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Pilot Study on Newly Developed

Highlights

Trial of Immunotherapy in HIV

Prevalence of Indigestible Foreign

Discovering Thoughts, Inventing Future

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Interprofessional Team Collaboration in Health Care

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Introduction- Health care is a multifaceted activity which requires health care professionals to work together for the patient or service users in a collaborative way to deliver the desired outcome. Hospitals are complex organisations humming with activities of heterogeneous groups of people such as doctors, nurses, paramedical and administrative staff, all working with a common goal of providing health care to service users (Kaini 2005, p.1). Health care professionals work together in a collaborative manner in various forms. It involves complex interactions between two or more members of different professional disciplines (Reel and Hutchings, 2007, pp.137). In a basic form, health care professionals consult their patients or service users and, each other as required, about the services needed by their service users. In more complex form of care, health care professionals work more closely, identifying together with service users what care services are required, who provides them and what adjustments need to be made to the health care plan and management. WHO (2010) asserts that 'it is no longer enough for health workers to be professional, in the current global climate, health workers also need to be interprofessional (WHO, 2010, pp.36).

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INTERPROFESSIONALTEAMCOLLABORATIONINHEALTHCARE

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Interprofessional Team Collaboration in Health Care

Bachchu Kailash Kaini

I. Introduction

Health care is a multifaceted activity which requires health care professionals to work together for the patient or service users in a collaborative way to deliver the desired outcome. Hospitals are complex organisations humming with activities of heterogeneous groups of people such as doctors, nurses, paramedical and administrative staff, all working with a common goal of providing health care to service users (Kaini 2005, p.1). Health care professionals work together in a collaborative manner in various forms. It involves complex interactions between two or more members of different professional disciplines (Reel and Hutchings, 2007, pp.137). In a basic form, health care professionals consult their patients or service users and, each other as required, about the services needed by their service users. In more complex form of care, health care professionals work more closely, identifying together with service users what care services are required, who provides them and what adjustments need to be made to the health care plan and management. WHO (2010) asserts that 'it is no longer enough for health workers to be professional, in the current global climate, health workers also need to be interprofessional (WHO, 2010, pp.36).

WHO (2010) further states that the world is facing a shortage of health workforce and policy makers are looking for new and innovative ways that can help them develop policies and programmes to bolster the global health workforce. Interprofessional team collaboration in health care is essential for the development of a collaborative practice friendly health work force, one in which all health care professionals work together to provide all kinds of services in a hospital. Different health care professionals have their own background, defined roles and responsibilities, code of practice and expertise. The objective of their presence in health care set up is only to offer the best possible service to alleviate or improve service users' health problem.

It was felt that the interactions between health care professionals in the past have been limited. Concepts of specialities and sub-specialties are emerging in health care. Most of the service users are aware of their treatment and care plans due to easy access of clinical and health care information. Different health care professionals such as nurses, doctors, bio-medical scientists, radiographers, pathology technicians etc are interdependent or associated to each other. Therefore, patient care in isolation is impossible. According to Parsell and Bligh (1999), the borders clarifying the rules, roles and responsibilities of different health care professionals are now less distinct due to the increasing similarity of knowledge and skill.

The range and complexity of factors that influence health and well-being, diseases and illnesses require health care professionals from all specialties and groups to work together in a comprehensive and collaborative manner (Canadian Nursing Association, 2005). For example, health service users need information about various health issues for prevention and treatment of diseases and illness, immunisation, screening for disease prevention, diagnosis of their health problems, continuous support for behavioural change and monitoring of management plans for long term health issues. Working together and collaboratively in an interprofessional care team and the combined knowledge, skills and expertise of health care professionals become a very strong tool to enhance the health of the entire population served (Canadian Nursing Association, 2005).

II. Interprofessional Team

Interprofessional involves joint working and interactions between health care professionals. It is a collaborative working (Leathard, 2003) in which health care professionals share a common purpose of developing mutually negotiated goals (Payne, 2000) which are achieved through agreed care plans, management and procedures (Colyer, 2012). For interprofessional care to happen in practice, health care professionals pool their knowledge, skills and expertise (WHO, 2010) and make joint decisions based upon the shared professional view points (Canadian Interprofessional Health Collaborative, 2010). Kane (1983) defines the term 'interprofessional team' as having a common objective, differential professional contributions and a system of communication.

Interprofessional care is the processes for providing the best health services to service users and helped to achieve the optimal desired outcomes and service users' satisfaction. The Health Force Ontario

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(2007) defines interprofessional care in its report '*Interprofessional Care: A Blueprint for Action in Ontario*' and states that it is 'the provision of comprehensive health services to patients by multiple health caregivers, who work collaboratively to deliver quality care within and across settings'. The Health Force Ontario (2010) further states that interprofessional care is 'a collaborative, team-based approach to providing optimal patient care and it benefits and empowers patients, and significantly improves health care provider satisfaction'.

III. Health Care Professionals

The health care team is composed of a number of professionals of different backgrounds, education, training, experiences and theoretical viewpoints. They differ not only in the resources they bring to the team, but also in role expectations, status, and the extent of their legal responsibility for the service users (Duncanis and Golin, 1979). Among the professions often represented on the team are medicine, nursing, allied health professions (AHPs) and health care management professionals. Duncanis and Golin further assert that each health care team is a unique blend of professional and personal characteristics of its members, its effectiveness determined largely by the dynamics of that configuration.

Moreover, different disciplines in health care have different philosophies and different problem solving styles. For the benefits of service users and health care professionals, they have to work on the team structure. Each health care team develops certain rules of operation, certain ways of proceedings to accomplish its task. These may range from unwritten or informal group norms of behaviour to formal written procedural manuals (Duncanis and Golin, 1979). Drinka and Clark (2000) assert that there are various specialties in health care and different types of technical skills to be learnt and knowledge to be acquired in health care. However, there are overlaps in some of the main bodies of knowledge and skills that underline different health professions. Collaboration is one of the characteristics of the team and team members' relationship depends on how they collaborate and who they work with.

a) Roles and Responsibilities of Health Care Professionals

Roles are associated with assigned tasks or behaviour that is expected to be performed by an individual or a team. Sullivan (1998) asserts that responsibilities refer to accepting accountability for views expressed, and ultimately for the decisions made. Without defined roles and responsibilities, health care professionals cannot perform effectively and that may create chaos in the complex work environment in health care organisations. Duncanis and Golin (1979) state that roles of team members are generally defined in terms of the particular professional competencies of each team member and the nature of the task to be done. In health care professionals' team, the roles that each member plays may be clinical and may serve a group dynamic function in the team. Each team member is assigned specific roles and responsibilities in the interprofessional care team.

