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MICROBAL DISTRIBUTION IN THE STOMACH OF CATTLE PROCESSED AND SOLD IN OIL PRODUCING ENVIRONMENT

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ABSTRACT

The components from the four stomach compartments of a ruminant animal(cattle) was assessed for microbal population present. Results from the laboratory analysis as observed in this research shows that various microbes namely bacteria and fungi were isolated with some having high frequency. These isolated microbes where identified using Gram stain and biochemical test methods of analysis and the result reveals presence of pathogenic microbes which includes; *Salmonella typhi*, *Salmonella enteridis*, *Enterobacteraerobacter*, *Salmonella typhimurim*, *Proteus mirabilis*, *Proteus vulgaris*, *Ctrobacterfreundis*, *Escherichia coli* and *Salmonella choleraesuis*. The presences of these pathogenic microbes infers that there is possible infection of human through consumption of improper processed and cooked intestine from ruminant animals with possible disease outbreak of typhoid due to the presence of *Salmonella typhi* bacterial. it is therefore of importance to consider practices that will eliminate some of this microbes before slaughtering of the animals to avoid food poising.

KEYWORD: The components *Ctrobacterfreundis*, *Escherichia coli* and *Salmonella choleraesuis*.

INTRODUCTION

Mammals, being the most developed organisms, possess highly sophisticated digestive system to feed on a variety of food types available in the world. Ruminants are mammals that are able to acquire nutrient from plant-based food by fermenting it in a specialized stomach prior to digestion, principally through bacteria and fungi actions. Ruminants pose a high level of importance for the mammalian and to the entire biosphere. They are animal protein source

and FAO recommends that every living being must consume an average of 58-65g of animal protein source per day, therefore making it as a must do condition to meet up protein intake. This has translated to high demands for animal protein source among which are ruminant animal such as the cattle, sheep and goat. Animal protein sources are in varied form such as our local diet (pepper soup) among others. The fact also exists that lot of cases of food poisoning has been recorded for consumption of these local diets.

The stomach of these animals contains high level of microbes which help in digestion and most of these microbes could be pathogenic to humans. Several cases of food poisoning recorded gives the indication that there is possible cross contamination of humans by microbes in the intestine of animals slaughtered for meat. There fore, there is an urgent need considering the level of sanitation standard in most of our slaughtering house and the so called abattoirs operated across the country. Hence, this research is geared towards identifying the possible microbes in the digestive tract of the ruminant animals (cattle) and where possible pathogenic ones are observed then there will be urgent need to treat the animals three weeks before slaughtering in other to break the link of infection between man and his consumable meat.

AIMS AND OBJECTIVES

- To assess the population of microbes present in the stomach of ruminant animals.
- To identify these microbes.
- To evaluate the possible diseases these microbes can cause to human.
- Possible ways of reducing the population of these microbes thereby reducing ability to cause disease condition in humans.

MATERIALS AND METHODS

Faecal Samples from the four stomach, petridish, test tubes, weighing balance, spatula, fail paper, cotton wool, sterile bottles, wire loop, laboratory lamp, pressure pot and stove.

Agars

Mac conkey, Agar, Nutrient Agar, Tripple sugar ion Agar, potato Dextrose Agar.

TREATMENT OF THE ANIMALS BEFORE COLLECTION OF THE SAMPLE.

- Kill the animal without roasting.
- Open up the stomach.

- Remove the intestine.
- Place on plate table.
- Identify the four stomachcompartments.
- Internal content from the four compartments were taken and sent for lab analysis.

PROCEDURE FOR ISOLATION AND IDENTIFICATION OF BACTERIA IN THE SAMPLES COLLECTED

FAECAL SAMPLING AND PROCESSING

Rectal Sample of faecal material (from the four stomach compartment) were taken manually with a glove. Faecal material was placed in labelled sterile bottles, closed tightly to exclude air, and kneaded for a few minutes to mix the digesta. Samples were brought to the laboratory and immediately processed into a dilution series for plating. 1g(wet weight) samples of faeces were placed in a test tube containing 9ml of sterile dilution solution. The test tube was shaken well to mix the sample and sterile water. A sub-sample of the solution was serially diluted further in another dilution solution and aliquites (0.5ml of 10^{-1}- 10^{-1})were used toinoculate various media.

CULTURE MEDIA

33.05g of MacConkey was measured using weighting beam and dissolved in 600ml of water. The solution was sterilised using pressure pot and stove for 1 hour 100^{o}c. After sterilising the MacConkey Agar, pour plate was done for each of the diluted sample (0.5ml of each solution was used). Same was done for nutrient Agar using 16.8g .The plates were inverted and sealed to avoid contaminations and was incubated at 37^{o}c for 24 hours. Characteristic colonies were picked from the plates and purified by repeated sub culturing. Pure colonies were streaked on nutrient agar slant in McCartney bottles as stock cultures for antimicrobial activity.

