ASSESSMENT OF DRINKING WATER AND WASTEWATER QUALITY IN DAIRY CATTLE FARMS AND ITS ADVERSE EFFECTS ON HOST CELL RESPONSE IN MICE

NAGACHANDRA RAO A/L GOPI NAIDU

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By

NAGACHANDRA RAO A/L GOPI NAIDU

Thesis Submitted to the School of Graduate Studies, University Putra Malaysia In Fulfillment of the Requirement of the Degree of Master of Veterinary Science

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DEDICATION

To my beloved family. Thank you for all the support, trust and encouragement throughout my study.

To my supervisory committee, especially to my supervisor, Dr. Jesse. Thank you for the kind words, unswerving support, trust and help given throughout this process. I could have never done this without your help.

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in Fulfillment of the requirement for the degree of Master of Veterinary Science

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Chairman : Professor Faez Firdaus Jesse Abdullah, PhD
Faculty : Veterinary Medicine

Water is an essential medium for animal metabolism as well as an important cleaning medium and it must be maintained at certain quality to avoid adverse effects on the animals. According to the National Water Quality Standards, water source with a classification of Class III and less is deemed suitable for animal consumption. The chances for water quality to be substandard is as the possibility of farmers using water from a questionable water source exists. Therefore, there is an urgent need to explore the possible adverse effects in the livestock from drinking the substandard drinking water. In our best knowledge, most cattle farms in Malaysia do not practice wastewater treatment prior releasing it into water bodies such as drains, rivers and lakes and this can lead to environmental pollution and adverse health effects to the fauna in the immediate surrounding. In this study 7 cattle farms were selected and both drinking water and wastewater samples were sampled for laboratory analysis. The water samples were analysed in-situ and in the lab for 15 water quality parameters which are dissolved oxygen, pH, salinity, electrical conductivity, turbidity, biological oxygen demand, chemical oxygen demand, total suspended solid, total dissolved solid, ammoniacal nitrogen, nitrate, phosphates, total coliform, iron and magnesium content. The results were then compared with the National Water Quality Standards and the overall classification for each water sample was decided. Then, the water classification was further narrowed to three categories for the purpose of this study, namely, good category (Class I and Class II), moderate category (Class III) and unsatisfactory category (Class IV and Class V). The results for drinking water were as follows; 1(14.29%) Class II, 2 (28.57%) Class III, 2 (28.57%) Class IV and 2 (28.57%) Class V. Meanwhile, all the wastewater samples in this study were categorized as Class V.
A total of 35 female mice were divided into 5 groups; Group 1, 2, 3, 4 and 5 with 7 mice in each group. All the mice were gavage fed with 0.25 ml of water samples three times daily for a period of 30 days. Group 1 was fed with sterile deionized distilled water as the negative control, Group 2 was fed with the water sample from the good

category, Group 3 was fed with the water sample from moderate category and Group 4 was fed with the water sample from unsatisfactory category. Meanwhile, Group 5 was fed with one of the wastewater samples. The mice were observed at regular intervals and any mice that require humane end-points were euthanized and blood was collected through cardiac puncture before post-mortem examination and preservation of visceral organs and the brain. At the end of the 30 days, all surviving mice were sacrificed and blood, visceral organs and the brain was preserved for serological and histological examinations.

All treatment groups showed weight loss in comparison to the control group with the group treated with the wastewater showed most significant weight loss ($p<0.05$). Mice in groups 1, 2 and 3 did not show any significant clinical signs changes throughout the experimental period. In this experiment, 57%(n=4) of the mice from group 4 exhibited moderate emaciation and mild ruffled fur at the end of the experiment while all the mice (n=7) from group 5 exhibited clinical signs of severe ruffled fur, dehydration and severe emaciation and were euthanized to minimize pain. No significant gross lesions were observed in mice from groups 1, 2 and 3. Only 57% (n=4) of the mice from group 4 showed signs of moderate dehydration while all the mice from group 5 showed signs of severe dehydration. 28.5% (n=2) of the mice from group 2 and 14.2% (n=1) of the mice from group 4 showed subcutaneous abcesses. Several coliform bacteria were isolated and identified from the abcesses.

Only mice from Group 5 showed significant decrease ($p<0.05$) in the serum estrogen levels in comparison to the control while no significant changes were observed in treatment groups in comparison to the control group for the serum progesterone levels.