Hornby and Atkins (2000) define role as a part to be fulfilled or carried by a health care professional or group to achieve shared goal and desired outcome which is essential for interprofessional care and collaboration between health care professionals. Roles and responsibilities of health care professionals are defined in their terms of contract and job description. They are bound to follow their professional norms, clinical practices, standards, organisational policies, procedures, protocols and guidelines. The Interprofessional Education Collaborative (2011) states that understanding of how professional roles and responsibilities complement each other in health care organisations are important part of their professional life.

Julia and Thompson (1994) describe two kinds of team roles – task and maintenance roles. They further mention that these two roles assumed by the members are characterised to assess the degree to which individual participation either facilitates or hinders team process; and the concept of role applied to team process provides a way for team members to symbolise the active participation of every other member in a team. Lister (1982) describes roles in the interprofessional team into personal roles and professional roles. Personal roles are based on the personality, socio-economic and cultural factors whereas professional roles derived from occupational status. Lister further states that professionals may assume other team function roles based on either professional or personal roles, further complicating the analysis of team role function typically seen in team behaviour.

It is expected that health care professionals are well informed of their roles, responsibilities and professional boundaries, but in reality, this may not always the case (Barrett and Keeping 2005). Overlapping roles and expertise, extended roles and cross-professional working practice are the factors that may shadow the clear definition of their roles. For example, roles of podiatry team and tissue viability nurse may be conflicting while offering services to a patient with heel pressure ulcers. Bliss *et al* (2000) state that lack of clarity and misunderstanding regarding the boundaries of professional roles may be a factor in restricting the utilisation of relevant professionals within interprofessional practice. Overlapping and blurring professional roles in interprofessional care team can result in feelings of insecurity and anxiety and can weaken professional confidence (Barrett and Keeping 2005; Loxley 1997 and Booth and Hewison, 2002). Farrell et al (2001) study informal roles in team

development stages as described by Tuckman (1965) in his team development model and conclude that informal role differentiation is observed at the beginning stages of team development and begins to diminish in the later stage.

Health care professionals and service users define their roles for themselves and other team members based on their experience, learning and the need of the services. Furthermore, they act within the defined and agreed roles in health care organisations and the society. Leiba (1994) states that health care professionals and service users must ensure flexibility and willingness to modify or even exchange their roles according to the needs of individual cases for effective interprofessional care and collaborative practice. The roles that a health care professional plays and the way people evaluate them in the society are important to maintaining a good self image. Hornby and Atkins (2000) assert that the self image of health care professionals and the image created by the society have a very strong impact on interprofessional care and collaboration.

Miller *et al* (2001) state that if health care professionals have detailed and accurate knowledge of other health care professionals' roles and boundaries, they are able to assess service users need when it is appropriate to refer to another member of the team for further treatment or assessment. It is argued that health care professionals should remain flexible at the professional boundaries of their roles in order to develop team knowledge and skills. Therefore, the requirement for health care professionals to be role flexible is fundamental to health service delivery.

Hidden roles create misunderstanding of professionals' roles and responsibilities. It may be due to lack of clarity of roles or unseen tasks that a health care professional is assigned to carry out. If health care professionals from two different teams or organisations work together, there may be different policies, protocols and practices in place. Such practices also create confusion in clarifying health care professionals' roles. Miller *et al* (2001) state that the differentiation of roles and the way in which non task based roles can develop are two factors to consider when examining the nature of other health care professionals' role contribution. Health care professionals get an opportunity to understand the roles of other professionals by working together in the close vicinity. Moreover, it makes interprofessional care more collaborative as everyone can easily engage in interaction and in-depth communication about specific issues and close observation of practices.

Health care professionals have to play non clinical roles in their day-to-day jobs. Non clinical roles include business planning, administrative and managerial, service development and improvement, commissioning, customer services, leadership, academic writings, teaching, tutorials, clinical governance and risk management, policy formulation and reviews, evaluation, monitoring etc. Understanding of non clinical roles helps to overcome divisions between health care professionals or different groups (Miller *et al,* 2001). Leathard (2003) asserts that health care professionals no longer enjoy the security of structured and defined traditional roles and changes have been noted from 'practice based training' to 'university based education' in nursing, therapy and social work.

The nature and complexity of the health issue of service users define the roles and tasks in which a group of health care professionals interacts and engages. A task for health care professionals can be an assessment, review, clinical judgement, intervention, clinical decision, referral, diagnosis, treatment or any other health services performed by them in relation to a service users' health issue. The Canadian Health Services Research Foundation (2006) states that the greater the interdependency of health care professionals, the higher the level of collaboration required to perform their tasks and to achieve the optimal desired outcomes. Miller *et al* (2001) assert that the role understanding is a complex issue as it consists of understanding others' roles, defining on how roles are achieved in daily job and understanding of the rationale behind a professionals' contribution.

Health care professionals have a shared goal of providing good care to all service users. However, in the practical scenario; the different roles, responsibilities and core values between health care professionals means the issues arising in day-to-day practice may vary (Reel and Hutchings, 2007, pp.144). Therefore, it is important to recognise and respect each other's roles, responsibilities, opinions, expertise and work stresses. This is required to play an effective role of a member of interprofessional care team.

Orchard *et al* (2005) suggest that members of a health care professional team should be aware of their role and expertise and they should be confident in their own capabilities, recognise the professional boundaries of their scope of clinical practice, be committed to the values and ethics of their own profession and be knowledgeable of their own practice standards. The Health Professions Regulatory Networks (2008) asserts that health care professionals must also be accountable for and committed to maintaining effective communications with other members of the interprofessional health care team, and promote team problem solving, decision making and collaboration by applying principles of group dynamics and conflict resolution.

Interprofessional Education Collaborative (2011) asserts that health care team member's roles and responsibilities vary within legal boundaries and actual roles and responsibilities change depending on

the specific care situation and sometimes as specified in the terms of references of the job. Many times health care professionals cannot communicate their own role and responsibilities to other colleagues properly. In such a condition, they cannot communicate others what they do, cannot understand what other professionals do and how others can help them to deliver an effective health services.

Health care professionals' roles evolved over time and it may be difficult to some health care professionals when other colleagues are taking on some of their roles and it may be relief for others as their colleagues helping them to perform their tasks (Reel and Hutchings, 2007, pp. 147). Gorman (1998) states that roles in a high performing team can be fluid and roles of health care professionals in an interprofessional team get passed back and forth, for example, leadership will shift from person to person as the circumstances demand. Roles of health care professionals in hospitals are limited by legal requirements and they have to exercise their professional skills and expertise with due care and diligence.

b) Skills and Competence for Interprofessional Collaboration

Health care professionals exposed in theoretical and practical education, training and personal development during their education and career in their own field and gain strong discipline based knowledge, skills and capability that give access to professional jurisdictions. Therefore, other health care professional groups may have limited understanding of the complexity of relationships between them (D'Amour *et al.* 2005).

