Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN: 0976-3376

Asian Journal of Science and Technology
Vol. 08, Issue, 12, pp.7099-7103, December, 2017

RESEARCH ARTICLE

BIOTYPING AND PREVALENCE OF RESPIRATORY AFFECTIONS IN GOATS

***Aulwar, B. S., Gangane, G.R., Birajdar, B.W., Moregaonkar, S.D., Suryawanshi, P. R. and Bhonsle, A.V.**

[1]Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Parbhani, Maharashtra Animal and Fishery sciences University, near futala lake, Nagpur, Maharashtra, India
[2]Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Udgir, Maharashtra Animal and Fishery sciences University, near futala lake, Nagpur, Maharashtra, India

ARTICLE INFO

Article History:
Received 11th September, 2017
Received in revised form
26th October, 2017
Accepted 09th November, 2017
Published online 30th December, 2017

Key words:

Prevalence, bacterial
Species identification by
MALDI-TOF, Age,
Genotype, goats.

ABSTRACT

The aim of this study was to investigate the respiratory affections in goats with special reference to bacterial infections through bacteriological techniques and MALDI-TOF. A total of 192 swabs from trachea and inner core of lung were collected aseptically. The generated data was basically pertaining to 60 died goats and 154 slaughtered goats which were screened during present study by bacteriological techniques and MALDI-TOF. After thorough post mortem examination of screened carcasses of goats, 192 amongst 214 were with either or mixed type of gross lesion targeting either or multiple organs of respiratory system. The overall prevalence of respiratory affections in screened goats having gross respiratory affections was found to be 89.72%. *Staphylococcus* species found to be highly prevalent than other bacterial Species recorded. The overall prevalence of respiratory affections in screened goats was found to be 89.72%. The goats aging 1 year showed 96.91% prevalence and goats ageing more than 1 year were with 71.92% prevalence of respiratory affections. *Staphylococcus* species found to be highly prevalent than other bacterial Species.

INTRODUCTION

India ranks second in goat population having 135.2 million goats (2012 Livestock census). Small ruminants assumes important position in livestock production. Unlike Cattle, small ruminants are capable of remarkable adaptability to diverse environmental conditions and are amenable ease of management (Amaravathi *et al.,* 2016). Respiratory diseases of small ruminants are multifactorial and there are multiple etiological agents responsible for the respiratory disease complex. Out of them, bacterial diseases have drawn attention due to variable clinical manifestations, severity of diseases, and reemergence of strains resistant to a number of chemotherapeutic agents. The respiratory diseases represent 5.6 per cent of all diseases in small ruminants (Chakraborty *et al.,* 2014). A number of pathogenic microorganisms have been implicated in the development of respiratory disease but the importance of environmental factors in the initiation and progress of disease can never be over emphasized. They irritate the respiratory tree producing stress in the microenvironment causing a decline in the immune status of the small ruminants and there by assisting bacterial, viral and parasitic infections to break down the tissue defense barriers (Rahal *et al.,*2014).

****Corresponding author:*** **Aulwar, B. S.,**
Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Parbhani, Maharashtra Animal and Fishery sciences University, near futala lake, Nagpur, Maharashtra, India

MATERIALS AND METHODS

Materials used: Tracheal, lung swabs, culture media, bacteriological reagents, Sugars, bacteriological media.

Screening of goat carcasses: The local slaughter places of goats in and around Parbhani and those goats died and presented to Department of Veterinary Pathology were screened during study period for noting respiratory affections with special reference to bacterial infections. Also, the slaughtered goats having grossly visible respiratory affection were also targeted for noting the prevalence of bacterial infection.

Collection of samples: The tracheal, lung swabs from goats suspected for respiratory affections were collected from carcasses of died and slaughtered goats in and around Parbhani. After aspect collection of these swabs, the same were subjected for isolation and identification studies and also an attempt was made for its biotyping.

