Scientific Research Publishing

Open Journal of Veterinary Medicine, 2020, 10, 39-54
https://www.scirp.org/journal/ojvm
ISSN Online: 2165-3364
ISSN Print: 2165-3356

Genital Infection and Associated Pathology in Red Sokoto and West African Dwarf Does in Makurdi

Ibrahim Garba[1*], Philip Makama Dawuda[1], Iyorhemba Utim Ate[1], Due Emmanuel Awai[2], Usman Adamu Rayyanu[3], Igah Eyitayo Olanrewaju[3], Akuchi Chidiadi Nwamo[3], Umbugadu Cletus Attah[4], Samuel Moses Abasiama[5], Jerry Ngutor Abenga[6]

[1]Department of Theriogenology, College of Veterinary Medicine, Federal University of Agriculture Makurdi, Makurdi, Nigeria
[2]Diagnostic Service Department, National Veterinary Research Institute, Nigeria
[3]Livestock Investigation Division, National Veterinary Research Institute, Vom, Plateau State, Nigeria
[4]Bacterial Research Division, National Veterinary Research Institute, Vom, Plateau State, Nigeria
[5]Central Diagnostic and Extension Division, National Veterinary Research Institute, Uyo Laboratory, Akwa Ibom State, Nigeria
[6]Department of Veterinary Pathology, College of Veterinary Medicine, Federal University of Agriculture Makurdi, Makurdi, Nigeria
Email: *garbaibrahim16@yahoo.com

How to cite this paper: Garba, I., Dawuda, P.M., Ate, I.U., Awai, D.E., Rayyanu, U.A., Olanrewaju, I.E., Nwamo, A.C., Attah, U.C., Abasiama, S.M. and Abenga, J.N. (2020) Genital Infection and Associated Pathology in Red Sokoto and West African Dwarf Does in Makurdi. *Open Journal of Veterinary Medicine*, **10**, 39-54.
https://doi.org/10.4236/ojvm.2020.104004

Received: November 8, 2019
Accepted: April 27, 2020
Published: April 30, 2020

Abstract

An abattoir survey of 84 genitalia of Sokoto (RS) and West African Dwarf (WAD) does was undertaken to investigate and compare bacterial isolates and associated genital disorders and conduct antimicrobial susceptibility of the isolates. Bacteriological examination showed that seven bacterial genera were identified from genital organs of RS and WAD does, respectively: *Escherichia coli* (64%, 63.2%), *Pseudomonas* spp (43.2%, 24.1%), *Klebsiella* spp (11.4%, 13.2%), *Proteus* spp (5.0%, 1.0%), *Staphylococcus* spp (5.0%, 8.0%) and *Citrobacter* spp (1.0%, 5.3%) and *Enterobacter* spp (in RS only) (2.0%). *Escherichia coli* and *Pseudomonas* spp were the dominant isolates. The rate of genital infection of RS and WAD does examined was highest with *Escherichia coli* (63.4%) and the pattern of bacterial isolation was high with *Escherichia coli*. There was no significant difference ($P > 0.05$) in the bacteria species colonizing the genital tracts of RS and WAD does. The relative risk (RR) for an infection of the uterus with *Escherichia coli* (1.08, 95% Confidence Interval (CI): 0.6588 to 1.769, $P > 0.7606$) was not significantly different in RS compared to WAD does. Bacteria were isolated from cases of endometritis, pyometra, postparturient metritis, mucometra, uterine congestion, melanosis, caruncular atrophy, salphingitis and cervicitis. Sensitivity test showed bacterial isolates were highly susceptible to Levofloxacin, Pefloxacin, Ciprofloxacin, Ofloxacin and Amoxyl. It was concluded that there was no difference in bacterial isolates in genital tracts of RS and WAD does and genital disorders could be

associated with bacterial infections in does. The potentials of these bacterial isolates for producing genital pathology in does are likely to be high in Makurdi, north-central Nigeria. Therefore, management of genital disorders associated with these pathogens can be achieved with proper use of these antimicrobial agents in does.

Keywords

Genital Infection, Pathology, Antimicrobial Susceptibility, Red Sokoto Doe, West African Dwarf Doe

1. Introduction

Goat plays an important role in developing countries because of its meat and milk production [1] and contributes to health and nutrition. Their potentially high reproductive rates and production efficiency has lead to an increased proliferation of small and medium scale goat farmers in Nigeria [2]. However, regular and successful reproduction is the key to profitable goat production. Poor fertility is a common problem in ruminants and it has been related to diseases involving different parts of the female genital tract [3]. This can be a complication of various infectious diseases that localize in the reproductive organ of goats with or without specific morphological defects [4] [5]. Such lesions may be part of a systemic disease or initiated as reproductive organ disturbances following breeding or obstetric interventions [6] [7] [8] [9]. Many of these reproductive pathologies are associated with diseases that lower the overall reproductive performance of the animals or cause fetal mortality and abortions [10].

The number of bacteria colonizing the reproductive tract is important determinants of uterine infections [9] [11]. Genital microflora are usually harmless until presence of predisposing factors such as trauma, systemic infection [12], lowered immune status during stress and cystic ovarian disease [13]. Genital microflora may act as opportunistic bacteria to cause genital infection that usually leads to reproductive failure in ruminants [4] [5] [14] [15] [16]. Varieties of bacteria have been isolated from the genitalia of the does and were shown to be associated with disease conditions of the genitalia [17] [18].

Various investigations have been carried-out and valuable suggestions made on sensitivity of bacterial species to different antimicrobial agents [19] [20] [21]. Antimicrobial agents are commonly used in the management of reproductive failures in livestock [22]. An insight on the microbes colonizing genital tract and their antimicrobial susceptibility will therefore demystify management of genital tract infections and abnormalities in does. The objective of this study therefore was to investigate and compare genital tract microbes and associated genital disorders in Red Sokoto and West African Dwarf does and performs antimicrobial sensitivity for an effective management of reproductive abnormalities in small ruminants.

2. Materials and Methods

2.1. Study Area, Animal and Sample Collection

The study was carried out in Makurdi, Benue state, north-central part of Nigeria on longitude 8.35˚E and latitude 7.44˚N with a radius of 16 km. The climate is tropical and the vegetation is predominantly guinea savannah with an annual rainfall of 1090 mm. This area is defined by two seasons, the rainy season (April to October) and dry season (November to March) with an average relative humidity of 26.3% to 71.3% throughout the year. The atmospheric temperature ranges from 27.38˚C to 34.09˚C [23].