Sub-culturing and streaking

Sub-culturing was carried out as agar slant using McCartney bottles. 3ml of the nutrient agar was poured in the bottles and slanted to solidify .Wire loop was sterilized and used to pick the different colonies from the isolated cultures. Inoculums from these cultures were streaked on the agar slants and the streaked agar bottles were then incubated at 37^{o}C for 24hrs. During the streaking, the environment was kept sterile using the flame. The wireloop used for streaking was sterilized at every interval to avoid cross contamination

COLONY COUNTS

Microscopic bacteria counts were done on each plate between 24 – 48 hours of incubation at 37^{o}c.

GRAM STAINING

Gram staining procedure was used to identify the isolated organisms. Heat fix smear of the organism was prepared on a glass slides, the glass slide was placed on the staining rack. The smears was flooded with crystal violet for 30 seconds and rinsed with water, covered with gram's iodine mordant for 15 seconds and rinsed with water.95% of ethanol was used to decolourized for 15 seconds and then rinsed with water. Saffranin was used as a counter stain for 30seconds then rinsed with water for 5seconds.Blot dry with air dry and then examine under a light microscope using oil immersion lens at 100x magnification.it was observed that the bacteria retained the pink colour of the counter stain, safranine. Hence, they were Gramm negative bacteria.

BIOCHEMICAL TEST

Triple Sugar Ion test.

This test is done to determine if the bacteria can ferment glucose, lactose, sucrose and check if hydrogen sulphide is produced in the process.64.5g of TSI was dissolved in 1Liter of distilled water warmed to dissolve and was mixed properly and poured into tubes sterilised using pressure pot and stove for 1hour at 100^{o}c.The medium was set in slant form allowed to cool and solidify the test organisms were then inoculated in it. A positive result is when there is change in the colour of the media in responds to fermentation. This procedure was followed for all isolates and results were recorded.

Catalase Test

The aim of this test is to distinguish those bacteria that produce the enzymes catalase from those that don't. The Sub Cultured organism from the different colony was picked from the petri dish using a wire loop and placed on a glass slide. The cell was mixed with a drop of 3% Hydrogen Peroxide (H_2O_2) and observed for the effervescence caused by evolution of oxygen as gas bubbles. This procedure was followed for all the isolates and the results were recorded.

METHYL RED (M.R) TEST

This test is used to determine if the bacteria can convert glucose to acidic products like lactate, acetate and formate. An isolat is inoculated into a broth tube with a sterile transfer

loop. The tube is incubated at 37^{o}C for 2days. After incubation 2.5ml of the medium was transferred to another test tube, five drops of the P^{H} indicator Methyl Red is added to the tube. The tube is gently rolled between the palms to disperse the M.R Test and observed for reaction. A red colour indicate a positive result. This procedure was followed for all the isolate and results were recorded.

IND0LE TEST

This test is used to identify bacteria which express the enzyme tryptophanase can hydrolyze the amino acid tryptophan to indole, pyruvic acid and ammonia. Aseptically, the labelled broth culture tubes with the test bacteria was inoculated and incubated for 24hours at 370c.0.5ml of Kavoc's reagent was added to the tubes and shaken gently. Positive results is when the indole reagent change colour to cerise red. This procedure was followed for all the isolates and the results were recorded.

OXIDASE TEST

The aim of the test was to differentiate organisms that produce certain cytochrome c oxidase from those that do not. Two drops of the oxidase reagent was applied onto piece of filter paper, bacteria from one colony with wire loop was transfer onto the spot with the oxidase reagent and observed for reaction. The formation of deep purple colour within 5-10 secs .indicated the positivity of the test organism. This procedure was followed for all the isolates and the results were recorded.

IDENTIFICATION OF FUNGI

Materials and method

PDA, petri dish, wire loop, spatula and weighing balance.

Procedure

3.9g of potato dextrose agar was measured using measuring balance and dissolved in 100ml of water.2g of antibiotic (gentamycin) was added to the solution before sterilization. After sterilization pour plate method was done from the dilution of sample. The petri dish was inverted and incubated at 27oc on work bench for 5days.

Identification

A drop of lacto phenol cotton blue was placed on the slide and a smear was made on the slide using sterile inoculating loop. Glass cover slipwas placed on the smeared potion of the slide.

Bubbles was avoided by carefully placing the cover slip. The edge of the slide was cleaned from excess phenol cotton blue stain and was masked on the microscope to view. The viewed fungus was identified by comparing with fungi atlas.