Isolation and identification of bacteria: A total of 192 swabs were collected from trachea and inner core of lung aseptically. All the samples were transferred to Bacteriology Laboratory of Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, MAFSU, Parbhani for isolation and identification of bacteria. The representative samples were for further bacterial genes, species confirmation subjected at (Rapid Diseases Diagnostic Centre For Small Ruminants, College of Veterinary and Animal Sciences, MAFSU, Udgir, Dist Latur/ MS). The broth cultured samples were incubated aerobically agitated thoroughly and mixed before overnight incubation. A loopful of broth culture was taken for streaking over an identified petridish plates containing nutrient agar and brain heart infusion agar. The remaining samples in the test tube were put as sample pool source inside a refrigerator at 4 ˚c till complete investigation process. From culture positive plates, representative colonies were further streaked on MacConkey agar. Isolation and identification of bacteria was done based on staining, colony characteristic, biochemical tests & Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) (Quinn P.J. 2006).

Considering the prevalence of respiratory affections in goats, the study on biotyping in respect of bacteria were conducted. The biotyping was attempted by employing bacterialogical techniques and representative samplrs were confirmed by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS).

Bacterial confirmation through Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (Maldi-Tof Ms):

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) has recently emerged as a powerful technique for identification of microorganisms, changing the workflow of well-established laboratories so that its impact on microbiological diagnostics has been unparalleled (De Carolis 2014).

- A small amount of bacteria was applied to the MALDI plate in a thin film as a direct method.
- *Ethanol Formic Acid Extraction:* A colony of bacteria was resuspended in 300 µl (Microliter) of water. Then, 900 µl of absolute ethanol was added to it. The mixture was centrifuged at 15,500 × *g* for 2 min, and the supernatant was discarded. Subsequently, 50 µl of formic acid (70% [vol/vol]) was added to the colony and mixed thoroughly by pipetting before the addition of 50 µl acetonitrile to the mixture. The mixture was centrifuged again at 15,500 × *g* for 2 min. One microliter of the supernatant was placed onto a spot of the steel target and air dried at room temperature.

Both the positive standard and the test supernatant were overlaid with 1 µl of matrix solution (saturated solution of HCCA [α-cyano-4-hydroxy cinnamic acid] in organic solvent [50% acetonitrile and 2.5% trifluoroacetic acid]) and air dried.
iii. MALDI-TOF Mass Spectrometry: 1 Measurements were performed on an Microflex LT MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Leipzig, Germany) equipped with a Smart beam laser. Spectra were recorded in the linear, positive mode at a laser frequency of 200 Hz (Hertz) within a mass range from 2,000 to 20,000 Da (Dalton). The IS1 (ionic state), IS2 and lens voltage were maintained at 20 kV (kilovolt), 18.6 kv and 6 kV, respectively whereas the extraction delay time was 40 ns (nanosecond) (Bruker Daltonic 2016). For each spectrum, 40 laser shots were collected and analyzed (10 × 50 laser shots from different positions of the target spot). The spectra were calibrated externally using the standard calibrant mixture (*Escherichia coli* extracts including the additional proteins RNase A and myoglobin; Bruker Daltonics). The calibration masses were as follows: RL36, 4,364.3 Da; RS22, 5,095.8 Da; RL34, 5,380.4 Da; RL33meth, 6,254.4 Da; RL32, 6,315 Da; RL29, 7,273.5 Da; RS19, 10,299.1 Da; RNase A, 13,682.2 Da; and myoglobin, 16,952.5 Da.

Data Analysis

For automated data analysis, raw spectra were processed using the MALDI Biotyper OC 3.1.66 software (Bruker Daltonics, Leipzig, Germany) at default settings. The software performed normalization, smoothing, baseline subtraction, and peak picking and created a list of the most significant peaks of the spectrum (*m/z* values with a given intensity, with the threshold set to a minimum of 1% of the highest peak and a maximum of 100 peaks). To identify unknown bacteria, each peak list generated was matched directly against reference libraries (4,623 species) using the integrated pattern-matching algorithm of the Biotyper OC 3.1.66 software (Bruker Daltonics, GmbH, Germany). The unknown spectra were compared with a library of reference spectra based on a pattern recognition algorithm using peak position, peak intensity distributions, and peak frequencies. A comparison between *Klebsiella Pneumonia* isolated from pneumonic cases in bovines and *Klebsiella* profile included in the Biotyper database for comparison purposes. Once a spectrum was generated and captured by the software, the whole identification process was performed automatically, without any user intervention (Bruker Daltonic 2016).