Hornby and Atkins (2000) assert that relational, organising and assessment skills are main three collaborative skills required for health care health care professionals. Relations skills are more about interaction and communication skills whereas organising skills are required for organising groups, meetings, setting up patient referral systems etc. Assessment skills are related to collecting, analysing and reflecting in evidence. Hammick *et al* (2009) suggest the following three categories of basic competencies for being an interprofessional practitioner.

Knowledge

- Understand the role and working context of other practitioners.
- Recognise the range of knowledge and skills of all other colleagues.
- Understand the principles and practice of effective teamwork.

Skills

- Apply sound verbal and written communication methods.
- Identify situations where collaboration is helpful or essential.
- Work collaboratively with service users and carers.
- Use interprofessional learning in work settings.

Attitudes

- Appreciate the value of interprofessional collboration.
- Acknowledge and respect others' views, values and ideas.

(Hammick *et al,* 2009; pp. 23)

Hammick and colleagues state that combining the knowledge, skills and attitudes enables a health care professional to be a competent practitioner. As health care professionals' careers develop and they move forward to more senior positions, their role require them to have more advanced interprofessional competencies. However, values for the interprofessional competencies such as respect for everyone, willingness to engage, a caring disposition towards colleagues and an appropriate attitude remain the same for all levels of professionals (Hammick *et al,* 2009, pp;23)

CHSRF (2006) asserts that integration of new health care professionals into clinical practice requires an orientation on the knowledge, skills, and attitudes needed for interprofessional care and teamwork, interactional factors and change management. A team development guidance or strategy that focuses on developing and sustaining capacity at the organisational and work or local level is also vital for the integration of health care teams into clinical practice.

Interprofessional Education Collaborative (IPEC, 2011) published an expert report -'*Core Competencies for Interprofessional Collaborative Practice: Report of an Expert Panel'* in 2011 and highlights the following competencies for interprofessional collaboration:

- Values/Ethics for Interprofessional practice.
- Roles/responsibilities.
- Interprofessional communication.
- Teams and teamwork.

Canadian Interprofessional Health Collaborative (CIHC, 2010) published '*A National Interprofessional Competency Framework*' and mentions the following six competency domains for collaborative practice:

- Interprofessional communication.
- Patient/client/family/community centred care.
- Role clarification.
- Team functioning.
- Collaborative leadership.
- Interprofessional conflict resolution.

These competencies focuses on the ability to integrate knowledge, skills, attitudes and values in arriving clinical judgements rather than relying on the demonstrated behaviours to demonstrate competence (CIHC, 2010). Engel (1994) highlights the ability to use an understanding of group dynamics, adapting change and participating in change, communication, understanding of how the interaction and productivity of the team as a whole tends to change over time as important competencies for interprofessional collaboration. Furthermore, Engel discusses managing self, managing with others, communication, negotiation, seeking and giving advice as other competencies for the same.

Health care professionals competencies gained through academic qualifications, training or experience may be diminished unless these skills are used frequently or at least practised intermittently in simulated situations (Engel, 1994; pp.72). Therefore, it is the responsibility of health care professionals, managers and leaders to arrange continuing professional and personal development to practice these skills and knowledge in different health care set ups. Hammick *et al* (2009) argue that health care professional understand the values, knowledge and skills of others in the health care team so that everyone can contribute in a harmonised and better way.

c) Impact of Interprofessional Collaboration

Health services are designed to provide the best possible care to service users and families, to improve the quality of life, to alleviate health issues and improve the health conditions. The main objective of IPC is to bring a broader scope of health care professionals' knowledge, skill and expertise to the efforts to improve the quality of care and clinical outcomes related to service users' health problems and issues. The main question of interprofessional collaboration is whether interprofessional care is benefiting patients, service users, their families, health care professionals and the health system. Interprofessional collaboration comes into practice to ensure that health care professionals can complete a care task or combination of tasks that they could not achieve effectively on their own (Reeves et al, 2010). According to Schmitt (2001), the impact of interprofessional collaboration should be assessed across the range of problems for which the health care team has been formed and operated. Effective health care cannot be achieved in isolation. The health care delivery system is based on a sequence of co-ordinated activities of professionals from various disciplines. According to Wanger (2004), it requires synchronised and rigorous efforts from all health care professionals and individuals and an appropriate care delivery system.

Some authors and researchers suggest that the advantages of effective interprofessional team collaboration can be significant. The outcome of effective interpersonal team collaboration is improved and better patient care (Leathard, 2003; Payne, 2000; Overtveit *et al*, 1997; Miller *et al,* 2001; Hornby and Atkins, 2000). Some of the reasons forbetter patient outcomes mentioned by those scholars are that collaborative practices and team approaches help team function better and make appropriate decisions for service users, co-ordinated and integrated action, capabilities to cope up with stressful and multifaceted environment, combined skills, knowledge and expertise for dealing with complex health problems and team synergy.

Barrere and Ellis (2002) confirm that interprofessional collaboration between doctors and nurses was a fundamental factor in positive patient outcomes regardless of the severity of a patient's condition. Weschules *et al* (2006) carried out a research in primary care and hospital set up and confirmed that improved patient outcomes have been demonstrated in studies of collaboration between pharmacists and physicians, and when pharmacists are included as part of the health care team. O'Brien-Pallas et al (2005) have also gathered the evidence of the positive outcomes of nurse-doctor collaboration in Canada. A report by Oandasan e*t al* (2006) '*Teamwork in health care: Promoting effective teamwork in health care in Canada: Policy Synthesis and Recommendations*' has also recommended interprofessional collaboration as an effective way to reduce stress, burnout among health care professionals, to improve the quality of care and enhance patient safety.

Various research findings have linked the outcomes of interprofessional collaboration with mainly service users, health care professionals and health care organisations or systems. The Health Professions Regulatory Network (2008) highlights the following outcomes associated with collaborative practice for service users, health care professionals and health care organisations:

Outcomes of collaborative practice for service users/ patients:

- Improved patient satisfaction.
- Improved patient transfer and discharge decisions.
- Improved patient care and outcomes.
- Decreased risk-adjusted length of stay for patients.
- Reduced medication errors.

