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EDITORIAL

REVIVING FORENSIC SPECIALITIES IN SRI LANKA

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Forensic Expert opinions have been useful unarguably worldwide to establish legal probanda, which otherwise would have been difficult, especially when eye witnesses are unavailable. This means that such forensic opinions can be of help to increase the probability of a fact in issue to be logically and of course legally in existence. Over the years, it is seen that many different forensic sciences contribute significantly to increase the evidentiary and probative value so that whichever side that embraces the opinion can comfortably maintain the line of argument towards their own ultimate probanda. Despite many criticisms they may have about forensic opinions ranging from them being 'unrealistic, unsubstantiated, opinionated, judgmental, confusing, disputed, logically implausible and the like', their use and contribution remains high.

While appreciating the many different forensic disciplines available world over today, this paper highlights a few key forensic domains that are essential to a country for its justice process. An equally significant aspect to mention here is that most of these essential disciplines in forensics are not developed except of course for forensic medicine and pathology. Accordingly, this paper argues that Sri

Lanka must pay attention to establish these disciplines to ensure justice and quality of law enforcement.

Forensic Medicine and Pathology is comparatively well established in Sri Lanka. It has its own well organized undergraduate teaching programs, organized post graduate training programs, boards of studies, a College and established departments in universities and specialized JMOs (Judicial Medical Offices) in hospitals. For many years this discipline has been in existence, of course, contributing immensely to ensure justice. However, most forensic medical units run with basic facilities and poor infra structure. Modern facilities such as emerging technology, specialized approaches in histopathology such as immune histochemistry or molecular pathology and biochemistry would be quite essential to upgrade their services in par with modern forensic investigations.

Given the growing demand and interest in Forensic Dentistry among many, Sri Lanka has introduced a master's degree program offered by University of Peradeniya and then another postgraduate program set to be offered from University of Colombo through the Post Graduate Institute of Medicine. These post graduate programs are in addition to

the teaching program offered in forensic dentistry at undergraduate level at University of Peradeniya. Notwithstanding the introduction of these academic programs, the commitment stake holders demonstrate to develop this discipline is not satisfactory. Although there are established units for forensic dentistry in Colombo and in Peradeniya, the infrastructure and facilities remain minimal.

Next important aspect is forensic Sciences. Despite the availability of a few trained personnel in the Government Analyst department, this country as a policy does not produce forensic scientists continuously. There are no academic programs in forensic sciences although a couple of sessions are taught in several degree courses. This means that the forensic sciences training programs are not well organized nor are they very specific in Sri Lanka. Officers in Government Analyst department become experts, ex-officio due to the recognition of this department by the law. The structure of the law is such that it more or less prevents budding experts in other units other than the government analyst department. Due to the very nature and tradition of the law enforcement relying heavily on the Government Analyst department owing to the recognition it has based on the substantive law, experts in other units for example in universities are sidelined although they may have acquired highly specialized qualifications and experience. There is a requirement at national level to provide defense opinions on all forensic aspects for example drugs or toxicology – and unfortunately in Sri Lanka many seem to solely rely on the Government

Analyst's report without it been reviewed by independent experts. This culture requires change to promote the development of science, fairness and justice. Fingerprint analysis is no different. The law itself identifies a police personal to be an expert while a university academic with high level of training and research in the field of forensic fingerprints may be sidelined.

Although there are a few people emerging with expertise with forensic entomology, anthropology, and the like there is no overall unit that can house all of them with a high level of coordination to facilitate a proper teamwork for forensic investigations. Forensic ballistics, forensic computing, forensic veterinary medicines are other areas that require further commitment, training, research, development and organization.

In order to develop these to full functioning entities in Sri Lanka, people with required qualifications should be given specialized training locally and abroad, familiarize them with forensic investigations and teamwork, teach them relevant law and providing expert opinions to court or law enforcement. This requires organized training programs, post graduate degrees, legal and government recognition of these disciplines, personnel and then a place where they can all converge as a team. Perhaps it may be a good idea to develop these disciplines in universities and converge them into a common centre when a case emerges which warrant their expertise. Certainly, research in all these areas is minimal in Sri Lanka. Most publications are case reports which are considered to be at the very bottom of research hierarchies. In short, these experts

must develop research while proving service function.

My contention is to identify these key forensic disciplines as essential for justice administration and develop organised training programs at post graduate levels coupled with long term training at a foreign unit for the

trainee and then utilise their services when and where necessary to deliver justice to the people of Sri Lanka. Indeed, it is important that their job security is maintained, terms of reference are designed, quality control mechanisms are in place and their discipline/expertise is recognised by law.

INCREASING THE FINE TO 25,000 RS. FOR DRINKING & DRIVING: INJUSTICE BY PREJUDICE IN MEDICO-LEGAL DIAGNOSIS

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INTRODUCTION

The Cabinet of Democratic Socialist Republic of Sri Lanka approved the increase of certain traffic fines to Rs. 25,000 on a memo presented by the Minister of Transport & Civil Aviation under the provisions of Motor Traffic Act. "Driving under the influence of liquor & drugs" is one of the offences for which the fine will be increased as such. This is a drastic increase by Rs. 23,000 from the existing fine.¹ However, there is evidence that increasing of fines for the traffic offences showed a marked reduction and effectively curbed crashes causing injuries and deaths in Sri Lanka.^{2,3} However, imposing a comparatively extraordinary fine in this regard should be without prejudice to the alleged driver. Therefore, we need a crystal clear legal framework which guide for a very solid diagnosis to the conclusion that the alleged driver has consumed alcohol/drug or not.

Key words: *drinking, driving, alcohol, clinical examination*

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1. Current legal status

According to the amendment act no. 31 of 1979 to the Motor Traffic Act of Sri Lanka, the relevant sub-sections of the section 151 states;

(1) No person shall drive a motor vehicle on a highway after he has consumed alcohol or any drug.⁴

2.1 Driving following consumption of alcohol

(1c) (a) Where a police officer suspects that the driver of a motor vehicle on highway has consumed alcohol he may require such person to submit himself immediately to a breath test for alcohol or an examination by a Government medical officer in order to ascertain whether such person has consumed alcohol and that person shall comply with any such requirement as the case may be.

(1D) (iii) Regulations may be made prescribing the mode and manner in which any examination may be conducted to ascertain whether a driver of a motor vehicle has consumed alcohol.

In the case of Sumanaratne vs. O.I.C. Police Station, Borella and Another, Justice A. De Z. Gunawardana held that the provisions in section 151 of the Motor Traffic Act does not take cognizance of the concepts "patient smelling of liquor" and "under the influence of liquor". He further stated that "what is required in a charge under section 151 of the Motor Traffic Act is to prove that the accused had consumed alcohol by adducing evidence that the concentration of alcohol in his blood is 80 mg/dl or more".⁵

2.2 Driving following consumption of drugs

Amendment no. 40 of 1984 to the subsection 151 (1C) (c) states, “Where a police officer suspects that the driver of a motor vehicle on a highway has consumed any drug, it shall be lawful for the police officer to produce such person before a Government medical officer for examination and that person shall comply with such requirement.”⁶

Sub-section (1D) (iii) states regulations may be made prescribing the mode and manner in which any examination may be conducted to ascertain whether a driver of a motor vehicle has consumed any drug.