Statistical Analysis

The data generated during present study was analysed by employing chi-square test (Snedecor G. M., and Cochran W.C. 1982).

RESULTS AND DISCUSSION

Overall prevalence of respiratory affections: During study period, 214 carcasses of varying aged goats were screened. Amongst those, 154 goat carcasses were screened from different local slaughter places available in and around Parbhani and 60 goat carcasses which were presented to Department of Veterinary Pathology, COVAS, Parbhani for conduct of post mortem examination during study period were examined. After thorough post mortem examination of screened carcasses of goats, 192 amongst 214 were with either or mixed type of gross lesion targeting either or multiple organs of respiratory system. The overall prevalence of respiratory affections in screened goats was found to be 89.72%. The respiratory system is consistently injured due to constant exposure to microbes particle fibers, pollutant, toxic gasses, vapors etc. present in the air. Also, the immune compromization due to managemental, productive and reproductive stress might be making the animal susceptible to injuries. This all could be making the respiratory system very much vulnerable to pathogens leading to high prevalence of respiratory affections. *Age wise prevalence of respiratory*

affections in goats: Table no.1 and 2 shows age wise prevalence of respiratory affections in goats. The data generated in respect of respiratory affections in goats was subjected to analyze it for age wise prevalence. This data was basically pertaining to 60 died goats and 154 slaughtered goats which were screened during present study. Amongst 60 goats, which were died and presented to Department of Veterinary Pathology, COVAS, Parbhani, 35 carcasses were of goats aging up to 1 year and 25 were of more than 1 year age. These carcasses were when screened for noting the gross respiratory affections revealed 97.14% prevalence in goats aging up to 1 year and this percent prevalence of respiratory affections in goats aging more than 1 year was 92.00%. The age wise prevalence of respiratory affections in slaughtered as well died goats didn't showed significant variation on its statistical analysis.

Prevalence of bacterial infections in lungs of goats: *Isolation and identification of bacteria through bacteriological techniques*: Table no 3 and 4 shows prevalence of bacteria isolated from lungs and trachea of screened goats. These bacteria were isolated by standard bacteriological technique. Amongst 214 goats screened, 192 goats were with either or mixed gross lesions. To know the prevalence of bacteria, 144 swabs from lungs and 48 swabs from trachea were collected and subjected to bacterial isolation and identification by following standard bacteriological techniques. Amongst 144 swabs collected from lungs, 137 swabs were examined through standard bacteriological techniques; however, 7 representative lung swabs were subjected to the MALDI-TOF-MS for confirmative diagnosis. On bacteriological studies of 137 lung swabs, *staphylococcus* species was isolated from 42 swabs showing 29.16% prevalence which was highest amongst all

Table 1. Age wise prevalence of respiratory affections in slaughtered goats

Category	No of goats screened	Positive	Percentage	X^2 Table			Significance
				X^2	1%	5%	
Goats up to 1 year	97	94	96.91	0.9462	6.64	3.84	Non-significant
Goats above 1 year	57	41	71.92	1.6069			
Total	154	135	87.66	2.5531			

Table 2. Age wise prevalence of respiratory affections in died goats

Category	No of goats screened	Positive	Percentage	X^2 Table			Significance
				X^2	1%	5%	
Goats up to 1 year	35	34	97.14	0.0169			
Goats above 1 year	25	23	92.00	0.023			
Total	60	57	95.00	0.0399	6.64	3.84	Non-significant

Table 3. Prevalence of bacteria isolated from lungs of screened goats

Sr. No	Isolated bacteria	No. of goats with gross lesion in lung	Positive	Percentage
1	*Staphylococci*	144	42	29.16
2	*E. coli*		32	22.22
3	*Bacilli*		32	22.22
4	*Proteus*		31	21.52
	Total	144	137	95.12

Table 4. Prevalence of bacteria isolated from trachea of screened goats

Sr. No	Isolated bacteria	No. of goats with gross lesion in trachea	Positive	Percentage
1	*Staphylococci*		10	20.83
2	*E. coli*		08	16.66
3	*Bacilli*		10	20.83
4	*Proteus*	48	10	20.83
	Total	48	38	79.15