Outcomes of collaborative practice for health care professionals:

- Improved job satisfaction.
- Decreased job associated stress.
- Lower nurse turnover rates.
- Improved communication among caregivers.
- Improved efficiency.
- Improved understanding of roles.

Outcomes of collaborative practice for health care organisations

- Decreased costs
- Improved efficiency of health care providers

(The Health Professions Regulatory Network, 2008; pp.3)

IV. Conclusion

The main objective of interprofessional care is to deliver the most optimal public health services, which requires looking atproblems from various medical and nursing perspectives and, hence, to make compromises (Pecukonis, *et al,* 2008). In terms of employment health care is one of the biggest industries. There is a considerable pressure as high costs involved with an increasing demand in an ageing society. In order to fulfil the demands and to provide high-level public health services, the medical and nursing staff need to share their learning and optimise their collaborative efforts. As various professions have different norms and habits collaboration is extremely vital for the delivery of efficient health services. Through collaborative practices, health care professionals are also able to learn from each other and to discover more about themselves and other colleagues. Sullivan (1998) asserts that health service delivery is an interactive process and requires coherent and aligned efforts to continuously review roles and responsibilities of health care professionals.

As health care workers professionals dedicate their time and efforts to provide the best possible care to patients and families to improve the quality of life, to alleviate health issues and improve the health conditions. Both from the perspective of their interest as health service providers and from the perspective of hospitals as places of learning, efficient teamwork and high quality health service provision are needed. There is emerging evidence that service users are benefiting from new ways of joint working and interprofessional team collaboration.

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A Hydrodynamics based Proposal to Substitute Heparin by Drag Reducing Polymers

By W. Lilienblum & A. Pinkowski

Introduction- Blood exhibits a non-Newtonian rheology, i.e., its shear-rate to shear-stress relationship is non-linear, i.e., one has to apply a threshold force, the so-called yield-stress before it moves at all. This particularity is due to the composition of blood and the particular qualities of its components (Boron et al., 2005). For our purpose we will consider that blood consists mainly of plasma with near-Newtonian flow properties and red blood cells (RBC) thus leading to a two-phase flow behavior where the plasma acts as the carrier phase and the RBC as suspended therein liquid-drop-like carried phase (Pinkowski, Lilienblum, 2015). At low shear rates (low velocity gradients) RBC tend to form rouleaux structures and these primary, randomly scattered rouleaux tend also to group together to form secondary rouleaux structures (Kulicke, 1986). Fibrinogen adhered to the vessel wall forms together with these secondary rouleaux fibrinogen filaments leading to increased viscosity at low shear rates. These fibrinogen filaments can be considered as precursors of blood clots. The key component in hemostasis is an elongated glycoprotein in the plasma that through activation by thrombin self-assembles into a first fibrin clot (Brown, J.H. et al. 2000).

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W. Lilienblum [α] & A. Pinkowski [σ]

I. Introduction

Blood exhibits a non-Newtonian rheology, i.e., its shear-rate to shear-stress relationship is non-linear, i.e., one has to apply a threshold force, the so-called yield-stress before it moves at all. This particularity is due to the composition of blood and the particular qualities of its components (Boron et al., 2005). For our purpose we will consider that blood consists mainly of plasma with near-Newtonian flow properties and red blood cells (RBC) thus leading to a two-phase flow behavior where the plasma acts as the carrier phase and the RBC as suspended therein liquid-drop-like carried phase (Pinkowski, Lilienblum, 2015). At low shear rates (low velocity gradients) RBC tend to form rouleaux structures and these primary, randomly scattered rouleaux tend also to group together to form secondary rouleaux structures (Kulicke, 1986). Fibrinogen adhered to the vessel wall forms together with these secondary rouleaux fibrinogen filaments leading to increased viscosity at low shear rates. These fibrinogen filaments can be considered as precursors of blood clots. The key component in hemostasis is an elongated glycoprotein in the plasma that through activation by thrombin self-assembles into a first fibrin clot (Brown, J.H. et al. 2000).

Heparin is used to treat and prevent blood clots in the veins, arteries, or lung, like venous thrombosis, pulmonary embolisms, coagulopathies and coronary artery clots. It is used also before surgery to reduce the risk of blood clots. Common side effects of heparin, however, are easy bleeding and bruising. Patients with renal failure have an increased risk of bleeding (Levine et al., 2001). Therefore it seems to be worthwhile to speculate about a possible replacement of heparin by another blood-thinning drug without the drawbacks of heparin mentioned.

Although it is commonly assumed that heparin produces its anticoagulent effect by inactivating thrombin and activated factor X through an antithrombin-dependent mechanism, a deeper knowledge of this noncoagulant action is considered still as very limited (Drewlo, 2013).

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However, from an hydrodynamic point of view the systemic (intravenous) administration of heparin will reduce the blood viscosity (Chandran, 2007). In other words the blood-thinning action of heparin consists also in facilitating the displacement of red blood cells (RBC) and inhibiting their clumping. This way the typically non-Newtonian flow pattern of blood becomes more Newtonian-like, i.e., more laminar. Hence the lower coagulation tendency due to heparin results in a better flowability of blood. At this pont it is important to point out that this better flowability tendency is valid also at rest of the blood flow, i.e., during the coagulation process. This means in turn that the coagulation process of any wound injury will slow down, i. e., heparin will deteriorate wound healing.

As was shown in detail in a previous publication (Pinkowski, Lilienblum, 2015) drag reducing polymers (DRP) in nanomolecular concentrations are capable to achieve the same effect of better flowability, i.e., they can smooth out local micro-turbulences and this way laminarize blood flow. However, it is crucial to stress at this point that this laminarizing effect of DRP vanishes at rest, i.e., the coagulation process will not slow down contrary to the action of heparin. Systemic administration of DRP into the blood circulation system have a great medical potential as was proved in vivo for many provoked lethal deseases. It was shown e.g. to be effective against atherosclerosis (Faruqui et al., 1987), and against provoked lethal hemorrhagic-shock in rats (Macias et al., 2004; Kameneva et al., 2004).

DRP injection was proposed as a novel hydrodynamic approach for the tratment of coronary artery disease (Pacella et al., 2006). Among the different polymers used for drag reduction the FDA approved water soluble polyethylene glycol (PEG) is clearly the favorite. It is also used as antifoaming agent in food (US Government, 2011). Its INS number is 1521 in the USA and E1521 in the EU resp. (Codex Alimentarius, 2012). The international nonproprietary name for PEG used in medicine is Macrogol.