Sample size was determined at 95% confidence level by using the formula of Thrusfield (1997):

$$N = \frac{Z^2 pq}{d^2}$$

where N = sample size, Z = appropriate value for the standard normal deviation for the desired confidence = 1.96, p = prevalence, $q = 1 - p$, d = level of significance (0.05) with assumption that 70% of the genitalia (non pregnant) of RS and WAD does will be infected with bacteria. A total of 100 (50 each from RS and WAD does) were from Wurukum and International cattle market abattoirs in Makurdi, respectively, from April to June, 2016. The genital tracts were collected twice weekly after evisceration and transferred on ice in clean labeled polyethelyne bags to the pathology laboratory. Ethical approval for this study was obtained from the College of Veterinary Medicine, Federal University of Agriculture Makurdi (FUAM).

2.2. Collection of Samples for Bacteriological Studies

Genital organ swabs from RS (44) and WAD (38) does were collected as per standard protocols described by [24] and [25]. Lesions were examined by their size, consistency, colour, shape, smell or location as described by [26]. Each reproductive tract was opened by cutting with a pair of sterilized scissors, starting from the vulva, into the vagina through the cervix and uterine body, into each horn, saphinx and finally the ovaries. Sterile cotton swab sticks (Nicecare®, Nigeria) was rolled over lesion-bearing and normal portions of the genital tracts accordingly. The genital swap samples were transported on ice in cold boxes to Central Diagnostic Laboratory, National Veterinary Research Institute (NVRI), Vom, in labeled Bijou bottles containing 2 ml nutrient broth.

2.3. Bacterial Culture, Isolation and Identification

Each swab sample was inoculated on to conventional culture media Blood agar (BA), MacConkey agar (MCA) and Nutrient agar (Oxoid, Basingstoke, UK), respectively, for the isolation and purification of bacteria. The media were prepared according to the manufacturer's instruction. Sterile wire loop was used to streak out the inoculums to allow spread of bacteria on the media to produce discrete

colonies. Discrete colonies on the culture media were examined and identified based upon colony morphology and pigment characteristics; a colony of each morphologic type was purified by a repeated subculture at 37˚C for 24 hours on to MCA, BA and Eosin methylene blue (EMB) [24]. The purified bacteria cultures were identified based upon reaction to Gram stain and microscopic characteristics (×100 lenses) as described by [27] and bio-chemical tests as described by [28].

2.4. Antibiotic Sensitivity Test

The antibiotic sensitivity was determined according to Kirby-Bauer (disc diffusion) method described by [29]. The [30] criteria was set to record the level of sensitivity. The diameter of the zone of inhibition was measured (mm): <10 mm = Resistant, >10 mm = Moderately sensitive and ≥15 mm = Sensitive.

2.5. Data Analysis

Descriptive statistics was used to represent the data generated. The distribution of the bacterial isolates was estimated as simple percentages. Relative risks (RR) for an infection with bacteria were analyzed. The pattern of bacterial isolation and rate of infection of genital tracts with bacteria was determined and expressed in percentage. All data analysis was subjected to Graphpad Prism Statistical Software [31] GraphPad Software Inc. (2016). GraphPad Prism Version 7.03. P-value was considered significant at <0.05.

3. Results

3.1. Distribution of Bacterial Isolates from Genital Tracts of RS and WAD Does

Seven genera of bacteria were identified from RS which includes *Escherichia coli*, *Staphylococcus* spp, *Pseudomonas* spp, *Klebsiella* spp, *Proteus* spp, *Enterobacter* spp and *Citrobacter* spp while six genera of bacteria were identified from WAD genitalia which includes *Escherichia coli*, *Staphylococcus* spp, *Pseudomonas* spp, *Klebsiella* spp, *Proteus* spp *and Citrobacter* spp (**Table 1**).

A total of 113 bacterial isolates were recovered from genital tracts of RS and WAD does, respectively (**Table 2**). Of the 59 bacterial isolates identified from RS (n = 44), 42 were recovered from the uterus: *Escherichia coli* 20 (46.0%), *Pseudomonas* spp 15 (34.0%), *Klebsiella* spp 3 (7.0%), *Staphylococcus* spp 2 (5.0%), *Proteus* spp 1 (2.3%) and *Citrobacter* spp 1 (2.3%). Vaginal cultures yielded 16 isolates: *Escherichia coli* 7 (16.0%), *Pseudomonas* spp 4 (9.0%), *Klebsiella* spp 2 (5.0%), *Enterobacter* spp 2 (5.0%) and *Proteus* spp 1 (2.3%). 1 (2.3%) isolate was recovered from the cervix while no bacteria were isolated from the salphinx (**Table 2**).

In the WAD (n = 38), 54 bacterial isolates were identified, out of which 37 were recovered from the uterus (**Table 2**): *Escherichia coli* 16 (42.1%), *Pseudomonas* spp 11 (28.0%), *Klebsiella* spp 2 (5.3%), *Proteus* spp 3 (8.0%), *Staphylo-*

coccus spp 3 (8.0%) and *Citrobacter* spp 2 (5.3%). The vaginal cultures yielded 14 isolates: *Escherichia coli* 7 (18.4%), *Pseudomonas* spp 4 (11.0%), *Klebsiella* spp 2 (5.3%) and *Proteus* spp 1 (3.0%). 3 isolates were recovered from the salphinx which includes *Escherichia coli* 1(3.0%), *Pseudomonas* spp1 (3.0%) and *Klebsiella* spp 1 (3.0%) while no bacteria were isolated from the cervix (Table 2).

3.2. Relative Risk (RR) Analysis

The relative risk for infection (RR) of the uterus with *Klebsiella* spp (1.30, CI: 0.2282 to 7.352, P = 0.7692) was higher in RS compared to WAD, while the RR for *Escherichia coli* (1.08, CI: 0.6588 to 1.769, P = 0.7606) and *Pseudomonas* spp (1.18, CI: 0.6174 to 2.237, P = 0.6177) were lower in RS compared to WAD. However, the RR for infection of the vagina with *Escherichia coli* (0.86, CI: 0.3328 to 2.241, P = 0.7631), *Pseudomonas* spp (0.86, CI: 0.2314 to 3.222, P = 0.8271) and *Klebsiella* spp (0.86, CI: 0.2282 to 7.692, P = 0.7692) were the same for RS and WAD. The RR for infection of the vagina and uterus between RS and WAD was not statistically significant (P > 0.05). The results are presented in Table 2.