Further, the sub-section 151 (1D) (iv) states, “Regulations may be made by prescribing the concentration of any drug in a person’s blood at or above which a person shall be deemed to have consumed any drug.”⁷

3. Contradictions in a medico-legal context

According to the judgment in Sumanaratne vs. O.I.C. Police Station, Borella and Another, the stipulated blood alcohol level can only be determined by carrying out the breathalyzer test or by carrying out a blood test of the alleged driver charged with this offence. When breathalyzers are available with Sri Lanka Police, this does not create a problem. However, the breathalyzers often go out of stock and in such circumstances, the driver is produced for a clinical forensic medicine examination for drunkenness. The Government medical officer who performs only the clinical forensic medicine examination without blood alcohol analysis cannot numerically opine on the blood alcohol concentration. However, blood alcohol analysis is not legally validated in the current Motor Traffic Law. Also, in Sri Lankan context, there are no legal regulations prescribing the mode and manner in which blood alcohol examination is conducted. Further, facilities for blood

alcohol analysis of the drivers has not been set up either in police stations or hospitals.

Further, it is quite unclear that which agency analyses the blood sample for alcohol. Availability of sampling bottles, procedure of dispatch and proper maintenance of chain of custody are some pertinent issues which should be streamline before increasing the fine for a fair administration of justice.

The conventional “Medico-Legal Examination Form” & “Medico-Legal Report” still use the terms “Smelling of liquor” & “Under influence of liquor”. These terms are now redundant following the amendment no 31 of 1979 of the Motor Traffic Act, which speaks “consumption of liquor/alcohol”.

According to the existing law when a driver is suspected for consumption of a drug, the only method of proving it, is by a clinical forensic medicine examination. It is extremely doubtful whether a mere clinical forensic medicine examination without blood/urine drug analysis is able to conclusively diagnose the consumption, differentiation and the exact blood concentration of the particular drug in the alleged driver.

In addition, there are no legal regulations prescribing the mode and manner in which blood drug examination is conducted. Further, regulations have not been made prescribing the legal limit (blood concentration) of any single drug.

CONCLUSION

The government medical officer’s opinion to the fact that consumption of alcohol, based only on clinical forensic medicine examination without blood alcohol analysis is redundant following the said amendment and the judgment of Sumanaratne vs. O.I.C. Police Station, Borella and Another.

Prior to implementing this law, following legal restructuring should be done.

- 1) Validate blood alcohol analysis under the section 151 of the Motor Traffic Act.
- 2) Validate blood drug analysis under the section 151 Motor Traffic Act.
- 3) Impose regulations under the subsection 151 (1D) (iii) Motor Traffic Act, prescribing the mode and manner in which any examination may be conducted to ascertain whether a driver of a motor vehicle has consumed alcohol or any drug.
- 4) Impose regulations under the subsection 151 (1D) (iv) Motor Traffic Act, prescribing the concentration of any drug in a person's blood at which a person shall be deemed to have consumed any drug.
- 5) The terms "Smelling of liquor" & "Under influence of liquor" in the conventional "Medico-Legal Examination Form" & "Medico-Legal Report" should be amended so that they fit with contemporary motor traffic law of the country. Without such legal restructuring, the implementation of this law invariably causes injustice.

ACKNOWLEDGMENT

Miss. Vinodani Dharmasena

REFERENCES

1. Motor Traffic (Amendment) Act No. 31 of 1979 (Sri Lanka) s 216. http://www.commonlii.org/lk/legis/num_act/mta31o1979279/s3.html
2. Jayatilleke AU, Darmaratne SD, Jayatillake AC. Increased traffic fines and road traffic crashes in Sri Lanka. *British Medical Journal*. 2012;18:A209.
3. Jayasekera SA. Traffic fines increased up to Rs. 25,000. Daily Mirror [Internet]. 2017 Aug 10 [cited 2017 Oct 09]. Available from: <http://www.dailymirror.lk/article/Traffic-fines-increased-upto-Rs--134486.html>
4. Motor Traffic Act No. 31 of 1979 (Sri Lanka) s 151(1). http://www.commonlii.org/lk/legis/num_act/mta31o1979279/s2.html
5. Sumanaratne v. O.I.C. Police Station, Borella and Another. In: *Sri Lanka Law Report*. Colombo: Ministry of Justice (Sri Lanka); 1991. p.345-350.
6. Motor Traffic (Amendment) Act No. 40 of 1984 (Sri Lanka) s 151(1C)(c). http://www.commonlii.org/lk/legis/num_act/mta31o1979279/s2.html
7. Motor Traffic (Amendment) Act No. 31 of 1979 (Sri Lanka) s 151(1D). http://www.commonlii.org/lk/legis/num_act/mta31o1979279/s2.html

DEATH DURING INDIGENOUS TREATMENTS: CHOKING DUE TO A HALF OF A LIME

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ABSTRACT

Thirty six years old woman, who was suffering from convulsions, decided to follow indigenous treatments. After beating with a coconut flower, when a lime was cut over the mouth, indigenous practitioner asked the patient to swallow the cut lime, put the cut pieces in to the mouth and pushed them deep using the cutting instrument. She struggled and found dead. Multiple injuries were found. Larynx was obstructed with half of a lime. This case shows that a conscious adult also may die due to choking if a food particle is forcefully pushed deep in to the mouth. It is an example for homicidal choking. Health education and regulations are necessary on indigenous treatments.

Key words: indigenous treatment, choking, lime

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INTRODUCTION

Fatal obstruction of the internal airway (choking) can occur between the pharynx and bifurcation of trachea. Food that can fill the pharynx, (such as bananas, marshmallows, or gelatinous candies) can be a danger for persons of any age¹, but commonly children² Choking due to a foreign object resulted in 140,000 deaths in 1990, which has increased to 162,000 deaths in 2013.³ Blood and dentures had also caused death due to choking. When adults die due to choking, usually there is a

contributory factor, such as alcohol or drug abuse and medicinal drugs such as tranquilizers in psychiatric patients.

CASE REPORT

Thirty six years old mother of one child was suffering from convulsions during last 10 years. She was under the treatments for epilepsy. To obtain a cure, she and her husband decided to follow indigenous treatments. One night, indigenous treatment started with a dance for the devil, followed by beating with a coconut flower, burning of feet, anal and genital area with a torch, and cutting of limes over various body parts. When a lime was cut over the mouth, indigenous practitioner instructed the patient to swallow the cut lime and put the pieces of lime in to the mouth and pushed the pieces down the throat with the cutting instrument. She struggled and became motionless. When she was brought to the hospital she was found to be dead.

Post mortem examination showed the following findings

- Multiple small abrasions around the mouth, hands and chest.
- Patterned contusions compatible with coconut flower on left loin.
- Contusions on the back and both loins.
- Black discoloration and blisters of skin around anus and feet.
- Burnt pubic hair.
- Fresh dislocations of lower central incisors teeth.
- Half of a lime obstructing pharynx.



Photo 01 : Patterned contusion of the face and lip contusions



Photo 02: Burn on sole



Photo 03: Patterned contusion by coconut flower on the left loin.



Photo 04: Half of the lime in pharynx

History provided by the husband suggested choking as the most possible cause of death. Postmortem examination revealed obstruction of the larynx with a half of a lime. Injuries were superficial and were not sufficient to cause death. There was no evidence of blood aspiration even though dislocated teeth were found. No postmortem changes of sudden natural death were found. Cause of the death was pronounced as;

1a- Asphyxia

1b- Chocking with a half of a lime

DISCUSSION

This is a rare form of fatal choking because the adult female had died due to forced insertion of a half of a lime down the throat when there is no factor for incapacitation. Forced insertion in to mouth by the cutting instrument had been witnessed by the husband and the injury pattern around mouth confirms the history.