In toto, 154 goats slaughtered at local slaughter houses in and around Parbhani were screened during present study. Amongst those, 97 goats were aging up to 1 year and 57 were of more than 1 year age. The goats aging 1 year showed 96.91% prevalence and goats above 1 year of age were with 71.92% prevalence of respiratory affections. Amongst 60 goats, which were died and presented to Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Parbhani, 35 carcasses were goats aging up to 1 year and 25 were of more than 1 year age. These carcasses when screened for noting the gross respiratory affections revealed 97.14% prevalence in goats aging up to 1 year and this percent prevalence of respiratory affections in goats aging more than 1 year was 92.00%. The age wise prevalence of respiratory affections in slaughtered as well died goats didn't showed significant variation on its statistical analysis.

bacteria species isolated. It was followed by *E. coli* (22.22%), *Bacillus* species (22.22%) and *Proteus* species (21.52%). *Staphylococcus* species and *E. coli* were confirmed biochemically by employing Catalase test and MR-VP test respectively. The forty eight tracheal swabs were collected from slaughtered and died goats which were with either or mixed type of gross lesions in respiratory tract. These were further subjected for isolation and identification of bacteria. The isolation and identification studies of bacteria recovered 20.83% prevalence of *staphylococci* species, followed by *Bacillus* species and *Proteus* species 20.83% each. However, *E. coli* were recovered from 16.66% tracheal swabs.

Isolation and identification of bacteria through MALDI-TOF-MS:

Table 5 indicates details of bacterial isolates recovered from tracheal and lung swabs of goats

Table 5. Bacteria isolated from lungs and trachea of goats by MALDI-TOF-MS

Analyte name	Analyte ID	Organism (Best match)	Score Value
C2 (+) (B)	1	*Staphylococcuschromogenes*	1.7
C3 (+) (B)	2	*Corynebacteriumefficiens*	1.7
C4 (++) (A)	3	*Acinetobacterpittii*	2.053
C5 (+++) (A)	4	*Escherichia coli*	2.381
C6 (+) (B)	5	*Alcalgenesfaecalis*	1.743
C7 (+) (B)	6	*Bacillus clausii*	1.72
C8 (++) (A)	7	*Escherichia coli*	2.243
C9 (+) (B)	8	*Corynebacteriumefficiens*	1.7
C10 (+) (B)	9	*Proteus hauseri*	1.991
C11 (+) (B)	10	*Bacillus cereus*	1.806
C12 (+) (B)	11	*Enterobacter cloacae and Enterobacterludwigii*	1.999
C13 (+) (B)	12	*Staphylococcus aureus*	1.7
C14 (++) (A)	13	*Comamonaskerstersii*	2.119
C15 (+) (B)	14	*Enterobacter cloacae*	1.94
C16 (+) (B)	15	*Staphylococcus sciuri*	1.7
C17 (++) (A)	16	*Enterococcuscasseliflavus*	2.097
C18 (+) (B)	17	*Lysinibacillusfusiformis*	1.99

Below figures showing mass spectra of bacteria those were isolated by MALDI-TOF MS:

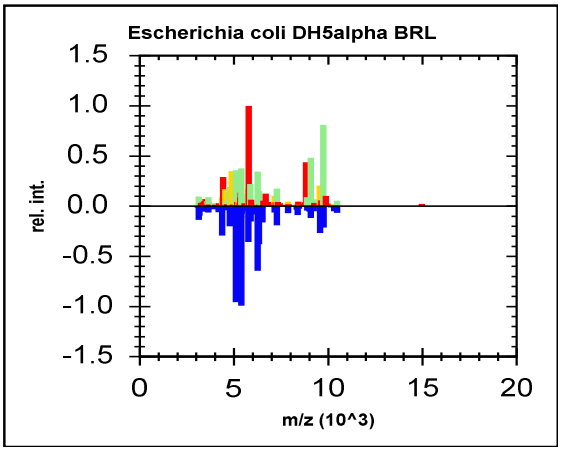

Fig. 1. ***Escherichia coli***

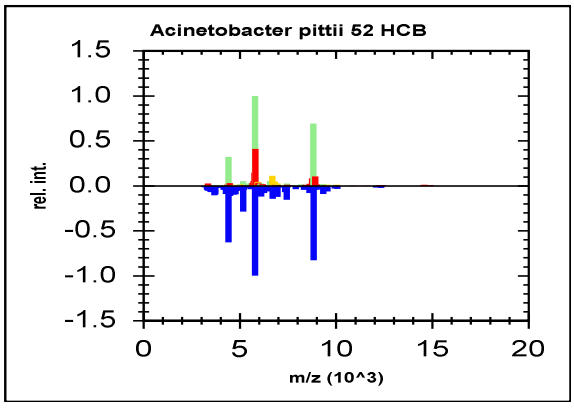

Fig. 2. ***Acinetobacter pittii***

In toto, 17 representative swabs (10 tracheal and 07 lung swabs) were subjected to MALDI-TOF-MS analysis for confirmation. Amongst those, 1 tracheal swabs showed mixed type of bacterial infection and rest 9 samples showed individual bacteria as mentioned in the table no. 5. However, 7 lung swabs on its screening through

MALDI-TOF-MS revealed two mixed type of bacterial infections and rest 5 samples showed prevalence of individual bacteria as shown in Table no 5.

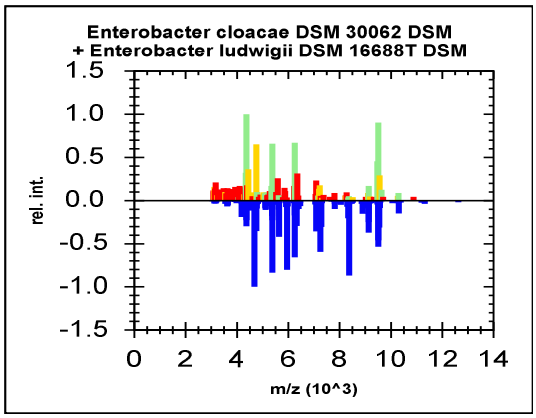

Fig. 3. ***Enterobacter cloacae + Enterobacter ludwigii***

Note: Meaning of Score Values:

Range	Description	Symbols	Color
2.300 ... 3.000	Highly probable species identification	(+++)	Green
2.000 ... 2.299	Secure genus identification, probable species identification	(++)	Green
1.700 ... 1.999	Probable genus identification	(+)	Yellow
0.000 ... 1.699	Not reliable identification	(-)	Red

Note: Meaning of Consistency Categories (A - C):

Category	Description
A	Species Consistency: The best match was classified as 'green'. Further 'green' matches are of the same species as the first one. Further 'yellow' matches are at least of the same genus as the first one.
B	Genus Consistency: The best match was classified as 'green' or 'yellow'. Further, 'green' or 'yellow' matches have at least the same genus as the first one. The conditions of species consistency are not fulfilled.
C	No Consistency: Neither species nor genus consistency (Please check for synonyms of names or microbial mixture).

The findings noted in respect of isolation and identification of bacteria by MALDI-TOF-MS are in consonance with the earlier reports of Ronald *et al.* (2004), Deborah *et al.* (2011) and De Carolis *et al.* (2014). These researchers recorded variable percent prevalence of bacteria isolated by the using MALDI-TOF-MS. Ronald *et al.*(2004) reported *Klebsiella pneumoniae* as major isolate followed by *Mannheimia haemolytica*, *Escherichia coli, Entero bacteraerogenes and Streptococcus* species from Bovine Respiratory Diseases. MALDI-TOF MS has successfully been used for the identification of a wide array of bacterial and fungal species Kumar *et al.*(2004). Earlier studies have recorded the use of MALDI TOF MS as a useful and rapid tool for identification of bacteria from the cultures especially when a good protein profile database allows a comparison of the profiles obtained with a large number of bacterial species and strains. Other studies have described an excellent correlation between MALDI-TOF MS identification and conventional microbiological identification in clinical bacterial isolates. In some cases, there are discrepancies between MALDI-TOF MS and conventional identification, as sometimes happens for the bacterial isolates specifically for *Streptococcus* species. MALDI-TOF MS seems to require high bacterial counts to be able to provide reliable scores. The result agrees with the results for the clinical samples, since only one out of thirty samples with bacterial count less than 5×10^5 CFU/ml,could

not form the peaks and reliable MALDI-TOF profiles within the specified incubation period.