In terms serum Ig G concentrations, only the wastewater treatment group showed significant elevation ($p<0.05$) in comparison to the control group. The treatment groups 3, 4 and 5 showed significant elevation ($p<0.05$) in comparison to the control group in serum Ig M concentrations. The treatment groups 4 and 5 showed significant elevation ($p<0.05$) in serum Il-12 concentrations in comparison to the control group.

Groups 3 and 4 showed significant elevation ($p<0.05$) in serum Hp concentrations in comparison to the control group while groups 3, 4 and 5 showed significant elevation ($p<0.05$) in SAA concentrations in comparison to the control group.

Histopathological analysis revealed that both the kidneys and the livers from the mice in group 3 showed moderate inflammation and degeneration cellular changes. Severe inflammation and degeneration were observed in both the kidneys and livers in mice from groups 4 and 5 while moderate inflammation were observed in uterus and ovaries of mice from groups 4 and 5. At the same time, mild presence of inflammatory cells and oedema was observed in groups 3, 4 and 5.

In conclusion, this study had proven that the drinking water provided for cattle consumption in most of the cattle farms are subpar as per the requirement set by the National Water Quality Standards. At the same time, due to a lack of wastewater treatment prior to its release to the environment, the quality of the wastewater is very poor and can become a source of pollution especially in cases where the farm is located nearby raw drinking water source areas. Based on the clinicopathological and serological symptoms exhibited by the mice in the treatment groups fed different qualities of drink water obtained from the dairy cattle farms, there is a high risk that there would be a negative impact in terms of production, reproduction and health from long term consumption of drinking water with subpar quality. Therefore, constant evaluation is required to ensure that the drinking water provided to the cattle in the dairy cattle farms are up to the standard for their consumption and the wastewater in the farms should be treated and evaluated before being released to the environment to avoid pollution.

Keywords: Drinking water; wastewater; National Water Quality Standards; dairy cattle farm, veterinary aspect, host cell response, mice model

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan untuk ijazah Master Sains Veterinar

PENILAIAN KUALITI AIR MINUMAN DAN AIR KUMBAHAN DI LADANG-LADANG LEMBU TENUSU DAN KESAN BURUKNYA DENGAN RESPON SEL HOS DI TIKUS

Oleh

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Air adalah medium yang penting di dalam metabolisme haiwan selain daripada peranannya sebagai medium pembersihan di dalam lading. Berdasarkan Piawaian Kualiti Air Kebangsaan Malaysia, apa-apa sumber air dengan kualiti keseluruhan Kelas III dan ke bawah adalah sesuai untuk digunakan sebagai air minuman haiwan ternakan. Kemungkinan untuk kualiti air yang kurang berkualiti adalah tinggi kerana kebanyakkan peladang menggunakan sumber air yang boleh dipersoalkan. Oleh itu, ianya adalah penting untuk mengkaji kemungkinan kesan buruk di dalam haiwan ternakan yang minum air daripada sumber yang boleh dipersoalkan. Kebanyakkan ladang lembu di Malaysia tidak merawat air kumbahan sebelum melepaskan air tersebut ke dalam badan-badan air seperti longkang, sungai dan tasik. Sebanyak 7 buah ladang lembu tenusus telah dipilih untuk kajian ini dan sampel air minuman dan air kumbahan telah dikumpul untuk analisis. Pemeriksaan sampel air tersebut telah dijalankan di ladang dan juga di makmal untuk 15 parameter berikut; oksigen terlarut, pH, kemasinan, kekonduksian elektrik, kekeruhan, keperluan oksigen biologi, keperluan oksigen kimia, jumlah pepejal tidak terlarut, jumlah pepejal terlarut, nitrogen ammonia, nitrat, fosfat, jumlah bakteria koliform, besi dan magnesium. Penemuan daripada penilaian di ladang dan makmal telah dibandingkan dengan Piawaian Kualiti Air Kebangsaan Malaysia dan pengkelasan keseluruhan untuk sampel-sampel air tersebut telah dibuat. Pada waktu yang sama, pengkelasan kualiti air tersebut telah diubahsuai untuk kajian ini kepada kategori bagus (Kelas I dan Kelas II), kategori sederhana (Kelas III) dan kategori tidak memuaskan (Kelas IV dan Kelas V). Keputusan penilaian kualiti air minuman adalah seperti berikut; 1(14.29%) Kelas II, 2 (28.57%) Kelas III, 2 (28.57%) Kelas IV dan 2 (28.57%) Kelas V. Kesemua sample air kumbahan pula telah dikelaskan sebagai Kelas V.