ANTIMICROBIAL ACTIVITY OF ISOLATED BACTERIA

The diffusion method was employed to test the antibacterial activity of the isolated organisms. The bacteria were cultured on nutrient agar prior to transfer into nutrient broth, a loopful of culturewas picked from the nutrient agar culture and inoculated into nutrient broth medium and incubated for 24hour at 37^0C. The cell suspension were transferred into sterile petri dishes aseptically, i.e 0.1ml of cell suspensions, and 15ml of Mueller Hinton nutrient agar was poured into the same places. This was allowed to gel and dry. Asterile forcep was used to pick antimicrobial disc and placed on the middle of the plate. This procedure was done for each isolate and the results were recorded.

RESULTS

Table 1a: Colony counts from the different four stomach compartment.

Dillutions	Rumen	Reticulem	Omasum	Abomasum
10^{-1}	TNTC	TNTC	TNTC	TNTC
10^{-2}	TNTC	TNTC	TNTC	162
10^{-3}	TNTC	TNTC	TNTC	62
10^{-4}	TNTC	TNTC	TNTC	37
10^{-5}	TNTC	TNTC	240	28
10^{-6}	246	TNTC	140	25
10^{-7}	154	TNTC	109	21 TFTC
10^{-8}	91	250	59	16 TFTC
10^{-9}	70	109	40	12 TFTC
10^{-10}	24 TFTC	106	23 TNTC	10 TFTC

TNTC; Too numerous to count.

TFTC; Too few to count.

Results of colony counts in the four stomach compartment in table 1a above, reveals high presence of microbes in the entire four compartments (Rumen, Reticulum, Omasum, Abomasum) of ruminates (cattle) with 10^{-1}-10^{-5}; 10^{-1}-10^{-7}; 10^{-1}-10^{-4} and 10^{-1} dilutions respectively. The distribution as shown implies more microbes in the Reticulum compartment as compared to Abomasum Showing TNTC counts at 10^{-1} dilution only.

Table: 1b Conversion table from colony count to percentage.

Percentage	Range
10	0-24(TFTC)

20	25-52.25
30	53.25-80.50
40	81.50-108.75
50	109.75-137.00
60	138.00-165.50
70	166.25-193.50
80	194.50-221.75
90	222.75-250.00
100	251.00-above(TNTC)

Table 1c: Table showing values in percentage of colony counts.

Dilution	Rumen	Reticulum	Omasum	Abomasum
10^{-1}	100	100	100	100
10^{-2}	100	100	100	60
10^{-3}	100	100	100	30
10^{-4}	100	100	100	20
10^{-5}	100	100	90	20
10^{-6}	90	100	60	20
10^{-7}	60	100	50	10
10^{-8}	40	90	30	10
10^{-9}	30	50	20	10
10^{-10}	10	40	10	10

Table 2a: Result of biochemical tests(triple sugar ion, indole, methyl red, catalyse, Gram stain, oxidase) as a confirmatory test.

Colonies	Butt	Slant	Gas	H_2s	Indole	M.R	Catalys	Gram stain	oxidase
1	Black	Red	_	+	_	+	+	_	_
2	Yellow	Yellow	+	_	+	+	+	_	_
3	Black	Red	+	+	_	+	+	_	_
4	Yellow	Red	_	_	_	_	+	_	_
5	Yellow	Yellow	+	_	_	_	+	_	_
6	Yellow	Red	+	_	_	_	+	_	_
7	Yellow/red	Red/yellow	+	_	_	+	+	_	_
8	Yellow/black	Black	+	+	_	+	+	_	_
9	Black	Red/yellow	+	+	+	+	+	_	_
10	Yellow	Yellow/red	+	_	_	+	+	_	_
11	Black	Yellow/red	+	+	_	+		_	_

Table2b: Showing colonies and probable organisms.

Colonies	Probable organisms
1	Salmonella typi
2	Escherichia coli
3	Salmonella enteridis
4	Shigellasonnei
5	Enterobacteraerobacter
6	Klebsiellapneumonia

7	Proteus mirabilis
8	Citrobacterfreundii
9	Proteus vulgaris
10	Salmonella choleraesuis
11	Salmonella typhimurim

Table 2aAS shown above represent results of biochemical test of each colonies as a confirmatory analysis test. it was observed that the organisms present in table 2b were the probableorganisms in each colony.

Table 3: Results from antimicrobial test (diffusion disc method) for some of the organisms, showing zone of inhibition in mm.