3.3. Rate of Genital Infection with Bacterial Isolates in RS and WAD Does

The rate of genital infection with bacterial isolates in RS and WAD does is shown in Table 3. Both RS and WAD genital organs were associated with *Escherichia coli* (63.4%) followed by *Pseudomonas* spp (31%) *and Klebsiella* spp (12.2%).

Table 1. Bacterial isolates from genital tracts of RS and WAD does.

Breed	Bacterial isolates	
	Genital tracts with no pathology	Genital tracts with pathology
RS	*Escherichia coli*	*Escherichia coli*
	Staphylococcus spp	*Staphylococcus* spp
	Klebsiella spp	*Klebsiella* spp
	Pseudomonas spp	*Pseudomonas* spp
	Proteus spp	*Proteus* spp
	Enterobacter spp	*Citrobacter* spp
	Citrobacter spp	
WAD	*Escherichia coli*	*Escherichia coli*
	Staphylococcus spp	*Staphylococcus* spp
	Klebsiella spp	*Klebsiella* spp
	Pseudomonas spp	*Pseudomonas* spp
	Proteus spp	*Proteus* spp
	Citrobacter spp	*Citrobacter* spp

Table 2. Distribution of bacterial isolates from different parts of the genital tracts of RS and WAD does.

Isolates	Breed				RR (at 95% CI)	Total
	RS (n = 44)		WAD (n = 38)			
	n	%	n	%		
Salphinx						
Escherichia coli	0	0.0	1	3.0		1
Klebsiella spp	0	0.0	1	3.0		1
Pseudomonas spp	0	0.0	1	3.0		1
Uterus						
Escherichia coli	20	46.0	16	42.1	(1.08, CI: 0.6588 to 1.769, P = 0.7606)	36
Staphylococcus spp	2	5.0	3	8.0	(0.58, CI: 0.1014 to 3.268, P = 0.5274)	5
Klebsiella spp	3	7.0	2	5.3	(1.30, CI: 0.2282 to 7.352, P = 0.7692)	5
Pseudomonas spp	15	34.0	11	28.0	(1.18, CI: 0.6174 to 2.237, P = 0.6177)	26
Proteus spp	1	2.3	3	8.0		4
Enterobacter spp	0	0.0	0	0.0		0
Citrobacter spp	1	2.3	2	5.3		3
Cervix						
Escherichia coli	1	2.3	0	0.0		1
Vagina						
Escherichia coli	7	16.0	7	18.4	(0.86, CI:0.3328 to 2.241, P = 0.7631)	14
Staphylococcus spp	0	0.0	0	0.0		0
Klebsiella spp	2	5.0	2	5.3	(0.86, CI: 0.2282 to 7.692, P = 0.7692)	4
Pseudomonas spp	4	9.0	4	11.0	(0.86, CI: 0.2314 to 3.222, P = 0.8271)	8
Proteus spp	1	2.3	1	3.0		2
Enterobacter spp	2	5.0	0	0.0		2
Citrobacter spp	0	0.0	0	0.0		0
Total	**59**		**54**			**113**

(n = 44, n = 38 is the total number of swabs collected from genital organs of RS and WAD respectively). RR is the relative risk for an infection with bacteria in RS compared to WAD does. P = Probability for infection (P > 0.05) is not significant. CI = Confidence interval. RS = Red Sokoto doe, WAD = West African Dwarf doe.

Table 3. Infection rate of bacterial isolates from genital tracts of RS and WAD does.

Breed	Bacterial isolates							Total
	Escherichia coli	*Staphylococcus* spp	*Klebsiella* spp	*Pseudomonas* spp	*Proteus* spp	*Enterobacter* spp	*Citrobacter* spp	
RS (n = 44)	28	2	5	19	2	2	1	59
WAD (n = 38)	24	3	5	16	4	0	2	54
Total (Infection rate)	52 (63.4%)	5 (6.1%)	10 (12.2%)	25 (31%)	6 (7.3%)	2 (2.4%)	3 (4.0%)	113

P > 0.05. (n = 44, n = 38 is the number of swabs collected from genital tracts of RS and WAD respectively). RS = Red Sokoto doe. WAD = West African Dwarf doe.

3.4. Pattern of Bacteria Isolation from Genital Tracts of RS and WAD Does

A total of 30 isolation patterns in RS and 23 patterns in WAD does were recorded from apparently normal genital tracts with *Escherichia coli* being the predominant while 12 isolation patterns in RS and 14 patterns in WAD does, respectively, were recorded from genital tracts with abnormalities with *Escherichia coli* being the predominant (Table 4).

3.5. Bacterial Isolates from Genital Tract Lesions in RS and WAD Does

Bacterial isolates from the genital tract of RS and WAD does associated with lesions are presented in Table 5. *Escherichia coli*, *Proteus* spp and *Staphylococcus* spp were isolated from cases of endometritis and uterine congestions while *Escherichia coli* and *Staphylcoccus* spp were isolated from pyometra, *Escherichia coli* and *Proteus* spp were isolated from case of uterine melanosis. *Escherichia coli*, *Staphylococcus* spp and *Pseudomonas* spp were isolated from postparturient metritis, while *Pseudomonas* spp and *Citrobacter* spp isolated from postparturient emphysematous metritis. *Escherichia coli*, *Proteus* spp and *Pseudomonas* spp were isolated from mucometra. *Klebsiella* spp and *Pseudomonas* spp were isolated from case of salphingitis. While *Escherichia coli* was isolated from acute cervicitis, *Proteus* spp was isolated from hemorrhagic necrotizing cervicitis.

Table 4. Pattern of bacterial isolation from genital tracts of RS and WAD does.