Sebanyak 35ekor tikus betina telah dibahagikan kepada 5 kumpulan; Kumpulan I, 2, 3,4 dan 5 dengan 7 tikus di dalam setiap kumpulan. Kesemua tikus tersebut telah digavaj dengan 0.25 ml sampel air tiga kali sehari selama 30 hari. Kumpulan 1 telah diberi air tulen steril sebagai kumpulan kawalan negatif, Kumpulan 2 telah diberi sampel air daripada kategori memuaskan, Kumpulan 3 telah diberi sampel air daripada kategori sederhana, Kumpulan 4 telah diberi sampel air daripada kategori tidak memuaskan dan Kumpulan 5 telah diberi sampel air kumbahan. Kesemua tikus tersebut telah diperhatikan dari masa ke masa dan mana-mana tikus yang mempamerkan gejala klinikal yang memerlukan euthanasia telah dimatikan dengan cara dislokasi tulang vertebra serviks dan sampel darah telah dikumpulkan melalui penusukan jantung sebelum pemeriksaan post-mortem dan organ yang penting telah dipelihara di dalam cecair formalin 10%. Pada akhir tempoh eksperimen, kesemua tikus yang masih hidup telah dimatikan dan sampel darah, dan organ telah dikumpul untuk pemeriksaan serologi dan histologi.

Kesemua kumpulan rawatan menunjukkan kekurangan berat berbanding dengan kumpulan kawalan negatif dengan kumpulan yang dirawat dengan air kumbahan menunjukkan kekurangan berat yang ketara ($p<0.05$). Tikus di dalam kumpulan 1, 2 dan 3 tidak menunjukkan tanda-tanda klinikal yang ketara sepanjang tempoh eksperimen. 57% (n=4) tikus daripada kumpulan 4 menunjukkan tanda-tanda kekurusan yang sederhana dan bulu tidak rata yang ringan. Pada masa yang sama, kesemua tikus daripada kumpulan 5 (n=7) menunjukkan tanda-tanda dehidrasi, kekurusan dan bulu tidak rata yang teruk sebelum mereka dimatikan. Tiada perubahan ketara yang telah dapat diperhatikan secara kasar pada pada kesemua tikus-tikus daripada kumpulan 1, 2 dan 3. Hanya 57% (n=4) daripada tikus-tikus daripada kumpulan 4 menunjukkan tanda-tanda dehidrasi yang sederhana manakala, kesemua tikus-tikus daripada kumpulan 5 menunjukkan tanda-tanda dehidrasi yang teruk. 28.5% (n=2) tikus-tikus daripada kumpulan 2 dan 14.2% (n=1) tikus-tikus daripada kumpulan 4 telah menunjukkan abses pada lapisan subkutaneus. Beberapa jenis bakteria jenis koliform telah berjaya diasingkan dan dikenalpasti daripada abses-abses tersebut.

Hanya tikus-tikus daripada kumpulan 5 menunjukkan penurunan yang ketara ($p<0.05$) dalam konsentrasi serum hormon estrogen berbanding dengan kumpulan kawalan. Dari segi konsentrasi serum hormon progesteron, kesemua kumpulan rawatan menunjukkan penurunan berbanding dengan kumpulan kawalan tanpa apa-apa penurunan ketara.

Hanya kumpulan yang dirawat dengan air kumbahan menunjukkan kenaikkan yang ketara ($p<0.05$) di dalam konsentrasi serum Ig G berbanding dengan kumpulan kawalan. Kenaikan di dalam konsentrasi serum Ig M di dalam tikus-tikus daripada kumpulan 3, kumpulan 4 dan kumpulan 5 adalah ketara ($p<0.05$) berbanding dengan kumpulan kawalan. Kenaikan konsentrasi serum Il-12 di dalam tikus-tikus daripada kumpulan 4 and 5 adalah ketara ($p<0.05$) berbanding dengan kumpulan kawalan.

Kumpulan 3 dan 4 menunjukkan kenaikan yang ketara ($p<0.05$) di dalam konsentrasi serum Hp berbanding dengan kumpulan kawalan. Kumpulan rawatan 3, 4 dan 5 menunjukkan kenaikan yang ketara ($p<0.05$) di dalam konsentrasi serum SAA berbanding dengan kumpulan kawalan.