Mechanisms of death in choking are hypoxia and neurogenic cardiac arrest.⁴ Hypoxia will produce postmortem features of asphyxia and neurogenic cardiac arrest will produce none. In this case, asphyxia features were not evident: therefore, the fatal mechanism is neurogenic cardiac arrest. When there is a doubt about the origin of the place of the food particle, whether from mouth or stomach, a litmus test is usually done. If the test shows acidic reaction, it indicates that the food particle had originated from the stomach. In this case, such a test is not valid, because the food particle was a half of a lime.⁵

A visit to the scene and history from eye witnesses and relations will help to find the circumstances of the fatal event. Usual

history is that after aspiration of the foreign-body, victims show symptoms of forceful cough, clutching of throat or chest, and then collapse. In this case, the victim had struggled and became motion-less. Manner of death in choking is usually accidental. It is rare, when a food particle is pushed down deep in to the mouth, as in this case, the event goes beyond a simple accident. With the knowledge and intention of the perpetrator, it could be an accident, medical negligence or homicide.

CONCLUSION

A conscious adult can also die due to choking if a food particle is forcefully pushed in to the mouth. Health education and regulations are necessary for indigenous treatments.

REFERENCES

1. Roy S. Swallow Safely: How Swallowing Problems Threaten the Elderly and Others. 2010.1st ed. Natick, USA: Inside/Outside Press. pp. 46–47.
2. Gregori D et al. Foreign bodies in the upper airways: the experience of two Italian hospitals. *Journal of preventive medicine and hygiene.* 2007 ;48(1):24-6.
3. GBD 2013 Mortality and Causes of Death, Collaborators. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet.* 2015;385: 117–71.
4. Saukko P, Knight B. *Knight's Forensic Pathology.* Philadelphia. CRC press.4th edi.2016; 362-364
5. Spitz WU. Asphyxia. In: Spitz WU editor. *Spitz and fisher's Medicolegal Investigation of Death.* Illinois; Charles C Thomas, 4th di.2004;824

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ABSTRACT

Innovation is a hallmark of advancement of science. Forensic professionals, together with other experts and equipment manufacturers, can help to design modern and novel equipment to enhance the quality of work and scientific validity. The major branches, such as Clinical Forensic Medicine and Forensic Pathology, need modern equipment as in other disciplines in Medicine. Creative ideas need support and link to the right individuals.

Key words: innovation, sexual assault forensic examination spectacles, forensic portable scanner, nanotechnology

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INTRODUCTION

Innovation is a hallmark of advancement in science. Developing countries may face limited resources in both materials and manpower. However, that should not prevent advanced thinking related to a particular field of interest. Ideas carry an extra source of power and the key foundation for greater projects.

Advancing the field of Forensic Medicine from any corner of the world could be done quite effectively due to the technological improvements in connectivity. Considering the need to take greater strides, intellectuals need to continuously work on innovative instruments effectively.

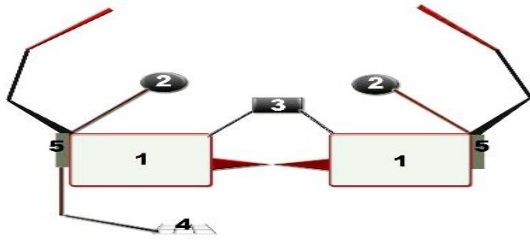
Conceptualization

Clinical Forensic Medicine and Forensic Pathology are the major fields in Forensic Medicine and the academia needs continuous flow of new information to feel that the field is still vibrant. The conceptualization of an instrument at times may look primitive, but it does pave a path for new ideas. The authors wish to present two innovative instruments, which they believe are useful if made available in day-to-day practice.

- A. Sexual Assault Forensic Examination Spectacles
- B. Forensic Portable Scanner

Sexual Assault Forensic Examination Spectacles

Clinical Forensic Medicine involves the examination of victims following sexual abuse. The interpretation of genital injuries, and proper recording for future reference and audit purposes are vital. Towards this line of thinking, an innovative instrument was designed with the existing ideas. This equipment will not be costly and is easily operable. It can be transported to selected places. The raw materials could also be easily accumulated.



- 1- Normal Glasses
- 2- Adjustable magnifying glass
- 3- Adjustable camera lens with light source
- 4- Foot pad and switch with adjustable antenna
- 5- External Memory Device

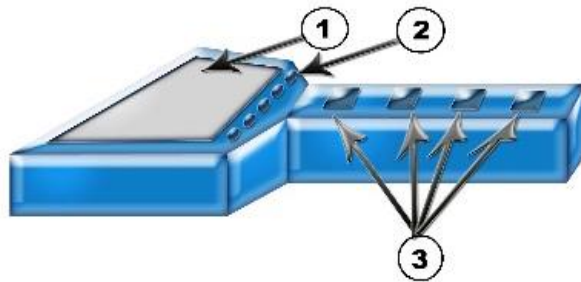
To do a proper genital examination both hands need to be free and mobile. Therefore, it is prudent that any instrument may be operated by using the foot to adjust the camera, light source and magnifying lens. The clicked images will go to the external memory device in the spectacles and can be later transferred to a permanent

storage device. The technology and expertise are already available but it is a matter of diverting the same to be effectively used in Forensic Medicine.

Forensic Portable Scanner

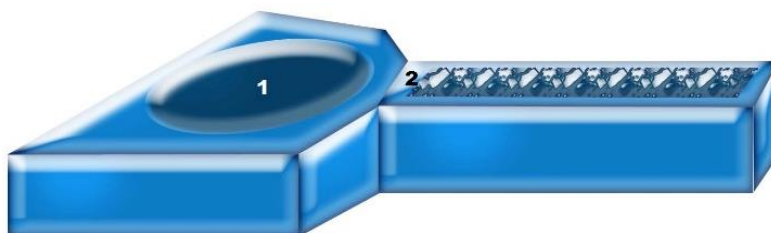
In developed countries, CT scanners are used as an alternative or together with routine autopsies to identify the cause of death and for various other medico-legal issues. There are autopsies, such as homicides following firearm injuries; deaths following explosions; death of a body packer and in the living; and those smuggling valuables inside body orifices (the so called 'mules'), in which the forensic portable scanner may become valuable to trace foreign material. The proposed scanner is portable; battery operated and contains functions to identify objects and substances of different density. It contains a screen for on the spot visualization and inbuilt memory device for long-term storage.

Portable Scanner (battery operated) Anterior view



- 1- Screen
- 2- Resolution buttons
- 3- Density buttons

Portable scanner (battery operated) Posterior view



- 1- Probe with sensors
- 2- Rechargeable battery

By using the probe on the body surface, the foreign objects or any other suspected material could be visualized and images could be captured. This can be used even during dissection to trace foreign materials, such as bullets, pellets, detonators following explosions, shrapnel and detached tip of a knife embedded inside a bone and etc. Modified nano-technology could be utilized to transfer waves to body surface and to retrieve them as images. This equipment would be an asset to Forensic Medicine if developed containing all the necessary components.

CONCLUSION

Modern and innovative instruments in Forensic Medicine will enhance the quality of work and the reliability of scientific findings. The forensic work will reach different levels throughout the world. The collaborative work between different specialists and equipment manufactures would soon become the order of the day.