	Bacterial isolates from genital tracts				
Breed	**Apparently normal genital tracts**	**Number (%)**	**Breed**	**Genital tracts with pathology**	**Number (%)**
RS (n = 31)	*Escherichia coli*	11 (35.5)	**RS (n = 13)**	*Escherichia coli*	6 (46.2)
	Staphylococcus spp	1 (3.2)		*Pseudomonas* spp	2 (15.4)
	Klebsiella spp	2 (6.5)		*Proteus* spp	1 (7.7)
	Pseudomonas spp	4 (12.9)		*Escherichia coli* + *Staphylococcus* spp	1 (7.7)
	Citrobacter spp	1 (3.2)		*Escherichia coli* + *Pseudomonas* spp	1 (7.7)
	Escherichia coli + *Pseudomonas* spp	8 (25.8)		*Pseudomonas* spp + *Klebsiella* spp	1 (7.7)
	Pseudomonas spp + *Enterobacter* spp	1 (3.2)			
	Pseudomonas spp + *Klebsiella* spp	1 (3.2)			
	Staphylococcus spp + *Proteus* spp	1 (3.2)			
WAD (n = 25)	*Escherichia coli*	9 (36.0)	**WAD (n = 13)**	*Escherichia coli*	4 (30.8)
	Staphylococcus spp	1 (4.0)		*Klebsiella* spp	1 (7.7)
	Klebsiella spp	1 (4.0)		*Escherichia coli* + *Staphylococcus* spp	4 (30.8)
	Pseudomonas spp	2 (8.0)		*Escherichia coli* + *Pseudomonas* spp	1 (7.7)
	Proteus spp	2 (8.0)		*Escherichia coli* + *Proteus* spp	1 (7.7)
	Escherichia coli + *Pseudomonas* spp	5 (20.0)		*Pseudomonas* spp + *Citrobacter* spp	1 (7.7)
	Pseudomonas spp + *Citrobacter* spp	1 (4.0)		*Pseudomonas* spp + *Klebsiella* spp	1 (7.7)
	Pseudomonas spp + *Klebsiella* spp	2 (8.0)		*Staphylococcus aureus* + *Proteus* spp	1 (7.7)

RS (n = 31), WAD (n = 25) is the number of apparently normal genital tracts of RS and WAD, respectively. RS (n = 13), WAD (n = 13) represents number of genital tracts of RS and WAD with pathology, respectively.

Table 5. Bacteria isolated from genital tract lesions in RS and WAD does.

Lesions	Bacteria isolated from lesions
Endometritis	*Escherichia coli* *Proteus* spp *Staphylococcus* spp
Pyometra	*Escherichia coli* *Staphylococcus* spp
Postparturient metritis	*Escherichia coli* *Pseudomonas* spp *Staphylococcus* spp
Postpaturient emphysematous metritis	*Pseudomonas* spp *Citrobacter* spp
Mucometra	*Escherichia coli* *Proteus* spp *Pseudomonas* spp
Uterine caruncular atrophy	*Klebsiella* spp *Pseudomonas* spp
Uterine congestion	*Escherichia coli* *Proteus* spp *Staphylococcus* spp
Uterine melanosis	*Escherichia coli* *Proteus* spp
Hemorrhagic necrotizing cervicitis	*Proteus* spp
Cervicitis (acute)	*Escherichia coli*
Salphingitis	*Klebsiella* spp *Pseudomonas* spp

3.6. Antimicrobial Susceptibility Test

The bacterial isolates (Gram –ve) show high susceptibility (77% - 94%) to Levofloxacin, Pefloxacin, Ciprofloxacin, Ofloxacin, Amoxyl and Gentamycin. The susceptibility to Amoxycillin clavulanate, Streptomycin and Ceporex were moderate (50% - 57%) while low rate was recorded for Ampiclox (34%) (**Table 6**). *Staphylococcus* spp showed high susceptibility (80% - 100%) to Levofloxacin, Ciprofloxacin and Amoxyl. The susceptibility to Norfloxacin, streptomycin, Gentamycin, Rifampicinand ampiclox were moderate (40% - 60%) while Chloramphenicol and Erythromycin showed low susceptibility (20%) (**Table 7**).

4. Discussion

Bacteria colonizing the vagina and uterus are likely to cause reproductive failure in domestic ruminants and are important determinants of uterine infections [9] [11] [32] [33]. Vaginal bacteria get access into the uterus during the peripartum period leading to metritis and endometritis and subsequent reduction in the reproductive capacities of these animals [4].

Out of the vaginal bacterial isolates recovered from RS and WAD in this study *E. coli* and *Pseudomonas* spp were the most common. The isolation rate of *E. coli* in RS and WAD (16% and 18.4%), respectively, was higher compared to the rate of for *Pseudomonas* spp similar to previous report [34] [35] [36] [37]. *Klebsiella* spp, *Enterobacter* spp and *Proteus* spp were also isolated with the later having the lowest isolation rate of 2.3%.

Table 6. Frequency of antibiotic susceptibility of bacterial isolates from genital tracts of RS and WAD does.

Antibiotics	Gram –ve bacterial isolates						Total (n = 113)
	Escherichia coli (n = 52)	*Klebsiella* spp (n = 10)	*Pseudomonas* spp (n = 25)	*Proteus* spp (n = 6)	*Enterobacter* spp (n = 2)	*Citrobacter* spp (n = 3)	
Ofloxacin	46 (86%)	8 (80%)	20 (80%)	6 (100%)	3 (100%)	2 (100%)	85 (87%)
Levofloxacin	50 (96%)	10 (100%)	23 (92%)	6 (100%)	3 (100%)	2 (100%)	94 (96%)
Pefloxacin	48 (92%)	9 (90%)	22 (88%)	6 (100%)	3 (100%)	2 (100%)	90 (92%)
Ciprofloxacin	47 (90%)	8 (80%)	21 (84%)	6 (100%)	3 (100%)	2 (100%)	87 (89%)
Amoxyl	42 (81%)	8 (80%)	20 (80%)	6 (100%)	3 (100%)	2 (100%)	81 (83%)
Gentamycin	43 (83%)	7 (70%)	18 (72%)	6 (100%)	2 (68%)	1 (50%)	77 (79%)
Augumentin	27 (52%)	5 (50%)	18 (72%)	3 (50%)	3 (100%)	1 (50%)	57 (58%)
Streptomycin	26 (50%)	5 (100%)	13 (52%)	3 (50%)	2 (68%)	1 (50%)	50 (51%)
Ceporex	26 (50%)	5 (50%)	12 (30%)	3 (50%)	2 (68%)	2 (100%)	50 (51%)
Ampiclox	15 (29%)	5 (50%)	9 (36%)	3 (50%)	0 (0%)	2 (100%)	34 (35%)

n = total number of Gram –ve bacteria isolated from genital tracts of RS and WAD does.