Analisa histopatologi telah mendedahkan yang hati dan buah pinggang daripada tikus-tikus daripada kumpulan 3 telah menunjukkan tanda-tanda keradangan dan degenerasi dari segi perubahan selular. Keradangan dan degenerasi yang teruk telah diperhatikan di dalam hati dan buah pinggang daripada tikus-tikus daripada kumpulan 4 dan 5 manakala keradangan yang sederhana telah diperhatikan di dalam uterus dan ovari daripada tikus-tikus daripada kumpulan 4 dan 5. Pada waktu yang sama, keradangan dan edema yang ringan thelah diperhatikan di dalam kumpulan rawatan 3, 4 dan 5.

Kesimpulannya, kajian ini telah membuktikan bahawa air minuman yang diberikan kepada lembu-lembu di kebanyakkan ladang-ladang lembu tenusu tidak mengikuti standard yang telah ditentukan oleh Piawaian Kualiti Air Kebangsaan Malaysia. Pada waktu yang sama, ketiadaan sistem rawatan air kumbahan sebelum pelepasan air kumbahan tersebut menyebabkan kualiti air kumbahan tersebut adalah sangat teruk dan ianya boleh menjadi sumber untuk pencemaran, terutamanya apabila ladang tersebut berada berdekatan dengan sumber bekalan air minuman mentah. Berdasarkan tanda-tanda klinikal, patologi dan serologi yang telah dipamerkan oleh tikus-tikus daripada kumpulan-kumpulan yang telah diberikan dengan air minuman yang berlainan kualiti daripada ladang-ladang lembu tenusu, mempunyai risiko yang tinggi untuk impak negatif dari segi produksi, reproduksi dan kesihatan di dalam lembu-lembu tenusu sekiranya pengunaan air minuman yang berkualiti rendah. Oleh itu, penilaian yang berterusan adalah perlu untuk memastikan bahawa kualiti air minuman yang dibekalkan kepada lembu-lembu di ladang lembu tenusu adalah sesuai dan air kumbahan daripada ladang-ladang ini dirawat dan dianalisa sebelum ianya dilepaskan ke persekitaran bagi mengelakkan pencemaran.

Kata kunci: Air minuman; air kumbahan; Piawaian Kualiti Air Kebangsaan Malaysia; ladang lembu tenusu, aspek veterinar, respon sel hos, model tikus

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Veterinary Science. The members of the Supervisory committee were as follows:

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LIST OF ABBREVIATIONS

ml	Mililiter
L	Liter
µl	Microliter
µg	Microgram
GAHP	Good Animal Husbandry Practices
SALT	Livestock Farm Accreditation Scheme
MyGAP	Malaysia Good Agricultural Practices
SALM	Good Agricultural Practices
SPLAM	Malaysia Aquaculture Farm Certification Scheme
DVS	Department of Veterinary Services
FAO	Food and Agriculture Organization of the United Nations
GAP	Good Animal Practices
CAP	Common Agricultural Policy
Il-12	Interleukin 12
Ig M	Immunoglobulin M
Ig G	Immunoglobulin G
Hp	Haptoglobin
SAA	Serum Amyloid A
DO	Dissolved Oxygen
BOD	Biological Oxygen Demand (BOD)
COD	Chemical Oxygen Demand
g	gram
TSS	Total Soluble Solid
TDS	Total Dissolved Solid

^{o}C	Degree Celsius
ELISA	Enzyme-linked immunosorbent assay
RIA	Radioimmunoassay
ppt	Parts per trillion
μS/m	Micro Siemens per meter
CFU	Colony forming unit
NTU	Nephelometric turbidity unit
ICP	Inductively coupled plasma

CHAPTER 1

INTRODUCTION

1.1 Background

Water makes up to 90% of all molecules in the body and about 70% of the live body weight. It is also considered to be the most important of all the nutrients as it is needed in the most regular basis and in the highest volume (Sejian, V *et al*, 2012). Domesticated animals can live about 60 days without food but they can only survive about 7 days without water (Church & Pond, 1974), therefore, it is advised for livestock to be given water in *ad libitum* as they can easily suffer from stress or dehydration due to lack of sufficient, clean drinking water (Sejian, V *et al*, 2012).

