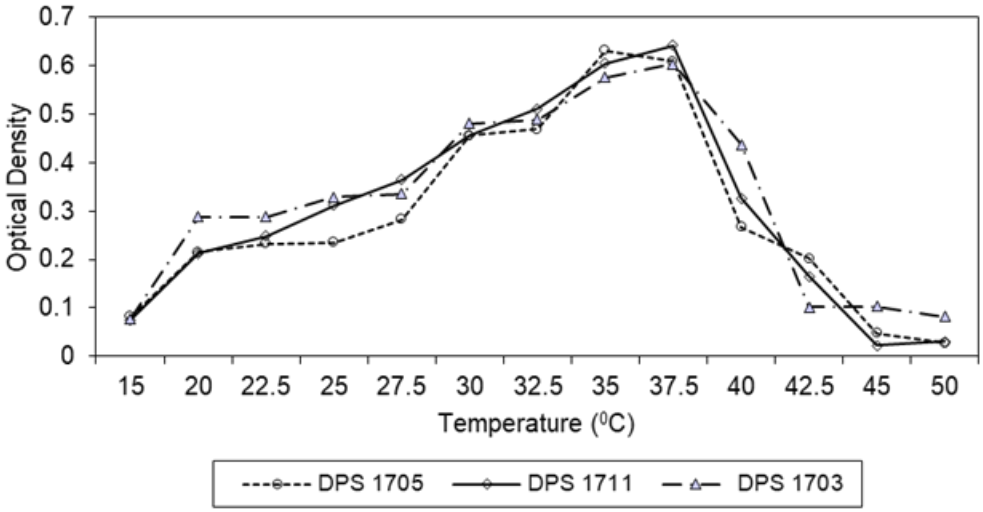

Figure 4. Effect of temperature on optical density of growth of DPS 1711, DPS 1705, and DPS 1703 (Postgate B, pH 5).

as crystalline violet-iodine ribonucleic acid.

Sulfate reducing bacteria (SRB) growth factors

The effect of temperature on SRB growth

Temperature is one of the important environmental factors that affect bacterial growth. Bacteria have specific temperature range of growth and optimal growth temperature. The minimum and maximum growth temperatures for microorganisms vary greatly, reflecting the range of temperatures in which they live (Païssé et al., 2013). Figure 3 shows the growth time range of DPS 1703, DPS 1705 and DPS 1711 isolates. At 20°C, SRB shows very slow growth, and a long acclimation time of 15 days is required. At room temperature, the growth of isolate was relatively rapid at 7 to 10 days, while at 45°C temperature the isolates did not grow. At the temperature range of 25 to 40°C, SRB Isolates showed the fastest growth especially DSS 1703 isolates. All isolates showed a similar growth time profile. The growth is also indicated by changes in the optical density of the media as a function of temperature (Figure 4). Figure 4 shows that

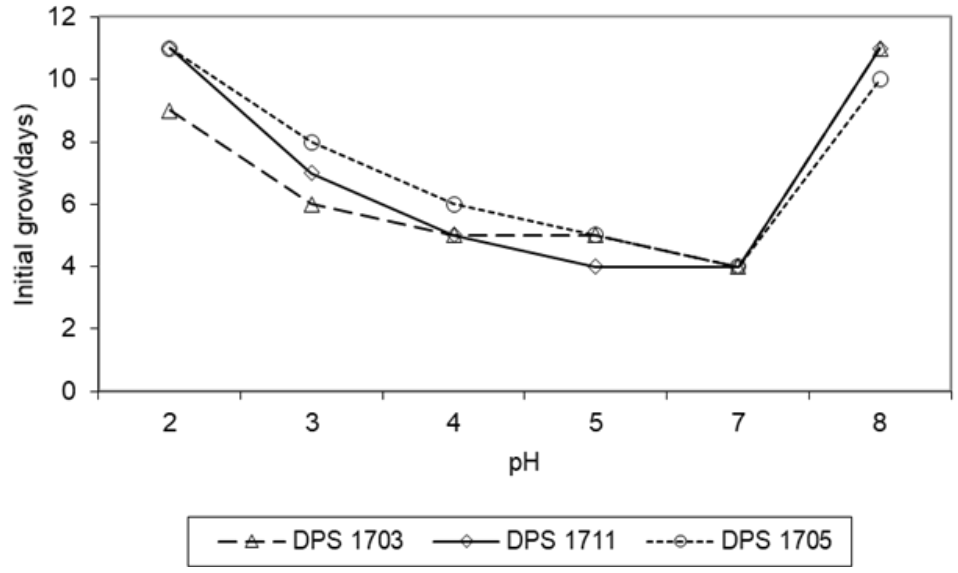

Figure 5. The curve of pH ratio and growing time (days) of DPS 1711, DPS 1705 and DPS 1703.