Table 7. Frequency of antibiotic susceptibility of *Staphylococcus* spp from genital tracts of RS and WAD does.

Antibiotics	Gram +ve bacterial isolates *Staphylococcus* spp (n = 5)
Norfloxacin	3 (60%)
Levofloxacin	5 (100%)
Ciprofloxacin	4 (80%)
Amoxyl	4 (80%)
Gentamycin	3 (60%)
Streptomycin	3 (60%)
Erythromycin	1 (20%)
Chloramphenicol	1 (20%)
Rifampicin	3 (60%)
Ampiclox	2 (40%)

n = total number of *Staphylococcus* spp isolated from genital tracts of RS and WAD.

The uterine bacterial isolates in the RS and WAD in this study are similar to those observed in the vagina. This was similarly reported previously [8] [9] [34] [36] [37]. *E. coli* was the common uterine bacterial isolate with an isolation rate of 46% and 42.1% in RS and WAD, respectively. *Citrobacter* spp and *Staphylococcus* spp were isolated while *Enterobacter* spp was not isolated. Bacteria isolated from the uterus were more than other portions of the genital tracts of both RS and WAD in this study similar to reports of [15] [38] [39]. *Klebsiella* spp and

Pseudomonas spp were isolated from the salphinx in WAD while none was recovered in the RS as previously reported [34]. These bacteria may be present as opportunist from the uterus as a result of ascending infections during clearance of bacteria from the uterine lumen [8] [36]. *E. coli* was recovered in the cervix only in the RS in this study.

The overall infection rate (63.4%) of the genital tract with *E. coli* in RS and WAD was higher than other bacterial isolates in this study. This agrees with the report of [37] that the overall weight of infection with *E. coli* (79%) was more than other isolates put together in cows and camels. However, in this study, the rate of infection with *E. coli* was higher in the uterus compared to other parts of the genital tracts contrary to earlier findings of [36] [37] who reported high rate of *E. coli* in the vagina compared to the uterus. The importance of *E. coli* as cause of genital disorders in animals cannot be ignored because it has been frequently reported as the most common isolate of the genital tract [34] [35] [36] [37].

Bacterial isolates from normal and pathological cases in genital tracts of ewes have been reported [18] [40]. Evidence implicating bacterial infections as causes of endometritis has been reported, and a variety of these bacterial species have been recovered from the uteri of infertile camelids [41] [42]. Previously, *E. coli* was thought to be a non-specific pathogen associated with endometritis in mares and cows [43]. The isolation of *E. coli* and *Staphylococcus* spp from endometritis in this study were similar to findings of [44] who reported *E. coli*, *Staphylococcus* spp and *Corynebacterium pyogenes* associated with endometritis in ewes.

Normal *E. coli* strains can cause relevant diseases as the pathogenic ones by producing cytotoxic necrotizing factors, verotoxins and *eae* gene isolated from healthy cows, sheep and goats [45] [46]. Specific strains of *E. coli* (EnPEC) have recently been shown to be pathogenic for the endometrium, causing pelvic inflammatory disease (PID) in cattle [47].

The pattern of isolation of *E. coli* with other bacterial isolates in this study is similar to the isolation pattern of *E. coli* observed with *Staphylococcus aureus*, *Bacillus* and *corynebacterium pyogenes* from cases of endometritis in does, camels, ewes and bitches [18] [44] [48] [49] [50] [51].

[52], reported post partum infections are eliminated within 2 - 4 weeks of parturition and some of the uterine pathogens persist to cause subclinical endometritis [53] [54]. In this study, *E. coli*, *Staphylococcus* spp and *Pseudomonas* spp were isolated in association with pyometra, post-parturient metritis and post parturient emphysematous metritis. This was similarly reported in repeat breeders with mucopurulent discharges in cows [55] and camels [51].

From the present study, it was observed that *E. coli*, *Staphylococcus* spp, *Proteus* spp and *Klebsiella* spp were isolated in association with uterine caruncular atrophy, uterine congestion and melanosis. This could arise from spread of infections from other pathological sites where different lesions are occurring on individual genitalia. Uterine congestions occurred with endometritis while caruncular atrophy and melanosis were observed as single cases in this study.

There is a continuous clearance and recontamination of the uterine lumen postpartum for up to 7 weeks [9], pathological changes and inflammatory responses can be triggered by some bacteria which persist in the uterus and delay uterine involution [56], thereby lowering infertility. The severity of these inflammatory conditions is likely to be influenced by these types of bacteria colonizing the uterus [9].

The occurrence of ovulation prior to the expulsion of exudates and debris from the uterus and during the postpartum period has been shown to favour heavy growth of bacteria in the uterine environment. This leads to the retention of the corpus luteum (CL) and consequent impairment of the ability of the uterus to secret PGF2α [13] [57]. The number of bacteria colonizing the uterus and the level of uterine immune response may portend a risk factor for lowered reproductive efficiency because of increased inflammatory reactions and possible damages to the uterine tissues by direct action of the bacteria or its toxins [9] [11] [57] [58].

From the result of this study, the susceptibility pattern of *E. coli* to antimicrobials showed that Levofloxacin, Pefloxacin, Ciprofloxacin, Ofloxacin, Amoxyl and Gentamycin are most effective. Moderately active are Amoxycillin clavulanate, Streptomycin and Ceporex. The suceptibility of *E. coli* to Ampiclox was low. This finding agrees with previous reports of antimicrobial susceptibility of bacterial isolates from genitalia of goats, ewes and cattle [5] [35] [36] [55] [59] [60], but in variance with previous observations made by [61] in goats in southern Nigeria, where they reported that Gentamycin, Ofloxacin, Streptomycin, Ampicillin and Amoxycillin clavulanate were highly effective against *E. coli*.

Staphylococcus spp was highly susceptible to Levofloxacin, Amoxyl and Amoxycillin clavulanate. This finding contradicts previous reports in cattle and sheep [55] who found Ciprofloxacin as one of the most effective antimicrobial agent against Staphyloccocal uterine infections in dairy cows. Ciprofloxacin, Norfloxacin, Gentamycin, Ceporex and Rifampicin were moderately effective against *Staphylococcus* spp. However, *Staphylococcus* spp showed low susceptibility to Chloramphenicol and Erythromycin.

There is an increasing antimicrobial resistance in animals, which is complicating empirical selection of antimicrobial agents in veterinary practice [36]. These complications are continuously evolving in relation to factors such as the site of isolation, sex, age, species of the animal [62] and location where different antibiotic agents varied in their activity against bacterial isolates in Nigeria [36] [63].