Antibiotics	*S.typhi*	*P.vulgaris*	*C.freundis*	*E.coil*	S.choleraesuis
SXT	R	15	15	20	R
CH	R	15(S)	20(S)	10(S)	R
SP	R	12	10	R	10
CPX	30(S)	20(S)	20(S)	10(S)	10(S)
AM	20(S)	15(S)	R	10(S)	10(S)
AU	17	15	R	10	17
CN	10(S)	18(S)	R	15(S)	R
PEF	30(S)	30(S)	R	30(S)	10(S)
OPX	20(S)	12(S0	R	10(S)	R
S	R	10	R	10	R

Antibiotics	*S.enteridis*	*E.aerobacter*	*S.typhimurim*	*P.mirabilis*
SXT	20	30	R	15
CH	20(S)	30(S)	R	20(S)
SP	10	30	R	20
CPX	30(S)	30(S)	R	10(S)
AM	10(S)	30(S)	R	10(S)
AU	10	25	R	10
CN	15(S)	20(S)	R	20(S)
PEF	30(S)	30(S)	R	30(S)
OPX	20(S)	30(S)	R	20(S)
S	20	30	R	20

Where;

R=Resistance

S=Sensitive

SXT=Septrin, CH=Chloramphenicol, SP=Sparflxacin, AM=Amoxacillin, AU=Augumentin, CN=Gentamycin, PEF=Pefloxacin, OFX=Tarivid and S=Streptomycin.

Table 3 shows result of antimicrobial test done on the following organisms which include; *Salmonellatyphi, Salmonella enteridis, Enterobacteraerobacter, Salmonella typhimurim, Proteus mirabilis, Proteus vulgaris, Ctrobacterfreundis, Escherichia coli*and *Salmonella choleraesuis,* and their zone of inhibition measured in milimeter (mm)..

Table 4: Shows isolated organisms,possible pathogenic effect and antimicrobial therapy agent.

BACTERIAL

Organisms	**Diseases**	**Antimicrobial agent**
Salmonella typhi	Enteric fever(typhoid)	Chloramphenicol,ceftriaxone.
Escherichia coli	Food poisoning	Azithromycin, ciprpfloxacin.
Salmonella enteridis	Food borne-illness	Ciprofloxacin, polymycin B.
Shigellasonnei	Dysentery	Trimethoprim sulfumethoxazole, ciprofloxacine.
Enterobacteraerobacter	Nocosomal and opportunistic infections	Fluoroquinolones, thrimethoprimsulfumethoxazole.
Klebsiellapneumoniae	Pneumonia	Aminoglycoside, carbapenems.
Proteus mirabilis	Urinary tract infection	Amoxicillin, polymycin B.
Citrobacterfreundii	Urinary tract and wound infection	Ciprofloxacin, ceftazidine.
Proteus vulgaris	Nosocomial infections	Thrd generation cephalosporin.
Salmonella choleraesuis	Spectima, acute enteritis	Ampicillin, trimethoprimsulfumethoxazole.
Salmonella typhimurim	Gastro enteritis	Third generation cephalosporin.

FUNGI

Organism	**Disease**	**Antimicrobial agent**
Candidiaalbican	Thrusth,vaginal infections	Fluconazole, itraconazole,amphotericin B
Microscoporus	Fungal infections of the skin	Itraconazole, griseofulvin.

DISCUSSION AND CONCLUSION

From the results, table1a shows that the stomach of ruminant animals has high population of microbes and it was observed that the reticulum and rumen has the highest population as shown in fig 4 which is in agreement with Williams et al (2010) who stated that "the reticulorumen is home to a population of microorganisms" thatinclude; bacteria, protozoa and fungi. It was observed that Bacteriahas the highest population compared to other microbes. Fungi has the least population in the four compartment, this could be attributable to the high P^{H}content of the stomach. Literature reveals that fungi survive more in low $P^{H.}$ The abomasum which is the "true stomach" has the lowest population of microbes, it produces hydrochloric acid making the environment more acidic for microbes as stated by Dijkatra, J

et al (2005). These microbes exist together in the stomach of the ruminant animals, ferment and breakdown plant cell walls into their carbohydrate fractions and produce volatile fatty acids such as acetate (used for fat synthesis), propionate (used for glucose synthesis) and butyrate from these carbohydrates. It was observed that the bacterial present in the stomach were Gamm negative bacteria under the family of Enterobacteriacae.

In conclusion, In a slaughter house or abattoir were the processing of these animals is not carried out in a hygiene or aseptic condition, the contain of the stomach which contain these microbes will be mixed with the meat and other part of the animal. This single act will make these microbes to gain access into the meat. When the meat or other parts of the animal is not properly cooked and consumed by humans, these microbes become pathogenic and cause disease to human. This is the reason why someone can eat a bowl of pepper soup and comedown with typhoid fever beause, pepper soup is prepared mostly from offals which is the intestines and other internal part of the stomach which is the main house of these microbes.

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