Conclusion

The results presented in this paper and past studies emphasize the utility of MALDI-TOF MS -based biotyping and prevalence of respiratory affections in goats. The overall prevalence of respiratory affections in screened goats was found to be 89.72%. The goats aging 1 year showed 96.91% prevalence and goats ageing more than 1 year were with 71.92% prevalence of respiratory affections. *Staphylococcus* species found to be highly prevalent than other bacterial species. MALDI-TOF MS is quick and reliable method for the identification of bacteria.

Authors' Contributions

ABS prepared the study design and carried out the research under the supervision and guidence of GRG. SDM suppurated during completion of research findings. ABS collected the samples and executed the isolation. ABS and AVB conducted the molecular part of the research.

The manuscript was drafted ABS and revised by GRG. BWB supported to BSA while whole research work. Present research has been carried under the guidance of GRG by ABS. SPR guided for microbiological part to BSA. All authors read and approved the final manuscript.

Acknowledgments

The study plan was approved by board of studies, Vet. Pathology, Maharashtra Animal & Fishery sciences, University, Nagpur, Maharashtra (grant number 02/17 dated 03-02-2017 of the Comptroller, MAFSU, Nagpur). The authors cordially thank to College of Veterinary & Animal Sciences, Udgir, dist Latur, Maharashtra, India, for MALDI-TOF MS for confirmation of bacterial isolation.

REFERENCES

Amaravathi, M.., Satheesh, K., Annapurna, P. and Subramanyam, K. V. 2016. Prevalence and aetiopathological studies of ovine interstitial pneumonia. *Int. J. Adv. Res. Biol. Sci.,* 3(3): 208-210.

Bruker Daltonic MALDI biotyper 2016 Veterinary Microbiology, (55)

Chakraborty, S., Kumar, A., Tiwari, R., Rahal, A., Malik Y., Dhama, K., Pal, A. and Prasad, M. 2014. Advances in diagnosis of respiratory diseases of small ruminants. Veterinary Me dicine International Volume 2014: 1 – 16. doi:10.1155/2014/508304.

Deborah S., Jan Verhaegen and Katrien Lagrou 2011. Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry for the identification of bacteria and yeasts in a clinical microbiological laboratory. *Acta Clinica Belgica*, 2011; 66-4.

Elena De Carolis, Antonietta Vella, Luisa Vaccaro, Riccardo Torelli, Teresa Spanu, Barbara Fiori, Brunella Posteraro, and Maurizio Sanguinetti 2014. Application of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. *JInfect DevCtries*., 8(9):1081-1088. doi:10.3855/jidc.3623.

Kumar, M. P., T. H. Menon, S. Shanmugasundaram & C.P. Girish Kumar 2004. Group A streptococcal infections of the pharynx in a rural population in south India. *Indian J Med Res* 119, pp 171-173.

Quinn P.J. 2006. Veterinary Microbiology and Microbial Disease. Blackwell science, (24).

Rahal, A., Ahmad, A.H., Prakash, A., Mandil, R., and Kumar, A.T. 2014. Environmental Attributes to Respiratory Diseases of Small Ruminants.Volume 2014, doi.org/ 10.1155/2014/853627.

Ronald, T. K. Pang, Philip J. Johnson, Charles M. L. Chan, Ebenezer K. C. Kong, Anthony T. C. Chan, Joseph J.Y. Sung, and Terence C.W. Poon 2004. Technical Evaluation of MALDI-TOF Mass Spectrometry for Quantitative Proteomic Profiling Matrix Formulation and Application. Clinical Proteomics, Volume 1, Issue 3–4, pp 259–270.

Snedecor, G. M., and Cochran, W.C. 1982. Statistical Methods (7th Edn.) Iowa state university press, Towa, 511.

****