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DECLARATION

The abstract was accepted for oral presentation at the INPALMS held in Bali, Indonesia. 2016

LIMITATIONS

The authors wish to state that the suggested equipment are merely conceptualized ideas done in the best interest to promote the forensic field. The technology and even the devices may be already available for different purposes.

REFERENCE

1. The virtopsy approach – 3D optical and radiological scanning and reconstruction in Forensic Medicine. Edited by Michael J. Thali, Peter Vock. 2009. CRC Press.
2. <http://www3.gehealthcare.com.au>, last accessed on 8.3.2017
3. <http://mius.org.uk/portable-ultrasound-scanners/>, last accessed on 8.3.2017
4. <http://www.theverge.com>, last accessed on 8.3.2017
5. <https://www.nlm.nih.gov/visibleproofs/exhibition/newscience.html>, last accessed on 8.3.2017

UNEXPECTED COMPLICATIONS FOLLOWING AN OCCUPATIONAL INJURY

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ABSTRACT

Introduction

Experts in forensic medicine are often requested to give an opinion on bodily damage resulting at work. General hazards in a work environment include electricity, fire, inflammable gases, high pressure gases and liquids, powerful or sharp moving machinery and fall. When an occupational trauma is complicated with an unexpected sequelae forensic pathologist is in a dilemma. We report a workman who was brought following blunt head and chest trauma complicated with a facial palsy and lobar pneumonia.

Case study

A 53 year old security officer was brought to the hospital following improperly fixed gate falling on to him while at work. He was recruited by the security company two months back and was appointed to this place without informing the condition of the gate. History revealed that he was unconscious for a few minutes and remained to have dizziness and vertigo. Next day he developed profound left lower motor neurone facial nerve palsy. He had fractures of the right petrous temporal bone, and a left scapula. Two weeks later he developed shortness of breath and fever, which was diagnosed as left sided lobar pneumonia.

Conclusion

Fracture of the bones and the facial nerve damage could be easily categorized as grievous injuries. Chest trauma may result in localized pulmonary contusion which can be complicated as an infection presenting late. The appreciation of the association between the infection and initial injury

needs thorough scientific basis since the category of hurt is crucial in formation of medico legal opinion.

Key words: Occupational injury, medico-legal opinion, unexpected complications, facial nerve palsy, chest trauma, lobar pneumonia.

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INTRODUCTION

Injuries due to workplace accidents are on the rise worldwide.¹ A survey done in year 2000 shows 15% of the injuries are due to occupational health hazards.² According to the statistics of the Ministry of Health regarding occupational health, out of the fatal accidents, 30% are from construction industry and 30% from electrocutions. Most of these hazards and accidents are preventable.²

An injury or pathological process that affects an employee as a result of an event at work can be claimed for compensation according to international laws.³ Experts in forensic medicine are often requested to give an opinion on bodily damage resulting at work. General hazards in a work environment include electricity, fire, inflammable gases, high pressure gases and liquids, hot gases and liquids, powerful or sharp moving machinery and fall. When an occupational trauma is complicated with an unexpected sequela the forensic pathologist

is in a dilemma. We report a man who was brought following a blunt head and chest trauma complicated with a facial palsy and lobar pneumonia as a consequence of occupational trauma.

CASE STUDY

A 53 year old security officer was brought to the hospital following an improperly fixed gate falling on to him while at work. He was recruited by the security company two months back and was appointed to this place without informing the condition of the gate. History revealed that he was unconscious for a few minutes and continued to have dizziness and vertigo. The next day, he developed profound left lower motor neurone facial nerve palsy. He was found to have fractures on the right petrous temporal bone, and left scapula. Two weeks later, he developed shortness of breath and fever which was diagnosed as left sided lobar pneumonia. Medico legal examination revealed that he was not under the influence of alcohol at the time. He is a chronic alcoholic and a smoker, but was previously healthy. He was reviewed on 17/12/2015, and the neurologist recommended a nerve conduction study and EMG, which indicated severe right facial nerve injury with no evidence of reinnervation and suggested nerve repair.

DISCUSSION

Anybody who faces an accident, or suffers from an injury or disease resulting in the total or partial disability of the workman for a period exceeding seven days³ due to their occupation, is eligible for compensation awards and medical care that is paid by the employer according to the workers' compensation laws of the country. The occurrence must be unexpected to be considered accidental in the context of compensation.⁴ However, if an injury is sustained as a result of conduct not expected from the employee, coverage under Workers' compensation will be denied.⁵ The fall of the gate in this case was unexpected and was accidental. This man

was unaware of the fact that the gate was not fixed and he was expected to operate the gate and was not under the influence of alcohol at the time of the incident. Thus, this is a case to be considered for payment of workman's compensation.

He was given inward treatment for 34 days where his earning capacity was totally affected (temporary total disability) during the period. He had an un-displaced fracture of the left petrous temporal bone and profound facial nerve palsy of the same side on the following day. The facial nerve palsy following trauma, is uncommon which occurs in 1.5% patients of skull base fractures.⁶ In most of the cases, spontaneous recovery follows. However, the opinion of the neurologist in this case was that the damage will be permanent. He will most probably be left with facial nerve damage resulting in paralysis of facial muscles and loss of sensation of anterior 2/3 of the tongue and loss of secret motor functions of the submandibular, sublingual and lacrimal glands. Complications of Bell's palsy include disfigurement or permanent contracture from irreversible facial nerve damage, eye damage (corneal ulcers and infections), chronic spasm of facial muscles and/or eyelids, and chronic taste abnormalities. Involuntary contraction of certain muscles may result from misdirected re-growth of nerve fibers. Psychological trauma may accompany facial disfigurement in some individuals.⁷ The onset of facial palsy may be immediate or delayed. Delayed facial paralysis occurs usually after 1-10 days of trauma and commonly due to entrapment of nerves within fibrous tissue or oedema, and chances of recovery are high. Immediate facial palsy occurs due to stretching/compressing/crushing/in the part of the nerve, where recovery chances are low and late.⁸ The facial nerve paralysis was observed in this man on the following day. However, before a conclusion is made regarding whether the damages are permanent, it is important to review him since there are some patients who had fully recovered after 6 months.⁹ The Workman Compensation Act of Sri Lanka includes

severe facial scarring or disfigurement as 75%, permanent total loss of vision as 50% and total loss of taste as 25% to loss of earning capacity.³

On the other hand, criminal negligence causing bodily harm is an indictable offence. In this case, the hospital authority is responsible for recklessly employing this man at this unfixed gate without ensuring his safety. Thus, forensic medical examination is required in categorizing the bodily harm or hurt. Fracture of the bones and facial nerve damage could be easily categorized as grievous injuries under limbs 'g' and 'f' of the section 311 of the penal code.¹⁰

This man developed a lobar pneumonia while being managed for the fractures in the ward. The appreciation of the association between the infection and initial injury needs scientific basis before concluding the category of hurt. Chest trauma, specially the fracture of the scapula, can result in contusion of the adjacent lung. It takes an average of six hours for the characteristic white region to show up on a chest X-ray, and sometimes contusion may not become apparent for 48 hours.¹¹ The initial chest X-ray of this man did not show any pulmonary contusions and further X-rays were not taken until he developed pneumonia in two weeks. However, considering the extent of the fracture, it is highly likely for him to get contusions of the adjacent lung.