Depending on the actual Reynolds numbers (Re) on distinguishes in blood rheology four different flow pattern: At high Re - turbulent flow (where the normally parabolic velocity profile becomes blunted), at medium Re - laminarity, at low Re - RBC-rouleaux, and at very low Re - the Fåhraeus-Lindqvist region (Fåhraeus-Lindqvist, 1931): in small vessels between 10 and 300 micrometers, the viscosity decreases with

decreasing tube diameter due to accumulation of RBC in the vessel center. This tendency of RBC accumulation in the vessel center leaving the plasma in the vessel wall region RBC depleted is also referred to as plasma skimming (Boron et al., 2005).

In turbulent flow the mass transfer is considerably enhanced and this is true also for local microturbulences in small vessels and at low blood flow velocity as was shown previously (Pinkowski, Lilienblum, 2015). Any blood agitation and increase in blood flowability however is counteracting the coagulation process. Despite the decreased Re numbers in small vessels the decreased blood flow favors RBC aggregation which in turn is a source of local vortices with enhanced mass transfer. Systemic administration of DRP inverses the flow situation thus favoring the anticoagulation. DRP act however only during flow which means that at rest the normal coagulation capacity of a patient will not be altered.

Due to the hypothetical character of the present proposal verification by animal models before any clinical trial are mandatory.

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Pilot Study on Newly Developed Botanical Larvicides and Repellents against *Aedes* Mosquitoes in Myanmar

By Htin Zaw Soe, Sein Min, Maung Maung Mya, Khine Khine Lwin, Aye Win Oo & Myat Khine

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Abstract- Dengue Haemorrhagic Fever (DHF) is one of the major public health problems in Myanmar. There are no effective vaccine and specific drug for DHF and its containment is totally based on vector Aedes mosquito control. Thus the present study was conducted with the general objective of developing innovative environment-friendly vector control tools mainly focusing on the plant sources. The test plants – Caesalpinia pulcherrima Linn. And Ervatamia coronaria (Jacq) Stapf. were locally searched in Magway – central Myanmar, extracted, screened and tested against Ae. Aegypti larvae and adults under the laboratory conditions, and in field trials preceded by animal acute toxicity and skin irritation tests in line with standard procedures and guidelines of WHO and OECD from August through September, 2015. Indepth interviews were undertaken among local residents to evaluate the public acceptance on new control tools. Test plant leaves contained some phytochemicals with larvicidal and repellent properties.

Keywords: *botanical larvicides, repellents, aedes mosquitoes.*

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Pilot Study on Newly Developed Botanical Larvicides and Repellents against *Aedes* Mosquitoes in Myanmar

Htin Zaw Soe [α], Sein Min [σ], Maung Maung Mya [ρ], Khine Khine Lwin [ω], Aye Win Oo [¥] & Myat Khine [§]

Abstract- Dengue Haemorrhagic Fever (DHF) is one of the major public health problems in Myanmar. There are no effective vaccine and specific drug for DHF and its containment is totally based on vector *Aedes* mosquito control. Thus the present study was conducted with the general objective of developing innovative environment-friendly vector control tools mainly focusing on the plant sources. The test plants – *Caesalpinia pulcherrima* Linn. And *Ervatamia coronaria* (Jacq) Stapf. were locally searched in Magway – central Myanmar, extracted, screened and tested against *Ae. Aegypti* larvae and adults under the laboratory conditions, and in field trials preceded by animal acute toxicity and skin irritation tests in line with standard procedures and guidelines of WHO and OECD from August through September, 2015. Indepth interviews were undertaken among local residents to evaluate the public acceptance on new control tools. Test plant leaves contained some phytochemicals with larvicidal and repellent properties. LC_{50} values (95% FCI) of crude ethyl acetate leaf extract larvicides of *C. pulcherrima* and *E. coronaria* against *Ae. aegypti* larvae were 3.21 (2.95 – 3.48) and 4.46 (3.16 – 6.05) mg/l respectively. Their repellent ED_{50} values (95% FCI) against *Ae. aegypti* adults were 0.02 (0.01 – 0.03) and 0.01 (0.005 – 0.02) mg/cm^2 respectively. Their repellent percentage protection (mean ± SD) was 88.4±13.3 (dose, 1.6 mg/cm^2) and 82.1± 6.4 (dose, 0.4 mg/cm^2) at 90 min post application respectively. The results of animal acute toxicity and skin irritation tests using test extract/repellents showed the safe use of new control tools by human. In field trials it was found that larval mortality was 100% in minor water containers treated with *C. pulcherrima* larvicide (dose, 7.2 - 14.4 mg/l) and *E. coronaria* larvicide (dose, 12.7 – 25.4 mg/l) separately in 24 hr. Their repellent percentage protection (mean ± SD) was 98.3±1.4 (dose, 1.6 mg/cm^2) and 97.8± 2.3 (dose, 0.4 mg/cm^2) in 90 min respectively. The local residents were interested in, accepted and demanded the new control tools. In conclusion the present study highlighted that newlarvicides and repellents were found to be very promising to be safely and effectively used to control *Aedes* mosquitoes.

Keywords: botanical larvicides, repellents, aedes mosquitoes.

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I. Introduction

Dengue and dengue haemorrhagic fever (DHF) is one of *Aedes* mosquito-borne diseases. Globally about 2.5 billion people live in more than 100 dengue endemic countries and there are approximately 50 million dengue infections annually. About 500,000 DHF cases required hospitalization each year and case fatality rate is 2.5%[1]. Each year hundreds of thousands of severe cases occur including 20,000 deaths, with 264 disability-adjusted life years (DALYs) per million population lost[2]. Reported cases and deaths in the South-east Asia Region are 232,530 and 2,031 respectively in 2009[1]. In Myanmar average annual reported cases and deaths of DHF were 14,739 and 111 respectively in the last decade (2005- 2014). Case fatality rate was under 1%. Up till now there is no reliable effective vaccine and specific treatment for DHF. Thus prevention and control measures are vitally important which are mainly based on vector control methods. The routine vector control methods currently used have several limitations, for example, labour-intensive. Thereforemethods which are locally available, feasible, cheap, ecofriendly and acceptable to the public are urgently needed and to be innovated. In Myanmar botanical larvicides and repellents are rarely studied. The present study was conducted with the general objective of developing innovative environment-friendly vector control tools mainly focusing on the plant sources.