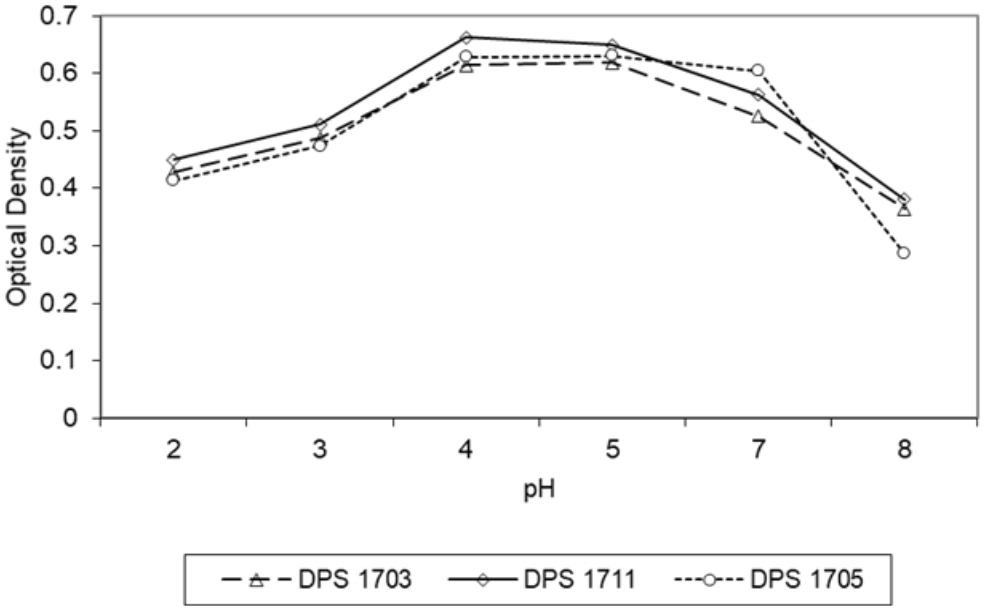

Figure 6. Effect of pH on optical density of growth of I DPS 1711, DPS 1705 and DPS 1703 (Postgate B, temperature 35°C).

the SRB isolates grow with optical density ranging between 0.30 and 0.65 at temperature range of 27 to 40°C with the best range of 35 to 37.5°C.

Effect of pH on growth of SRB

Microorganisms have a certain pH range for their growth, with optimum pH range of 5 to 9. Only certain species are able to grow under extreme conditions such as below pH 2 or above pH 10. Comparison of the activities of all SRB isolates at various pH, with a pH range of 2 to 9, is shown in Figure 5. At pH 2 and pH 8 the growth time of the three isolates was very slow and at pH 9 did not show growth at all. Optimal growth time occurs at pH 4 to 7 and fastest at pH 7. Figure 6 shows the optical density of DPS 1711, DPS 1705 and DPS 1703 isolates at various pH. Optimal optical density occurred at pH range of 4 to 5. The growth

Table 6. Sulfate reduction by DPS 1711, DPS 1705 and DPS 1703 at various initial sulfate concentrations.

[$SO_4^=$] Initial (ppm)	Sulfate reduction					
	DPS 1703		DPS 1705		DPS 1711	
	$SO_4^=$	Red (%)	$SO_4^=$	Red (%)	$SO_4^=$	Red (%)
500	53.07[a]	89[a]	21.54[b]	95[b]	13.48[c]	97[c]
1000	69.40[a]	93[a]	63.68[b]	94[a]	51.06[c]	95[bc]
2000	431.04[a]	78[a]	209.48[b]	89[b]	79.25[c]	96[c]
5000	1302.11[a]	74[a]	1071.50[b]	78[b]	922.54[c]	82[c]
8000	1704.32[a]	78[a]	1346.16[b]	82[b]	1309.41[c]	83[b]

of the isolates at low pH is the ability of SRB to interact with acidic environment to achieve optimal environment for its growth. Such interaction conditions and abilities vary for each microorganism. The time taken for SRB isolates to grow at lower pH is longer, which is in line with the length of time required to achieve optimum pH. Tolerance to low pH is only performed by extra cellular interaction, while intra-cellular activity requires a higher optimal pH condition.