Pulmonary contusion is associated with complications including pneumonia¹² and acute respiratory distress syndrome, and it can cause long-term respiratory disability. As many as 20% of people with pulmonary contusion develop pneumonia.¹³ Infection that occurs within 72 to 96 hours of injury is termed 'early onset pneumonia'. Both early onset as well as late onset pneumonias (as in our case) are reported following pulmonary contusions.¹⁴ The risk of pneumonia in lung contusion begins almost immediately after acute traumatic damage. Both gram-positive and gram-negative bacteria are responsible for pneumonia in

trauma patients. However, a late pneumonia has an equal mix of gram-positive and gram-negative bacteria as the offending microbe that is isolated on the culture.¹⁵ Contused lungs are a good culture media for growth of bacteria. Contusions induce inflammation with high local concentrations of pro-inflammatory mediators stimulating chemotaxis and activation of neutrophils.¹⁶ Further, impaired chest movements, and cough reflex also predispose the infection. In patients with trauma, significant risk factors for pneumonia included age, gender, pulse rate, systolic blood pressure, obesity, Glasgow Coma Scale motor score, and ventilation on admission.¹⁷

The pneumonia in this man was a left lower lobar pneumonia which was developed two weeks after the chest trauma. By definition, this can also be considered as a nosocomial or hospital acquired pneumonia, which is contracted by a patient in a hospital at least 48–72 hours after being admitted. It is usually caused by bacterial infections. Common risk factors include, mechanical ventilation, old age, decreased filtration of inspired air, disease states that result in respiratory tract obstruction, trauma, (abdominal) surgery, medications, diminished lung volumes, or decreased clearance of secretions. However, the pneumonia in this man was a left lower lobar pneumonia which is the area that could have been badly affected by the trauma on the lower scapular area suggesting the relationship to the trauma.

In addition to the chest trauma, this 53 year old man had other risk factors to develop pneumonia which include chronic smoking and chronic alcoholism leading to chronic pulmonary damage and consequently immune suppression. However, this man was previously healthy and acquired all these complications following the traumatic event.

Lobar pneumonia needs proper anti-bacterial therapy. The mortality rate of hospital acquired pneumonia remains high at 30% even with treatment.¹⁸ This

indicates the seriousness of the condition. However, since the condition is a remote complication of the initial trauma and it is contributed by his existing weaknesses, the category of hurt was concluded as endangering life.

CONCLUSIONS

This man was an unfortunate victim of blunt force trauma associated with two uncommon complications following an accident at the work place. The case highlights the importance of proper scientific investigations considering the differential diagnosis in establishing the causal relationship in remote complications associated with medico legal trauma in proper administration of justice.



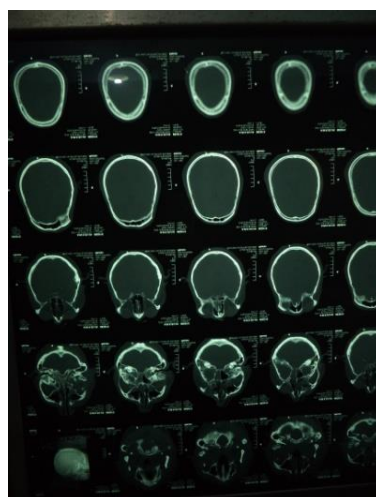
Lower motor facial nerve palsy



Abrasion on the back of the chest



Left scapular fracture



CT scan showing petrous temporal bone fracture



CXR showing left lobar pneumonia

REFERENCES

1. Statistics of occupational injuries. Sixteenth International Conference of Labour Statisticians. Geneva. 6-15 October 1998. International Labour Organization.
2. www.health.gov.lk/OccupationalHealth
3. Workmen's Compensation ordinance. Vol. viii. Laws of Sri Lanka. www.srilankalaw.lk/...VIII/workmen-s-compensation-ordinance.html
4. Accidental injury under the New York Workmen's Compensation Law. 28 Fordham L. Rev. 322 (1959)
5. Thomas D. Terry, Workmen's Compensation - Statutory Requirement of Injury by Accident, 2 Wm. & Mary L. Rev. 289 (1959), vol2,iss1, 144
6. PP Devriese. Facial paralysis after trauma of the skull. In, Samii M and Brihaye J (eds). Traumatology of the skull base. Springer Verlag New York 1983:155-63
7. Bell's Palsy." MayoClinic.com. 24 Sep. 2004. Mayo Foundation for Medical Education and Research. 1 Jul. 2009 <<http://www.mayoclinic.com/invoke.cfm?objectid=2B73952A-337E-4AB7-95368E5C254B2D65&dsection=1>
8. K. Puvanendran, M.Vitharana and P. K.Wong. Delayed facial palsy after head injury Journal of Neurology, Neurosurgery and Psychiatry. 1977. 40; 342-350
9. Keki E Turel, Nootan Kumar Sharma, Joy Verghese, Sanjeev Desai. Post Traumatic Facial Paralysis Treatment Options and Strategies. Indian Journal of Neurotrauma (IJNT) 2005, Vol. 2, No. 1, pp. 33-34
10. Legislative enactment of Ceylon. Penal Code [Sri Lanka], Ch; 19 Section 311
11. Wanek S, Mayberry JC. Blunt thoracic trauma: Flail chest, pulmonary contusion, and blast injury. *Crit Care Clin* 2004;20(1):71-81.,Allen GS, Coates NE. Pulmonary contusion: A collective review. *Am Surg* 1996;62(11):895-900.
12. Croce MA, Fabian TC, Waddle-Smith L, Maxwell RA. Identification of early predictors for post-traumatic pneumonia. *Am Surg* 2001; 67:105-10
13. Tovar JA. "The lung and pediatric trauma". *Seminars in Pediatric Surgery* (2008)17 (1): 53-59. doi:10.1053/j.sempedsurg.2007.10.008.
14. [Hafen GM](#), [Massie J](#). Lung contusion from focal low-moderate chest trauma [Pediatr Pulmonol](#). 2006 Oct;41(10):1005-7
15. Dolgachev VA, Yu B, Reinke JM, Raghavendran K, Hemmila MR. Host susceptibility to gram-negative pneumonia after lung contusion. *The journal of trauma and acute care surgery*. 2012;72(3):614-623. doi:10.1097/TA.0b013e318243d9b1.
16. Vladislav A. Dolgachev, Julia M. Reinke, Krishnan Raghavendran, R. Hemmila, Host susceptibility to gram-negative pneumonia after lung contusion. *J Trauma Acute Care Surg*. 2012 Mar; 72(3): 614-623. doi: 10.1097/TA.0b013e318243d9b1
17. Janus TJ, Vaughan-Sarrazin MS, Baker LJ, Smith HL. Predictors of pneumonia in trauma patients with pulmonary contusion. *J Trauma Nurs*. 2012 Jul-Sep;19(3):139-47. doi: 10.1097/JTN.0b013e318261d1e2.
18. Crouch Brewer S, Jones CB, Leeper KV, Wunderink RG. Ventilator-associated pneumonia due to *Pseudomonas aeruginosa*. *Chest* 1996;109:1029

DOES DURATION OF COOKING HAVE AN EFFECT ON PCR DETECTION OF MEAT FOR FORENSIC PURPOSES?

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ABSTRACT

Although conventional Polymerase Chain Reaction (PCR) is one of the best methods used for meat identification for forensic purpose, some samples of cooked meat presented to Veterinary Research Institute for meat species identification have not responded in conventional PCR. Objective of this research was to exclude one of the possible reasons that would have caused this problem. To identify the effect of different cooking time on Deoxyribo Nucleic Acids (DNA) extraction and PCR, beef was used as the meat type, since very often the suspicious sample is claimed to be 'beef'.