II. Materials and Methods

a) Test plants

Plant species *Caesalpinia pulcherrima* Linn. and *Ervatamia coronaria* (Jacq) Stapf. are found to have larvicidal and repellent activities against *Aedes* mosquitoes[3]. They are growing in and outside the compound of University of Community Health, Magway, Myanmar and authenticated at Department of Botany, Magway University for botanical names.

b) Extraction and screening of test plants

Thoroughly washed test plant leaves were separately shade dried at room temperature 32 ± 4C°. Each dried leaf powder sample (100 g) was separately mixed with the sufficient amount of solvent ethyl acetate

in Soxhlet apparatus and evaporated by a rotary evaporator at 70 - 80°C in Pharmacology Research Division, Department of Medical Research (DMR), Yangon.Finally left crude extract residue was taken, placed in a porcelain dish and stored in the desiccator.Theirdried leaves were screened by phytochemical methods[4]to investigate presence of bioactive compounds.

c) Test mosquitoes

Aedesaegypti Lin. were reared in the insectary of Medical Entomology Research Division(MERD), DMR and kept at 26 ±2 C° and relative humidity (RH) 70 - 80 % with a photoperiod of 10 hr light and 14 hr dark.

d) Larvicidal bioassays

Larvicidal activities of plant extracts were tested against *Ae. aegypti* larvae using the methods recommended by WHO[5] in MERD, DMR.*C. pulcherrima* crude extract material (0.2 g) was taken and mixed with 20 ml of acetone to get stock solution (1%). Appropriate volumes of stock solution were dropped into each of five glass beakers (250 ml) containing 200 ml of tap water to make serial concentrations of 1.563, 3.125, 6.25, 12.5 and 25 mg/l. Only acetone (1 ml) was put into sixth beaker containing tap water 200 ml as control. Each batch of 25 third and fourth instar larvae was gently introduced into all beakers. Dead and moribund larvae were counted as dead at 24 hr (25 – 26 C°/RH 72 – 74%). Six replicates of similar procedure were made and LC_{50} and LC_{90} values with 95% fiducial confidence intervals (FCI) were calculated using probit analysis. The same procedure was also carried out with *E. coronaria*crude extract.

e) Repellent bioassays

i. *For finding ED_{50} and ED_{90}*

To find out effective dose (ED) of *C. pulcherrima*crude extract against *Ae. aegypti* female adults, its stock solution (1%) was used with WHO guidelines[6]. Firstly four volunteers including one female from DMR were thoroughly explained about procedure of bioassays and their informed consent was obtained. They were instructed not to use cosmetics/perfumes/ scented soap and not to smoke one day before the bioassays. Those with history of allergy and serious reactions by mosquito bite were excluded. Before the test volunteers' forearm areas from wrist to elbow were measured. Average area of four volunteers was 501.1 ± 33.5 cm^2. Next their forearms were thoroughly washed and cleaned with tap water. Secondly the left forearm as control of one volunteer was evenly applied with 1 ml of diluent acetone using a glass rod (30 cm). His hand was protected with a soft plastic glove not to bite the mosquitoes. The diluent was air dried for one min and the forearm was then introduced into a stainless steel cage (30 cm × 30 cm ×30 cm) containing fifty 3- 4 day-old, one day-starved, nulliparous female *Aedes* mosquitoes. The numbers of mosquito landing/ probing on the exposed skin were counted during 30 sec. Thirdly the control forearm was withdrawn and evenly applied with 1 ml of 1% stock solution (extract 0.01 g/ml) as treated forearm and air dried for one min. Afterwards treated forearm was introduced into the same cage and mosquitoes landing/probing were counted during 30 sec. Then additional 1 ml of 1% stock solution was applied on that treated forearm and tested by same procedure till the treated forearm was applied five serial double the concentration doses cumulatively (ie. 0.01, 0.02, 0.04, 0.08 and 0.16 g/ml). Fourthly volunteer's right forearm applied with 1 ml acetone was inserted into the cage again as control. Finally percentage protection (p) was calculated using the formula $p = (C - T) / C \times 100$ where C is number of mosquitoes landing/probing on control forearm and T is on treated forearm (26 – 28C°/RH 70 – 79%). The same procedure was performed two replicates per volunteer by four volunteers. ED_{50} and $ED_{90}$with 95% FCIwere calculated usingprobit analysis. The same procedure was also conducted for *E. coronaria.*

ii. *For finding percentage protection*

Percentage protection of *C. pulcherrima* crude extract against female *Aedes* mosquitoes was investigated in line with WHO guidelines[6]. Time of the test was between 0800 hr and 1600 hr. Firstly the left forearmas control of one volunteer was evenly applied with 1 ml of diluent acetone. The diluent was air dried for one min and the forearm was then introduced into a stainless steel cage (30 cm × 30 cm ×30 cm) containing fifty 3- 4 day-old, one day- starved, nulliparous female *Aedes*mosquitoes. The numbers of mosquito landing/ probing on the exposed skin were counted during 3 min. Secondly his right forearm was evenly applied with 1 ml of 40% stock solution to get extract 0.4 g/ml (ie. approximately double the dose – 0.2 g/ml of its ED_{90}) and air dried for one min. Afterwards treated forearm was introduced into the same cage and mosquitoes landing/probing were counted during 3 min period. Next the forearm was withdrawn and introduced again into the same cage after 30 min interval. Similar procedure was performed for the period of 150 min. Control forearm was inserted into the same cage every time just before the treated forearm was inserted(25 – 28C°/RH 70 – 78%). The same procedure was performed two replicates per volunteer by four volunteers. The percentage protection (p) was calculated using the same formula $p = (C - T) / C \times 100$. Similar procedure was performed for the extract 0.8 g/ml (ie. double the first dose). The same procedures were undertaken for the *E. coronaria* crude extract at 0.1g/ml (ie. approximately double the dose – 0.05 g/ml of its ED_{90}) and 0.2g/ml (ie. double the first dose).

f) Animal acute toxicity tests and skin irritation tests

The tests were conducted at Laboratory Animal Service Division, DMR according to guidelines of Organization for Economic Cooperation and Development (OECD) 420[7]. For animal acute toxicity tests, 10 week old female mice *Musmusculus* (irc strain) were used (25 – 26C°/RH 72 – 74% with photoperiodicity 12 hr light and 12 hr dark). Each of six healthy test mice was fed with *C.pulcherrima* crude extract at 2000 mg/kg with the polyoxyethylene (20) sorbitanmonolaurate ('Tween' 20) as a vehicle. Each of six control mice was provided with 1 ml 'Tween 20'. Then all mice were watched for 14 days for signs of mortality and toxicity. Similar procedure was conducted for *E.coronaria*. For skin irritation tests,lab-reared 4 month old female guinea pigs *Caviaporcellus* were used. Hairs on the area (4 cm × 4 cm) of the backside of each of three healthy guinea pigs were removed by shaving. The resultant bare areas were applied with *C.pulcherrima* crude extract prepared with acetone at 1.6 mg/cm^2(ie. approximately four times its ED_{90}). One control guinea pig was applied with 1 ml of acetone. Next they were monitored whether they developed skin reactions in 72 hr (25 – 26C°/RH 72 – 74%). Similar procedure was performed with *E.coronaria* at 0.4 mg/cm^2(ie. approximately four times its ED_{90}).

g) Field trials

Study area and period: Trials were conducted in Ward *Aungmingala* purposively selected inMagway Townshipin August -September, 2015 because of its highest proportion (35.7%) of DHF cases (10 cases) in 2015. House Index, Container Index and Breteau Index of the ward in July 2015 were 18%, 23% and 82 respectively.