The decrease in sample size in this study was as a result of damage incurred during the course of transport to the laboratory. The isolates were not identified at the *specie* level which is certainly a major limitation in this study. Also a reproductive history of live animals in correlation with clinical parameters would have elucidated on the importance of the isolates. Where possible, gynaecological evaluations in live animals should proceed with proper history, clinical and laboratory examination in order to determine specific aetiologies and generate useful data for effective diagnosis and management of genital disorders.

5. Conclusion

Bacteria colonizing the genital tract are similar in RS and WAD does. *E. coli* was the dominant bacteria isolated. *E. coli* and other isolates were found to be associated with genital disorders does. The bacterial isolates were found to be susceptible to antimicrobial agents tested. The potentials of these genital pathogens producing genital pathology and infection in goats are likely to be high but treatment could be achieved with the proper use of antimicrobials tested in this study. This could help in improving goat fertility, viable and productive small ruminant farm enterprise, increase in dietary protein and healthy wellbeing of the populace at large.

Acknowledgements

The assistance rendered by the people in sample collection and laboratory processing of the samples for this study is gratefully acknowledged. The authors are thankful to the Directors, Veterinary Teaching Hospital, FUAM and NVRI, Vom, for providing necessary facilities for this work.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Williamson, G. and Payne, W.J. (1984) An Introduction to Animal Husbandry in the Tropics. 4th Edition, ELBS and Longman, Essex.

[2] Lawal-Adebowale, O.A. (2012) Dynamics of Ruminant Livestock Management in the Context of the Nigerian Agricultural System. In: Javed, K., Ed., *Livestock Production*, IntechOpen, London, 61. https://doi.org/10.5772/52923

[3] Palmieria, C., Schiavia, E. and Della, L.S. (2011) Congenital and Acquired Pathology of Ovary and Tubular Genital Organs in Ewes: A Review. *Theriogenology*, **75**, 393-410. https://doi.org/10.1016/j.theriogenology.2010.09.020

[4] Levinson, W.E. and Jawetz, E. (1994) Medical Microbiology and Immunology. 3rd Edition, Prentice-Hall Int. Inc., Englewood Cliffs, 20-23.

[5] Martins, G., Figueira, L., Penna, B., Brandão, F., Varges, R., Vasconcelos, C. and Lilenbaum, W. (2009) Prevalence and Antimicrobial Susceptibility of Vaginal Bacteria from Ewes Treated with Progestin-Impregnated Intravaginal Sponges. *Small Ruminant Research*, **81**, 182-184. https://doi.org/10.1016/j.smallrumres.2008.12.003

[6] Garg, G.M., Vadnere, S.V. and Sharma, V.D. (1982) Immunology and Infectious Diseases. *Indian Journal of Comparative Microbiology*, **3**, 103-104.

[7] Patgiri, G.P. and Uppal, P.K. (1983) Mycoflora of Bovine Female Genital Tract Affected with Various Reproductive Disorders. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases*, **4**, 19-22.

[8] Tibary, A. and Anouassi, A. (2001) Uterine Infections in Camelidae. *Veterinary Science Tomorrow*, **1**, 1-12. http://dspace.library.uu.nl:8080/handle/1874/28883

[9] Singh, J., Murray, R.D., Mshelia, G. and Woldehiwet, Z. (2008) The Immune Status of the Bovine Uterus during the Peri-Partum Period. *The Veterinary Journal*, **175**, 301-309. https://doi.org/10.1016/j.tvjl.2007.02.003

[10] Kassim, F., Abdul-Kareem, A., Al-Mayah, A.S. and Kaisar, D. (2007) Abnormalities of Reproductive Organs in Ewes: A Prospective Histopathological Study. *Basrah Journal of Veterinary Research*, **6**, 97-107. https://doi.org/10.33762/bvetr.2007.58644

[11] Azawi, O.I. and Al-Mola, M.K. (2010) Effect of Season and Mating System in Awassi Ewes Superovulated with FSH on Fertilization Rate and Embryo Recovery. *Iranian Journal of Veterinary Surgery*, **24**, 75-79. https://doi.org/10.33899/ijvs.2010.5590

[12] Atwa, E. and Rady, F. (2007) Bacteria and Fungi Associated with Abortion in Sheep and Goat in Menoufiea Governorate. *Assiut Veterinary Medical Journal*, **53**, 326-349.

[13] Kaneko, K., Nakamura, M. and Reiichiro-Sato, R. (2013) Influence of *Trueperella pyogenes* in Uterus on Corpus Luteum Lifespan in Cycling Cows. *Theriogenology*, **79**, 803-808. https://doi.org/10.1016/j.theriogenology.2012.12.007

[14] Hirsh, D.C. (1990) The Genital Tract as a Microbial Habitat. In: *Review of Veterinary Microbiology*, 243-244.

[15] Shallali, A.A., Hussein, A.M., Salih, M.M and Dafalla, E.A. (2001) A Preliminary Report on Bacteria Isolated from the Female Genital Tract of Sudanese Sheep and Goats. *The Sudan Journal of Veterinary Research*, **17**, 55-63.

[16] Bukar, K.Y.M., Amin, J.D. and Zaria, L.T. (2007) Bacteria Flora of the Anterior Genitalia of the Sahelian Doe in Maiduguri-Borno State, Nigeria. *Nigerian Veterinary Journal*, **28**, 60-62. https://doi.org/10.4314/nvj.v28i2.3558

[17] Safiriyu, I.O., Waliu, A.S. and Gabriel, E. (2006) Exfoliative Vaginal Cytology during the Estrus Cycle of West African Dwarf Goats. *Reproduction Nutrition Development*, **46**, 87-95. https://doi.org/10.1051/rnd:2005067

[18] Sulake Fadhil, A., Shaimaa, O.H. and Hammedh, H.A. (2013) Isolation and Identification of Microflora Species at Different Level of the Ewe Genital Tract. *Journal of Agricultural and Veterinary Science*, **6**, 2319-2380. https://doi.org/10.9790/2380-0635457

[19] Singh, K., Bhargava, D.N., Kumar, A. and Shrifi, D. (1992) A Bacteriological Study of Non-Surgical Wounds in Bovine. *Indian Veterinary Journal*, **69**, 291-293.