Total of 18 samples of beef from 3 different commercial sources were used. Samples (n=6) from each source were cut in to equal sizes and cooked separately to minimize contamination. They were cooked at 20 min, 40 min and 60 min cooking periods by adding equal amounts of commercially available products of turmeric powder, curry powder, chillie powder, salt powder and water. Samples were kept separately until DNA extraction. Forward and reverse primers were used for DNA amplification of bovine cytochrome b gene. The samples were subjected to DNA quantification by using the nanodrop spectrophotometer. Change in absorbance by DNA samples was used to quantify the DNA samples.

The results of gel electrophoresis revealed that the samples were positive in all 3 cooking conditions with bands of ~ 272 bp equivalent compared to ladder and the positive control sample. Statistical analysis of DNA quantities revealed that even

though the cooking time (up to 60 min) had no effect on the extracted DNA for species identification of beef samples as mentioned above, the DNA samples extracted from beef samples at 60 minutes resulted in high absorbance values indicating possible denaturation and fragmentation of DNA.

Key words: *Meat identification, cooking time, PCR*

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INTRODUCTION

Taste and nutritional value makes meat a main component of human diet. In Sri Lanka, meat is supplied by government slaughter houses in Kandy and Colombo and Island wide slaughter houses maintained by the 'Pradeshiya Sabhas'. Some authorized private companies such as "Prima", and "Crysbro" etc. also supply meat to the market. Though the authorized bodies play a major role in supplying meat, 'bush meat' is also present in the market. Increase in bush meat and illegal killing of animals can be due to uncontrollable access to wild life, lack of education, poverty, unemployment etc.

Bush meat and the illegal slaughtering of animals have led to many problems in religious, legal, ethical, health and economic sectors of the country and also it is a threat to the wild life. In Sri Lanka, many wild

animal species belonging to the 'Cervidae' family are illegal to be killed. Ceylon spotted deer (*Axis axis ceylonensis*), Ceylon hog deer (*Axis porcius oryzus*), Ceylon Sāmbhar (*Cervus unicolor unicolor*) and barking deer (*Muntiacus muntjak malabaricus*) are considered as protected animals (Fauna and Flora protection Ordinance 1937; Rajapaksha *et al.*, 2003). Buffaloes (*Bubalus bubalis*) are also banned to slaughter in Sri Lanka according to the Animal act no 29 of 1958. In order to discourage illegal hunting, to protect wild life, and to avoid substitution of meat species, forensic identification of meat is important. Though there are regulations stipulated to conserve and protect animals and to prevent selling their meat, implementations of the legislations is limited due to the failures of identification of meat (Rajapaksha *et al.*, 2001).

In Sri Lanka meat samples are submitted by the court and the wild life department to the Veterinary Research Institute (VRI), Gannoruwa for species identification. Most common claim for the suspicious meat sold is 'beef'. If the laboratory findings do not prove that the received meat sample as 'beef' then specific species identification is required for further investigation. There are various methods used for identification of species origin of meat such as sensory analysis, anatomical variation of species, and histological differentiation of the hair on meat, tissue fat properties, level of glycogen in muscle tissue, electrophoresis and DNA hybridization. Methods such as Agar Gel Precipitation Test (AGPT), dot blot assay, or counter immunoelectrophoresis are also being used for meat species identification (Dissanayaka *et al.*, 2001). Detection of the specific meat proteins, which may be denatured or destroyed during processing and process of rotting, is the basis of these methods. Other than that Polymerase Chain Reaction (PCR), Restricted Fragment Length Polymorphism (RFLP) and Random Amplified polymorphic DNA (RAPD) techniques are used for meat species identification (Ilhak and Arslan, 2006). Out of these numerous

methods mentioned above, VRI uses conventional PCR for meat species identification. PCR easily amplifies the target regions of template DNA even in the presence of a small quantity within a much shorter time. When compared with protein, DNA is more stable and resistant to factors such as high temperature, chemicals and pressure. Because of these properties identification of different meat species including both raw meat as well as the meat subjected to thermal treatments is possible at present (Spychaj *et al.*, 2009).

However, the extraction of DNA for species identification of meat is not possible from all the samples presented to the laboratory which may be due to the nature of the samples. The majority of the samples received by lab are either rotten or processed (Rajapaksha *et al.*, 2003). Some samples are stored and transported under different conditions and some are already cooked samples. Cooked samples can vary with cooking conditions depending on duration of cooking (being overcooked, under cooked), cooking temperatures, amount of water and spices added etc. For an example usually curry powder, chillie powder, turmeric powder, salt are added during beef cooking. Other than that, sera (*Cymbopogon citratus*), rampe (*Pandanus amaryllifolius*), ginger (*Zingiber officinale*) and tamarind (*Tamarindus indica*) are added during meat cooking to increase flavor and moisture. Due to the effect of one or more of the factors mentioned above only some samples respond to the PCR while some do not.

Failure of species identification results in failures in administering justice. Though it is considered that the PCR technique is successful at identification of meat, lack of sensitivity of PCR assay for cooked meat of members of Cervus family was reported by Rajapaksha *et al.*, 2002 and same was reported for boiled horse meat by Mastunaga *et al.*, 1999.

Exclusion of the factors that do not influence on the DNA extraction of meat is

useful in determining the sensitivity of the method and narrowing down the investigations on the possible causative factors for PCR failures. Therefore, the aim of this study was to analyze the effect of time duration of cooking on the DNA quantity yield and its effect on the sensitivity of PCR by taking beef as a convenient sample. The study compared three different cooking times viz 20min, 40 min and 60min with the DNA extraction of meat (beef).

MATERIALS AND METHODS

1. Sample Collection and Preparation

Fresh beef samples were obtained from 3 different commercial sources. Each Sample was washed properly and labeled as A, B, and C. Each sample was cut into 6 pieces each of approximately 2cm x 2cm x 2cm. The 6 pieces from each sample were put into separate clay pots. Following amounts of commercially available spices and water was added to each pot just before cooking.

Recipe:

*Curry powder	1 tsp
Chillie powder	1 tsp
Salt powder	½ tsp
Turmeric Powder	¼ tsp
Water	1 cup

*Consistency of curry powder: coriander, cumin, fennel, turmeric, cardamom, cloves, Fenagreek, Cinnamon, Rampe and curry leaves. Consistency of salt: Edible salt, KI
All ingredients were mixed well with meat pieces. The initial temperature was recorded at each cooking session. The samples were cooked in a closed container under relevant cooking conditions on a hot plate. Two pieces were taken out at the time intervals of 20 min as follows:

20 min- 2 pieces
40 min- 2 pieces
60 min- 2 pieces

The samples were wrapped in sealant bags and grouped according to the time duration of cooking and kept in the freezer at -20°C until DNA extraction.

2. DNA Extraction

Approximately 50 mg of each tissue sample was measured and the following procedure was adopted for each sample separately.

Each sample was crushed gently using a tissue grinder. Digestion buffer (600µl) was added to each sample placed in labeled endpordorf tubes.

Digestion buffer preparation

3M Nacl	800
1MTris-Hcl	250
0.5 M EDTA	1.25 ml
10% SDS	1.25 ml
(pH 8.4)	
Proteinase k enzyme	100µg/ ml
Filled up to 25 ml with distilled water.	