Reducing sulfate of SRB

SRB has the ability to transfer electron or hydrogen to sulfate which acts as terminal electron acceptor. From the process of the redox reaction, the sulfate is reduced to sulfide. Sulfate reduction occurs in anaerobic conditions (Rückert, 2016). The main product of sulfate reduction depends on the substrate used. If the substrate used is hydrogen, then the product is hydrogen sulfide. When simple organic materials such as primary lactates are the electron donors, then the product is sulfide (Paulo et al., 2013). In Table 6 it was shown that the ability of the isolates to reduce sulfate for DPS 1703 (74 to 93%) is highest at the initial sulfate content of 1000 ppm, for DPS 1705 (78 to 95%) the highest is when the initial sulfate content is 500 ppm and for DPS 1711 (82 to 97%) the highest reduction percentage is under the initial sulfate content of 500 ppm. DPS 1711 has the highest sulfate reducing ability compared to the other isolates at all initial sulfate contents.

SRB uses sulfate (SO_4^{2-}), thiosulfate ($S_2O_3^{2-}$), sulfite (SO_3-) and other reducible sulfur ions as terminal electron acceptor in the respiration of its metabolism. In the presence of an organic compound or H_2 as an electron donor under anaerobic condition, the sulfate ion is reduced to sulfide following the equation (Torres-Alvarado et al., 2016):

$$2CH_2O + SO_4^{2-} \longrightarrow H_2S + 2HCO_3^-$$

$$5H_2 + SO_4^{2-} \longrightarrow H_2S + 4H_2O + 2e$$

Sulfate reduction can occur under wide range pH, pressure, temperature and salinity intervals. In a natural environment, sulfate can be a limiting factor for SRB activity, while simple organic compounds are available in the presence of other bacterial activity (Müller et al., 2014)

Conclusions

The selected bacteria namely (DPS 1711, DPS 1705 and DPS 1703) Sulfate reducing bacteria are identified as *D. orientis.* These bacteria exhibit wide range temperature and pH tolerance thus could be applied for treating highly polluted waste such as acid mine waste.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest

REFERENCES

Atlas RM (1993). Handbook of Microbial Media. CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida 33431. 556 p.

Barton LL, Faugue GD (2009). Biochemistry, physiology and biotechnology of sulfate-reducing bacteria. Advances in Applied Microbiology 68:41-98.

Dennis E, Julia G (2014). Corrosion of iron by sulfate-reducing bacteria: New Views of an Old Problem. Applied and Environmental Microbiology 80(4):1226-1236.

Kato S (2016). Micribial extracellular electron transfer and its relevance to iron corrosion. Microbial Biotechnology 9(2):141-148.

Müller AL, Kjeldsen KU, Rattei T, Pester M, Loy A (2014). Phylogenetic and environmental diversity of DsrAB-type dissimilatory (bi)sulfite reductases. International Society for Microbial Ecology Journal 9(5):1152-1165.

Païssé S, Ghiglione JF, Marty F, Abbas B, Gueuné H, Sanchez JM, Muyzer G, Quillet L (2013). Sulfate-reducing bacteria inhabiting natural corrosion deposits from marine steel structures. Applied Microbiology and Biotechnology 97:7493-7504.

Paulo RDM, Diogo R, Marcos ACB, Carlos MG, Angela B, Mariana VP, Patricia do RD, Vânia AV, Chapaval P (2013). Occurrence of sulfate reducing bacteria (SRB) associated with biocorrosion on metallic surfaces in a hydroelectric power station in Ibirama (SC)-Brazil. Brazilian Archives of Biology and Technology 56:5.

Rückert C (2016). Sulfate reduction in microorganisms—recent advances and biotechnological applications. Current Opinion in Microbiology 33:140-146.

Shiqiang C, Peng W, Dun Z (2014). Corrosion behavior of copper under

biofilm of sulfate-reducing bacteria. Corrosion Science 87(5):407-415.
Torres-Alvarado MR, Calva-Benítez LG, Álvarez-Hernández S, Trejo-Aguilar G(2016). Anaerobic microbiota: spatial-temporal changes in the sediment of a tropical coastal lagoon with ephemeral inlet in the Gulf of Mexico. Revista De Biologia Tropical 64(4):1759-1770.
Xu D, Li Y, Song F, Gu T (2013). Laboratory investigation of microbiologically influenced corrosion of C1018 carbon steel by nitrate reducing bacterium Bacillus licheniformis. Corrosion Science 77:385-390.

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