[20] Nizamani, A.W. (1999) Studies on the Bacterial Flora of Uteri of Slaughtered Goats. M.Sc. Thesis, Department Veterinary Microbiology, Sindh Agriculture University, Tando Jam.

[21] Rind, R. and Khan, T.S. (2000) Antibiogram Sensitivity of Bacterial Organisms Identified from Surgical and Non-Surgical Wounds of Animal. *Pakistan Journal of Biological Science*, **3**, 1719-1723. https://doi.org/10.3923/pjbs.2000.1719.1723

[22] Drillich, M. (2006) An Update on Uterine Infections in Dairy Cattle. *Slovenian Veterinary Research*, **43**, 11-15.

[23] Omudu, E.A. and Amuta, E.U. (2007) Parasitology and Urban Livestock Farming in Nigeria: Prevalence of Ova in Faecal and Soil Samples and Animal Ectoparasites in Makurdi. *The South African Veterinary Association*, **78**, 271-278. https://doi.org/10.4102/jsava.v78i1.285

[24] Amin, J.D., Zaria, L.T. and Malgwi, R.M. (1996) Vaginal Aerobic Bacterial Flora of Apparently Healthy Cattle in Various Stages of the Reproductive Cycle in the Sahel Region of Nigeria. *Bulletin of Animal Health and Production in Africa*, **44**, 15-18.

[25] Cheesbrough, M. (1985) Culturing of Anaerobes. In: *Medical Laboratory Manual for Tropical Countries*, Butterworth Co., Kent, 248-264.

[26] Assey, R.J., Kessy, B.M., Matovelo, J.A. and Minga, U. (1998) Incidence of Gross Reproductive Abnormalities in Small East African Zebu Cattle. *Tropical Animal*

Health and Production, **30**, 361-368. https://doi.org/10.1023/A:1005144721298

[27] Cowan, S.J. and Steel, K.J. (1993) Manual of Identification of Medical Bacteria. Third Edition, Cambridge University Press, Cambridge, 22-126.

[28] Forbes, B.A., Sahm, D.F. and Weissfeld, A.S. (2007) Bailey and Scotts' Diagnostic Microbiology. 12th Edition, Elsevier, Amsterdam.

[29] Bassiri, E. (2013) Antibiotic Sensitivity Testing. Microbiology; Boil 275. University of Pennsylvania, Philadelphia.

[30] NCCLS (1984) Performance Standers for Antimicrobial Disc Susceptibility Tests: Approved Standard M2-A3. Villanova.

[31] GraphPad Software Inc. (2016). GraphPad Prism Version 7.03. http://www.graphpad.com

[32] Zaid, N.W. (2009) Vaginal Flora of Iraqi Sheep and Goats during Different Reproductive Stages. *Al-Anbar Journal of Veterinary Sciences*, **2**, 25-30.

[33] Silva, V.F., Damasceno, T.E.F., Souza, N.J.D., Franco, I. and Costa, M.M. (2011) Microbiotacérvico-vaginal de ovelhasmestiças e suasusceptibilidadeaosantibióticos. *Pesquisa Veterinária Brasileira*, **31**, 586-590. https://doi.org/10.1590/S0100-736X2011000700007

[34] Saad, M.A. (2014) Isolation and Identification of Some Aerobic Bacterial Flora from Female Genitalia in Goats in Babylon City. *Journal of Agriculture and Veterinary Science*, **7**, 19-22. http://www.iosrjournals.org https://doi.org/10.9790/2380-071021922

[35] Ogunbodede, M.A., Oladele, G.M., Ode, O.J. and Ubah, S.A. (2014) Survey of Gross Abnormalities and Microbial Load on the Female Reproductive Tract of Maradi Goats Slaughtered at Bodija Abattoir, Nigeria. *Advanced Journal of Agricultural Research*, **2**, 1-7.

[36] Mshelia, G.D., Bilal, V.T., Maina, V.A, Okon, K., Mamza, S.A., Peter, I.D. and Egwu, G.O. (2014) Microbiological Studies on Genital Infections in Slaughtered Ewes from Tropical Arid Zone of Nigeria. *Sokoto Journal of Veterinary Science*s, **12**, 18-22. https://doi.org/10.4314/sokjvs.v12i1.3

[37] Mshelia, G.D., Okpaje, G., Voltaire, Y.A.C. and Egwu, G.O. (2014) Comparative Studies on Genital Infections and Antimicrobial Susceptibility Patterns of Isolates from Camels (*Camelus dromedarius*) and Cows (*Bos indicus*) in Maiduguri, North-Eastern Nigeria. *Springerplus*, **3**, 91. https://doi.org/10.1186/2193-1801-3-91

[38] Bhuiyan, M.J., Hossain, M.I., Moslehuddin and Shahasuddin, M. (1998) Female Reproductive Disorders in Black Bengal Goats of Bangladesh. *Bangladesh Veterinarian*, **15**, 49-50.

[39] Rahman, M.H., Chowdhury, E.H., Saha, S.S., Islam, A. and Alam, M.G.S. (2008) Abattoir Study of Reproductive Diseases in Goats. *The Bangladesh Veterinarian*, **25**, 88-91. https://doi.org/10.3329/bvet.v25i2.4623

[40] Tibary, A., Fite, C., Anouassi, A. and Sghiri, A. (2006) Infectious Causes of Reproductive Loss in Camelids. *Theriogenology*, **66**, 633-647. https://doi.org/10.1016/j.theriogenology.2006.04.008

[41] Zina, B.A. and Hameedah, H.A. (2015) Isolation and Identification of Bacterial Flora from Vagina in Normal Ewes (Slaughter and Living Ewes). *Journal of Pharmacy and Biological Sciences*, **10**, 2319-7676.

[42] Wernery, U. and Kumar, B.N. (1994) Reproductive Disorders in Dromedary Camels Due to Infectious Causes and Its Treatment. *Journal of Camel Practice and Research*, **1**, 85-87.