The sample was incubated at 35°C for 3 hrs. Equal volume (600µl) of Phenol : chloroform : iso amyl alcohol (25:24:1) in which pH was 7.9 was added to it. The sample was mixed well using vortex and centrifuged at 14000rpm for 5 minutes at room temperature. The supernatant was taken in to a separate tube and 60µl of 3M Nacl was added to each sample. Then 1.2 ml of Ethanol was added and vortexed well. The samples were kept at -20°C in freezer for an overnight. After that the samples were centrifuged at 14000rpm in 10 minutes at room temperature.

The supernatant was discarded and 120µl of 70% Ethanol was added to the remaining pellet, centrifuged at 14000 rpm for 5 minutes at room temperature, the supernatant was removed and pellets were air dried. 100 µl of DNA free H₂O was added and samples were kept at -20°C until used.

3. PCR technique

Each of 20 samples (including positive and negative control samples) were prepared for the PCR as shown in table 1.

Table 1: PCR constituents

Constituent	Amount(μ l)
PCR buffer	3
dNTP	0.6
P ₃ Primer	1
P ₆ Primer	1
Taq Polymerase	0.3
DNA	1
DNA free water	18.1

The samples were loaded in to 500 μ l eppendorf tubes and the PCR was performed under following PCR conditions:

95 °C – 3 min (pre dwell)
95 °C – 1 min (denaturation)
55 °C – 1 min (primer annealing)
72 °C – 1 min (polymerization)
72 °C – 7 min (post dwell)

Mitochondrial cytochrome b sequence was amplified using following two forward and reverse primers. This was used because mitochondrial DNA is more stable under various conditions compared to nuclear DNA.

Forward:

5'/GACCTCCCAGCTCCATCAAACATCT
CATCTTGATGAAA3'

Reverse :

5'/CTAGAAAAGTGTAAGACCCGTAAT
ATAAG3'

4. Gel electrophoresis

Gel electrophoresis was performed to separate and visualize the amplified PCR products according to the size. To prepare the gel, 1.5 g Agarose and 150 ml of TBE buffer was added and kept in microwave oven for 2.5 min to dissolve. The liquid was allowed to cool inside a biosafety cabinet. 12 μ l of ethidium bromide was added, mixed

and poured on a chilled tray. It was let to set for 40 min. Loading mix was prepared and loaded to relevant well.

Ladder	loading buffer	2 μ l
	Ladder	5 μ l
Sample	Loading buffer	2 μ l
PCR product		6 μ l

Electrophoresis was done on agarose gel at 100 V for 45 minute resulting gel was visualized using a UV trans illuminator. The results were compared with the DNA ladder.

DNA quantification and the statistical analysis of the data obtained were performed. DNA concentration can be assessed using methods such as absorbance, agarose gel electrophoresis, fluorescent DNA binding dyes, etc. Most common methods are using spectrophotometer and the agarose gel electrophoresis. An increase in absorbance at 260 nm of the DNA solution is because of the denaturation of double stranded DNA to single stranded DNA. This is due to the increase in DNA yield.

RESULTS

1. Results of Gel Electrophoresis

All the 18 samples, 6 in each time period (20 min, 40 min, 60 min) of 3 beef sources gave bands equivalent compared to that of ladder and the positive sample as shown in figure 1,2 and 3.

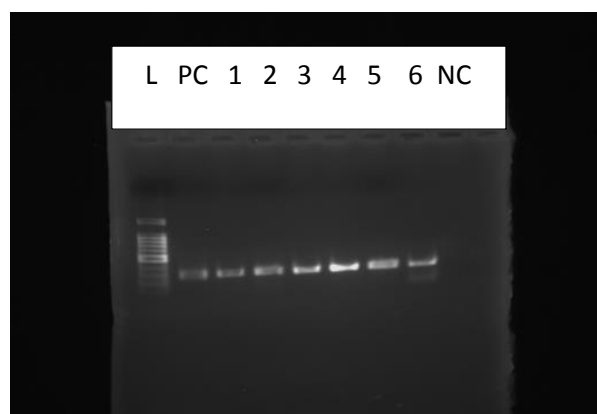


Figure 1 : Gel electrophoresis of the beef samples cooked for 20 minutes (PC: DNA extracted previously from meat and used as positive control, NC: Negative Control without DNA)

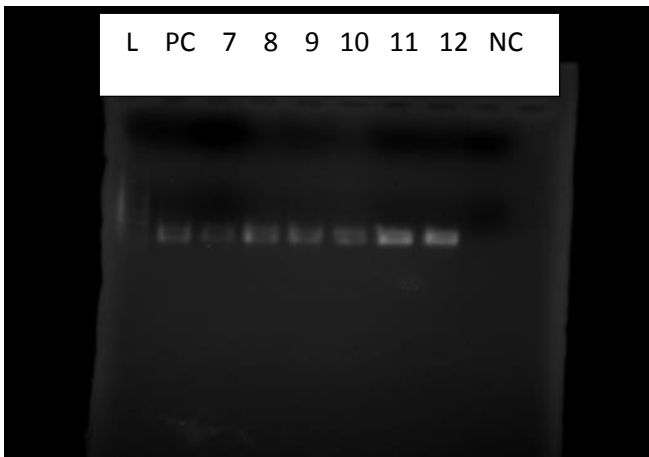


Figure 2 : Gel electrophoresis of the beef samples cooked for 40 minutes

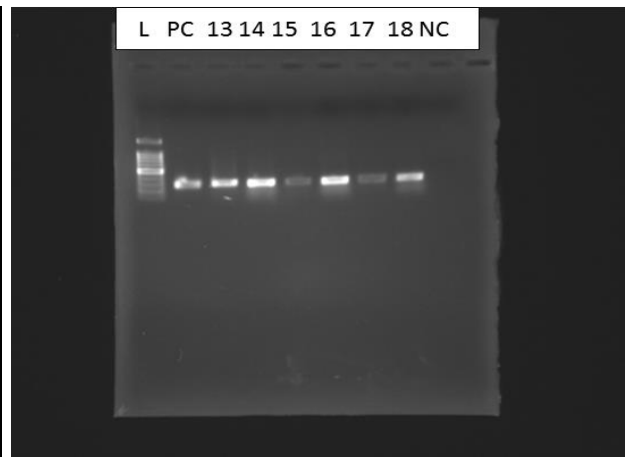


Figure 3: Gel electrophoresis of cooked beef samples at 60 minutes

When DNA was amplified a band of about 272 bp was observed for all the meat samples of different cooking time periods. Note that the bands of sample no 15 and of 17 were light (Figure 3). There was no band for negative control (NC; Figure 1, Figure 2, and Figure 3).

2. Quantification of DNA using nanodrop method

Table 2: Mean DNA quantities according to time

Cooking Time (min)		DNA quantity (ng/µl)		Mean DNA quantity
20	A ₂₀	317.1	A ₂₀	275.35
	A ₂₀	233.6		
	B ₂₀	583.5	B ₂₀	340.25
	B ₂₀	603.9		
	C ₂₀	1050	C ₂₀	1031.15
	C ₂₀	1013		
40	A ₄₀	559.9	A ₄₀	961.45
	A ₄₀	1363		
	B ₄₀	732.9	B ₄₀	1018.95
	B ₄₀	1305		
	C ₄₀	294.3	C ₄₀	808.8
	C ₄₀	1029		
60	A ₆₀	372.85	A ₆₀	1557.42
	A ₆₀	2742		
	B ₆₀	1414	B ₆₀	2851
	B ₆₀	4288		
	C ₆₀	3280	C ₆₀	2228
	C ₆₀	1176		

3. Analysis of Data

Data obtained was analyzed using Minitab 15 software version to detect the effect of cooking time on the DNA quantity of the relevant samples.