Physiologically, water plays an important role in the transport of nutrients between cells as well as a vital medium for intracellular metabolism (Gropper *et al.*, 2009). There were some studies carried out and had shown that there are positive correlation between regular access to clean drinking water and performance such as growth, reproduction and milk production (Ensley *et al.*, 2000; Schutz *et al*, 2012). Animals that has access to sufficient clean water are also usually less prone to illness and diseases due to reduced contact with pathogens and toxins in the water (Schutz *et al.*, 2012). Till date there is still no study was carried out in Malaysia to determine the quality of drinking water in ruminant livestock.

The common water sources in livestock farms are tap water, underground water, surface water such as lakes, ponds, rivers, swamps and drains as well as feed that is high in moisture such as green chop, pasture and silage (usgs.gov, 2005). Underground water typically contain less particulate matter such as leaves, soil and bugs as the grounds acts as a fine filteration mechanism as compared to the surface while on the other hand, it will also have a higher concentration of dissolved substances as the chemicals and minerals are leached off the ground as the water moves through it (usgs.gov, 2016). The natural composition of both ground water and surface water can be altered due to pollution through human activities, causing the infiltrations of contaminants such as pesticide, fertilizers, animal wastes and chemicals (Sasakova *et al.*, 2018). The quality of the tap water follows the standards of drinking water quality that is safe for human consumption as set by the various governments (moh.gov.my, 2004). Currently, there is a minimum drinking water quality that had been set by the National Water Quality Standards that is considered safe for livestock consumption in Malaysia (WEPA, 2006). This, in turn, raises a need to assess the quality of the ground water and surface water sources before they are deemed suitable for livestock consumption.

In the United Kingdom, United States and Canada, there are strict guidelines available for drinking water quality in livestock industry as well for sampling and testing guidelines (Higgins *et al*, 2008; Wright, 2007). This is because most cattle farmers used ground water and surface water sources as drinking water to their livestock without any treatment, raising a need to check for the suitability of the water sources for livestock consumption. . Treatment such as filtration, chlorination or reverse osmosis must be performed in cases of substandard drinking water sources before it is deemed safe for consumption (Qadir *et al*, 2007)

Wastewater is water that has been adversely affected in quality through anthropogenic influence that originates from domestic, industrial, commercial or agricultural activities, surface runoff or stormwater and from sewer inflow or infiltration (WHO, 2014). Water is also used extensively in most livestock farming for the purpose of cleaning, animal cooling and sometimes for manure transfer leading to the production of copious amount of wastewater (FAO, 2019). Water is also used extensively in most livestock farming for the purpose of cleaning and this wastewater has been released into the rivers, drains and lakes without any proper treatment. These practices will lead to environmental pollution and as a medium of disease transfer. The proximity of the wastewater to the drinking water sources may increase the chances of polluting the drinking water. Currently, there is a severe lack of information on the drinking water and wastewater quality in the ruminant livestock industry of Malaysia.

Oral inoculation of contaminated water into an animal model such as mice and rats had been done in many instances in order to determine the possible effects from the consumption of the water (O'Donnell *et al.,* 2015). The clinicopathological effects from drinking the different water qualities can be an important indicator for the possible effects that the animals might suffer from the consumption of these water samples. For host cell responses such as acute phase proteins, antibodies, proinflamatory cytokines and reproductive hormones due to the effect of water contaminants in animals has not been studied till date. Therefore this study was designed to determine the quality of drinking water and wastewater in selected dairy cattle farms and to elucidate the clinicopathological and host cell responses due to oral inoculations of different qualities drinking water and wastewater using animal models.

1.2 Problem Statement

Till date, there are still lack of study related to determining quality of drinking water and wastewater in livestock farming in Malaysia. Therefore, this study was designed to fill up the gap knowledge of water quality research in the ruminant livestock industry in Malaysia.

1.3 Objectives

The objectives of this study are:

1. To determine the water quality of the drinking water and the wastewater from selected dairy cattle farms.
2. To determine the clinical responses in mice inoculated with three different quality of water samples; namely good, moderate and unsatisfactory quality, from the assessment of objective (1) and a wastewater sample.
3. To determine the host cell responses in mice model from orally inoculated with three different quality of water samples; namely good, moderate and unsatisfactory quality, from the assessment of objective (1) and a wastewater sample focusing towards assessment of female reproductive hormones, acute phase protein and immunoglobulin concentration responses.

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