[43] Sokkar, S.M. and Kubba. M.A. (1980) Pathological Studies on the Fallopian Tubes of Ewes. *Zentralbl. Veterinarmed. Reine A*, **27**, 118-122. https://doi.org/10.1111/j.1439-0442.1980.tb01677.x

[44] Adams, N.R. (1975) A Pathological and Bacteriological Abattoir Survey of the Reproductive Tracts of Merino Ewes in Western Australia. *Australian Veterinary Journal*, **51**, 351-354. https://doi.org/10.1111/j.1751-0813.1975.tb15945.x

[45] Orden, J.A., Ruiz-Santa-Quiteria, J.A., Cid, D., Díez, R., Martínez, S. and De la Fuente, R. (2001) Quinolone Resistance in Potentially Pathogenic and Non-Pathogenic *Escherichia coli* Strains Isolated from Healthy Ruminants. *Journal of Antimicrobial Chemotherapy*, **48**, 421-424. https://doi.org/10.1093/jac/48.3.421

[46] Afset, J.E., Bergh, K. and Bevanger, L. (2003) High Prevalence of Atypical Enteropathogenic *Escherichia coli* (EPEC) in Norwegian Children with Diarrhoea. *Journal of Medical Microbiology*, **52**, 1015-1019. https://doi.org/10.1099/jmm.0.05287-0

[47] Sheldon, I.M., Rycroft, A.N., Dogan, B., Craven, M., Bromfield, J.J., Chandler, A., Roberts, M.H., Price, S.B., Gilbert, R.O. and Simpson, K.W. (2010) Specific Strains of *Escherichia coli* Are Pathogenic for the Endometrium of Cattle and Cause Pelvic Inflammatory Disease in Cattle and Mice. *Microbiology*, **37**, 3437-3442. https://doi.org/10.1371/journal.pone.0009192

[48] Cockcroft, P.D. (1993) Urine Retention and Abdominal Straining in a Ewe with a Pyometra and a Retained Corpus Luteum. *Veterinary Record*, **132**, 115-116. https://doi.org/10.1136/vr.132.5.115-a

[49] Moghaddam, A. and Gooraninejad, S. (2007) Abattoir Survey of Gross Abnormalities of the Ovine Genital Tracts in Iran. *Small Ruminant Research*, **73**, 259-261. https://doi.org/10.1016/j.smallrumres.2006.10.022

[50] Gamcik, P., Nemes, D. and Schvare, F. (1975) Bacteriological Findings in the Genital Organs of Sheep in the Course of Oestrus Synchronization, Pregnancy and the Puerperium. *Folia Veterinaria*, **19**, 359-366.

[51] Ali, A., Hassanein, K.M., Al-Sobayil, F.A., Tharwat, M., Al-Hawas, A. and Ahmed, A.F. (2010) Relationship between Characters of Vaginal Discharges and Uterine Bacterial Isolates Recovered from Repeat Breeding Female Camels (*Camelus dromedarius*). *Journal of Agriculture and Veterinary Science*, **2**, 87-97.

[52] Hussain, A.M., Daniel, R.C.W. and O'Boyle, D. (1990) Post-Partum Uterine Flora Following Normal and Abnormal Puerperium in Cows. *Theriogenology*, **34**, 291-302. https://doi.org/10.1016/0093-691X(90)90522-U

[53] Fourichon, C., Seegers, H. and Malher, X. (2000) Effect of Disease on Reproduction in Dairy Cows: A Meta-Analysis. *Theriogenology*, **53**, 1729-1759. https://doi.org/10.1016/S0093-691X(00)00311-3

[54] Heuwieser, W., Tenhagen, B.A., Tischer, M., Luhr, J. and Blum, H. (2000) Effect of Three Programmes for the Treatment of Endometritis on the Reproductive Performance of a Dairy Herd. *Veterinary Record*, **146**, 338-341. https://doi.org/10.1136/vr.146.12.338

[55] Gani, M.O., Amin, M.M., Alam, M.G.S., Kayesh, M.E.H., Karim, M.R., Samad, M.A. and Islam, M.R. (2008) Bacterial Flora Associated with Repeat Breeding and Uterine Infections in Dairy Cows. *Bangladesh Journal of Veterinary Medicine*, **6**, 79-86. https://doi.org/10.3329/bjvm.v6i1.1342

[56] Williams, E.J., Fischer, D.P., Pfeiffer, D.U., England, G.C.W., Noakes, D.E., Dobson, H. and Sheldon, I.M. (2005) Clinical Evaluation of Postpartum Vaginal Mucus Reflects Uterine Bacterial Infection and the Immune Response in Cattle. *Theriogenology*, **63**, 102-117. https://doi.org/10.1016/j.theriogenology.2004.03.017

[57] Palmer, C. (2003) Post-Partum Metritis in Cattle: A Review of the Condition and the Treatment. *Large Animal Veterinary Rounds*, **3**, 13-15.

[58] Sheldon, I.M. and Dobson, H. (2004) Postpartum Uterine Health in Cattle. *Animal Reproductive Science*, **82-83**, 295-306. https://doi.org/10.1016/j.anireprosci.2004.04.006

[59] Martins, L.T., Neto, P.C.S., Neto, S.G., Rauber, L.P., Bertolini, M., Vieira, A.D. and Mezzalira, A. (2010) Microbiological and Functional Evaluation of an Alternative Device (OB) for Estrous Synchronization in Ewes. *Ciência Rural*, *Santa Maria*, **40**, 389-395. https://doi.org/10.1590/S0103-84782010000200021

[60] Khan, A.Z., Hayat, C.S., Munir, Z. and Ayaz, U. (2004) Prevalence of Mastitis in Buffaloes and Antibiotics Sensitivity Profiles of Isolates. *Pakistan Journal of Life and Social Sciences*, **2**, 73-75.

[61] Goncuoglu, M., Ormanci, F.S.B., Ayaz, N.D. and Erol, I. (2010) Antibiotic Resistance of *Escherichia coli* O157:H7 Isolated from Cattle and Sheep. *Annals of Microbiology*, **60**, 489-494. https://doi.org/10.1007/s13213-010-0074-8

[62] Hoesktra, K.A. and Paulton, R.J.L. (2002) Clinical Prevalence and Antimicrobial Susceptibility of *Staphylococcus aureus* and *Staphylococcus intermedius* in Dogs. *Journal of Applied Microbiology*, **93**, 406-413. https://doi.org/10.1046/j.1365-2672.2002.01708.x

[63] Emikpe, B.O., Oyero, O.G. and Akpavie, S.O. (2009) Isolation and Antibiogram of Aerobic Nasal Bacterial Flora of Apparently Healthy West African Dwarf Goats. *Revue d'elevage et de Medecine Veterinaire des Pays Tropicaux*, **62**, 17-21. https://doi.org/10.19182/remvt.10089