Larval survey and introduction of test larvicides: Larval survey was conducted at 50 randomly selected houses.Next out of 48 randomly chosen *Ae.aegypti* larva-positive minor water containers (flower vases and spiritual bowls) in and around the surveyed premises, 24 containers were marked and treated with *C. pulcherrima*larvicide at 7.2 – 14.4 mg/l (ie. its LC_{90} to twofold dose) and remaining 24 containers with *E. coronaria*larvicide at 12.7 – 25.4 mg/l (ie. its LC_{90} to twofold dose). All treated containers were checked for larval mortality at 24 hr.

Percentage protection of test repellents: Field trials were conducted for two days using the methods by Choochote W et al for percentage protection[8]with the help of four well-trained male volunteers from Medical Entomology Section of Health and Disease Control Unit, Nay Pyi Taw during 0800 – 1600 hr. Each volunteer had to sit indoors and catch/count *Aedes* mosquitoes in nine assigned houses at least 10 metres apart from each other. Using mosquito coils, burning thrash and smoking in and around the premises by householders were not allowed. Firstlyvolunteer's legs were thoroughly washed and cleaned with tap water and right leg was treated with *C. pulcherrima* crude extract (dose:1.6 mg/cm^2). Control left leg was treated with acetone 1 ml. Areas of both legs above knees and below ankles were covered with short trousers and socks respectively to prevent the mosquito bites. The volunteer had to sit indoors and count/catch mosquitoes landing/probing on exposed areas of both legs within 10 min with mouth aspirators. The mosquitoes caught were kept in a paper cup for species identification and calculation of landing/ biting rate. After 10 min at first house volunteer moved to his second assigned house and took the similar functions for 10 min. This procedure was completed in 2 hr. Volunteers performed their second replicate in different houses. Then percentage protection within 90 min exposure was calculated using $p = (C - T) / C \times 100$. Next day the same procedure for two replicates was carried out for *E. coronaria*(dose: 0.4 mg/cm^2) in different houses (28 – 34 C°/RH 48 - 72%).

h) Indepth interviews

Ten local residents were recruited at Day 7 of field trials and Principal Investigator (PI) disseminated field trial results. Next indepth interviews were performed by PI himself. Their opinions on results of new larvicides and repellents in their ward were mainly elicited and their actual wordings were recorded, transcribed and translated into English.

i) Statistical analysis

LC_{50}, LC_{90},ED_{50} and ED_{90} were calculated by probit analysis using SPSS version 16.0. Chi-squared test was used to find out homogeneity of test mosquitoes and paired t test to find out significant difference between landing/biting rates at significance level 0.05.

j) Ethical considerations

Research proposal was submitted to Ethical Review Committee of University of Community Health, Magway and ethical clearance was obtained. Informed consent from study volunteers in laboratory and field trials was also received.

III. Results

a) Laboratory results

Preliminary phytochemical tests on dried leaf powder of *C. pulcherrima* showed that it contained carbohydrates, α-amino acids, phenolic compounds, tannins, saponins, steroids, alkaloids, glycosides and reducing sugar. Those of *E. coronaria* also had similar compounds except saponins (Table 1).

Table 1: Results of preliminary phytochemical tests on dried leaf powder of *C. pulcherrima* and *E. coronaria*

Test for	Extract	Test reagent	Observation	Results*	
				C.pulch-errima	*E.coro-naria*
Carbohydrates	H_2O	10% α-napthol + concentrated H_2SO_4	Pink ring	+	+
α-Amino acids	H_2O	Ninhydrin reagent	Red	+	+
Phenolic compounds	H_2O	Ferric chloride solution	Deep brown	+	+
Flavonoids	Methanol	HCl/Mg	No colour	-	-
Tannins	H_2O	Ferric chloride solution	Blue black	+	+
Saponins	H_2O	Distilled H_2O	Frothing	+	-
Steroids	Petroleum ether	Acetic anhydride + concentrated H_2SO_4	Deep green	+	+
Alkaloids	10% acetic acid and EtOH	(i) Mayer's reagent (ii) Dragendroffs reagent	White precipitate Orange precipitate	+	+
Glycosides	H_2O	10% Lead acetate	White precipitate	+	+
Reducing sugar	Diluted H_2SO_4 + 5N NaOH	Benedict's solution	Brick red precipitate	+	+
Cyanogenic glycoside	H_2O	H_2SO_4 + Sodium picrate solution	No colour	-	-

* + = present, - = absent

Table (2) shows larvicidal activity of crude ethyl acetate extracts of *C. pulcherrima* and *E. coronaria*a gainst *Ae. aegypti* under laboratory conditions. Test mosquitoes were not in heterogeneity in the former (p = 0.577) and in heterogeneity in the latter (p = 0.009).

Table 2: Larvicidal activity of crude ethyl acetate extract of *C. pulcherrima* and *E.coronaria* against *Ae. Aegypti.*

Concentration (mg/l)	*C. pulcherrima*		*E.coronaria*	
	Mean mortality ± SD (%)	LC_{50} and LC_{90} (95% FCl) (mg/l)*	Mean mortality ± SD (%)	LC_{50} and LC_{90} (95% FCl) (mg/l)**
1.563	12.7 ± 5.9	3.21 (2.95-3.48)	6.0 ± 3.3	4.46 (3.16-6.05)
3.125	50.0 ±10.4	7.2 (6.42-8.29)	34.7 ± 7.9	12.71 (8.81-25.03)
6.25	82.7 ±7.4		74.0 ± 20.2	
12.5	99.3 ± 1.6		88.7 ± 10.3	
25.0	100.0 ± 0.0		96.0 ± 3.8	
Control	1.3 ± 2.1		1.3 ± 2.1	

* p = 0.577, ** p = 0.009

Table (3) shows repellent activity of crude ethyl acetate extracts of both test repellents against *Ae. aegypti* female adults under laboratory conditions.