One way ANOVA was performed to evaluate the difference of mean DNA quantities extracted according to the cooking time. The results indicated a statistical significance ($p < 0.05$) for the DNA quantity measured in terms of absorbance and this was mainly due to the difference observed in the mean absorbance for meat cooked for 60 minutes compared to the means observed for 20 min or 40 min samples (Figure 4).

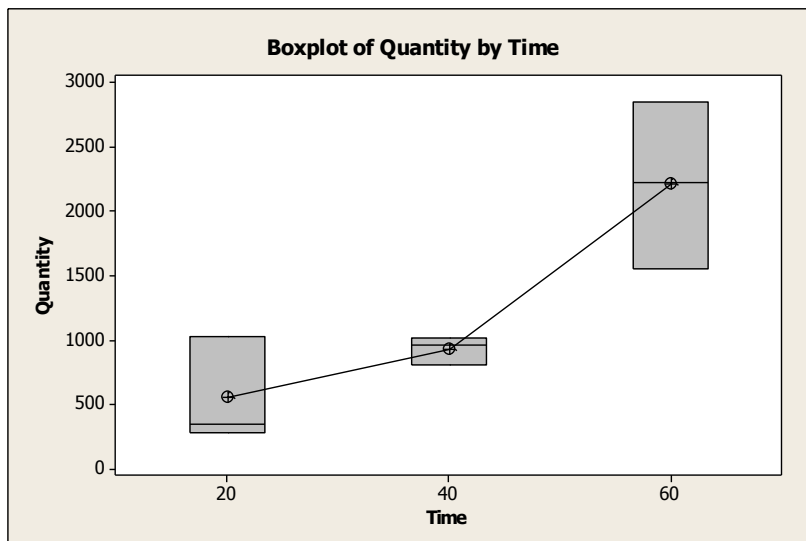


Figure 4: Box plot of DNA quantity according to cooking time

The box plot in figure 4 shows that there is less variation between the mean DNA quantities between samples cooked for 20 minutes and 40 minute, while the mean DNA quantity of the sample cooked for 60 minutes has a wide variation. Purity of the samples also was checked and contamination was ruled out.

DISCUSSION

As all the samples cooked up to 60 minutes gave bands in gel electrophoresis, successful DNA extraction (Figure 1, Figure 2 and Figure 3) was indicated and results indicate that conventional PCR method can be used to identify DNA extracted from the beef samples cooked up to 60 minutes. For the successful amplification of DNA purity and the quality of DNA template and the heating process has a greater impact.

The absorbance of DNA samples at Nano drop spectrophotometer indicates the DNA quantity. When the samples were cooked for longer time (60 minutes) the absorbance of the DNA sample has been increased exponentially. This resulted in a statistically significant difference in the mean DNA quantity at 60 minutes compared to 20 minutes or 40 minutes cooking time. This could be due to the release of more DNA from cells followed by denaturation and fragmentation of DNA released with prolonged cooking. The relatively high variation between the mean DNA quantity and lighter bands in gel electrophoresis of 60 minutes cooked beef sample

may be due to the high temperature exceeding boiling temperature of water (100°C) at the time of 60 minutes resulting in DNA fragmentation (Musto *et al.*, 2010). Caution is required when the fragments become smaller which affects the sensitivity of PCR technique. However up to 60 minutes of cooking all the beef samples were able to produce a positive band at ~272bp level on the ladder.

CONCLUSION

PCR and gel electrophoresis can be used effectively to identify beef samples cooked for 20 min, 40 min and 60 min time in species identification of meat for forensic purpose. High mean quantity of DNA was

yielded from samples cooked for 60 min when compared with the quantity of DNA of 20 min and 40 min of cooking. This indicates possible DNA fragmentation at 60 minutes of cooking. However, this did not affect the sensitivity of the test. Therefore, it can be concluded that the species identification of beef is possible in conventional PCR method if they are cooked under the conditions as in this experiment and cooked up to 60 minutes.

REFERENCES

1. Animals act No. 29 of 1958
2. Dissanayaka, S. M., A.Horadagoda, S.B.P. Athauda, and Welihena, S. Rapid and accurate identification of meat by a simple dot blot assay. Proceedings of 53rd Annual Convention of the Sri Lanka Veterinary Association; 2001.
3. Fauna and Flora protection Ordinance. No 2 of 1937
4. Ilhak O.I., ArslanA. Identification of Meat Species by Polymerase Chain Reaction(PCR) Technique. Turkish Journal of Veterinary and Animal Science. 2006;31 (3): 159-163
5. Musto M. DNA quality and integrity of Nuclear and Mitochondrial Sequences from Beef Meat as Affected by Different Cooking Methods. Food Technol. Biotechnol. 2011; 49 (4): 523–528
6. Rajapaksha, W. R. A. K. J. S., Thilakaratne, I. D. S. I. P., Chandrasiri, A. D. N. and Niroshan, T. D. Development of PCR Assay for Differentiation of Some Important Wild Animal Meat of Sri Lanka. Journal of Veterinary Medicine Series B. 2002; 49: 322–324.
7. Rajapaksha, W. R. A. K. J. S., Thilakaratne, I. D. S. I. P., Chandrasiri, A. D. N. and Niroshan, T. D. Development of PCR Assay for Identification of Buffalo Meat. Asian-Aust. J. Anim. Sci. 2003; 16(7): 1046-1048
8. Spychaj, Anita, Mozdziak, P.E. and Pospiech. PCR methods in meat species identification as a tool for the verification of regional and traditional meat products. Acta Scientiarum Polonorum Technologia Alimentaria. 2009; 8(2): 5-20

INSTRUCTIONS TO AUTHORS

Sri Lanka Journal of Forensic Medicine, Science & Law (SLJFSL) publishes original research papers, reviews, points of view, case reports, technical notes and letters to the editor, in all areas of Forensic Medicine, Forensic Sciences & relevant Law & Ethics.

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A soft copy of the manuscript, including figures and tables, should be submitted to the editor in Microsoft Word format: Dr. Induwara Gooneratne, Editor, SLJFMSL, Dept. of Forensic Medicine, Faculty of Medicine, University of Peradeniya (induwarag@yahoo.com). The paper should be typesetted with double spacing. All pages should be numbered. The manuscript should be divided into the following sections, each of which should begin on a separate page: Title Page, Summary/ Abstract, Text, Acknowledgements, References, Tables, Figures and Legends. Authors are encouraged to email or e-submit (through the webpage) articles in the above format. The title page should give the full title, names of authors with qualifications, posts held during the study, department(s) and institution(s) where the work was carried out, and the name and full address (including telephone number, emails) of the author for correspondence.

The summary/abstract should not exceed 250 words and should illustrate what was done, the main findings and conclusions. Up to five Key words should be given under the Summary.

The text of full papers should be divided into Introduction, Materials and Methods, Results, and Discussion. Only generic names of drugs should be given, if applicable. Abbreviations should be spelt out when first used in the text. Scientific measurements should be given in SI units. Statistical methods should be specified in the methods section and any term which is not in common usage should be referenced.

Tables and figures should be referred to in the order of appearance in the text in Arabic numerals within parentheses, e.g. (Fig. 1). Tables with brief titles should be typed on separate pages. Figures should be used only when data cannot be expressed clearly in any other form. Photographs should clearly show the figure number and caption, and attached as jpg files or inco-operated into the MS word document.

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2. The submission file is in Microsoft Word format

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