Table 3: Repellent activity of crude ethyl acetate extracts of *C. pulcherrima* and *E.coronaria*against *Ae. aegypti*

Test repellent	ED_{50} (95% FCl)*	ED_{90} (95% FCl)*
C. pulcherrima	0.02 (0.01 – 0.03)	0.48 (0.28 – 1.35)
E. coronaria	0.01 0.005 – 0.02)	0.12 (0.08 – 0.16)

*mg extract / cm^2 skin

Table (4) expresses percentage protection of crude ethyl acetate extracts of both test repellents against *Ae. aegypti* under laboratory conditions.

Table 4: Percentage protection of crude ethyl acetate extracts of *C. pulcherrima* and *E. Coronaria* against *Ae. aegypti*

Test repellent	Concentration (mg/cm²)	% protection (mean ± SD)					
		Time post application of repellent (min)					
		0	30	60	90	120	150
C. pulcherrima	0.8	78.1± 13.3	67.8± 20.3	54.7± 21.0	43.7 ± 26.0	35.2 ± 24.3	32.2± 17.7
	1.6	94.4± 6.9	91.3± 9.7	88.7± 14.8	**88.4**± 13.3	**84.6**± 18.9	**84.3**± 21.4
E. coronaria	0.2	88.9± 4.8	63.1± 13.8	58.4± 17.0	51.4± 25.3	50.1± 26.9	50.0± 24.5
	0.4	93.3± 3.3	87.7± 4.9	83.4± 8.8	**82.1**± 6.4	**82.1**± 9.6	76.7± 10.8

All mice tested with both plant extracts were still alive and active at Day 14 without any toxic signs. Similarly in skin irritation tests there were no signs of irritation, erythema,escharand oedema formations in all tested guinea pigs at 72 hr.

b) Field trial results

Test larvicides of ethyl acetate extract of *C. pulcherrima* and *E. coronaria* were introduced into larvainfested minor water containers in surveyed houses separately andall larvae in treated containers were found to be dead at 24 hr. Total number of mosquito species caught during two days was 154 [*Ae. aegypti* (89.6%), *Ae. albopictus* (8.4%), *Culex quinquefasciatus* (1.6 %) and *Anopheles vagus* (0.7 %)]. Mosquito landing/biting rates were much lower in repellent treated skin than control and it was statistically significant ($p \leq 0.05$). Percentage protection was 98.3% by *C. pulcherrima* repellent and 97.8% by *E. coronaria* repellent during 90 min (Table 5).

Table 5: Repellent activity of crude ethyl acetate extracts of *C. pulcherrima* and *E. coronaria* against *Aedes* speciesin field trials

Test repellent/control	Concentration (mg/cm²)	Mosquito landing/biting rate per man-hr (mean ± SD)	% protection (mean ± SD)
C. pulcherrima	1.6	0.75 ± 0.69*	98.3± 1.4
Control		33.42 ± 21.19	
E. coronaria	0.4	0.83 ± 0.79**	97.8 ± 2.3
Control		34.59 ± 18.78	

Different from control: * marginally significant ($p = 0.05$), **significant ($p < 0.05$)

c) Indepth interviews

Ten local residents (two ten-household leaders, three housewives, two dependents, one businessman, one labourer and one basic health staff midwife) wereindepth interviewed at ward religious centre.

One of two ten-household leaders stated his opinion like:

'DHF is caused by mosquito bite. This year about 10 – 15 children in our ward were affected by DHF. We need more drugs to prevent mosquito bite. The currently tested larvicides and repellents are known to be effective in mosquito control. We want to use them.'

(60 year old male ten-household leader)

One of three housewives expressed as follow:

'DHF is a mosquito-borne disease. We use mosquito coils and bednets to avoid mosquito bite. When we know the good effect of currently tested larvicides and repellents, we want to use them at our homes.'(35 year old housewife).

IV. Discussion

The present pilot study is the first and foremost study of its kind ever in Myanmar. *C pulcherrima* Linn. and *E coronaria* (Jacq) Stapf. - were searched locally, collected and investigated for their larvicidal and repellent activities under laboratory and field conditions followed by evaluation of public acceptance on the use of these botanical control tools in a selected community.

C. pulcherrima (Family *Fabaceae*) known as *Seinbangale* is cultivated as ornamental trees in and around human dwellings and has several medicinal properties, for instance, anti-inflammatory. It has more than fifty chemical compounds like β-pinene and γ-terpinene. In the present study nine secondary metabolites were detected as a qualitative determination in its leaves including saponins which are anti-feedant and toxic to cold blooded organisms and insects[9]. Another test plant *Ecoronaria* (Family *Apocynaceae*) called *Zalat* is grown as an ornamental and fragrant flower in gardens. It contains 66 alkaloids and medicinal

properties such as antioxidant and anti-infection in animal model[10]. In the present study its leaves also contained same secondary metabolites as in *C. pulcherrima* except saponin as a qualitative determination. These metabolites have larvicidal properties damaging the tissues of mid gut and cuticle of mosquito larvae. Plantphenolics, terpenoids, alkaloids and saponins are larvicidal against *Aedes* mosquitoes and also have pesticidal actions.They also has repellent action against mosquito by acting locally or at distance from the human body by molecules that alter the functioning of mosquito's sensory motor systems and block its sense of smell from the host or have neurotoxic effects[11].

In larvicidal bioassays LC_{50} of *C. pucherrima* extract (3.21 mg/l) against *Aedes* larvae was lower than that of *E. coronaria* (4.46 mg/l). It may be due to presence of saponins in the former. When compared to other studies, LC_{50} values (mg/l) were 97.53 for ethyl acetate extract *E. coronaria* and 144.67 for ethyl acetate extract *C. pucherrimia*[12]. Therefore LC_{50}values of two test extracts of the present study were lower than those repellents. In repellent bioassays, ED_{50} of *C. pucherrima* extract (0.02 mg/cm^2) against female *Aedes* adults was higher thanthat of*E. coronaria*(0.01 mg/cm^2). Therefore the latter is more effective than the former. Regarding percentage protection, *C. pucherrima* repellent and *E. coronaria* had 88.4% at 1.6 mg/cm^2 and 82.1% at 0.4 mg/cm^2 respectively at 90 min post application. In this case the latter is also more effective than the former in terms of the dose at 90 min. When compared to 25% DEET (*N,N*-diethyl-3-methylbenzamide)its complete protection time at 0.83 mg/cm^2(25mg/30cm^2) was for 6.25 hr[13].In animal acute toxicity tests and skin irritation tests due to the lack of toxic symptoms till 14 day observations and no skin adverse effects till post application 72 hr the test plant extracts are considered safe for human use.

In field trials larvicidal efficacy of both test larvicides are satisfactory as the result of 100% mortality at 24 hour of *Aedes* larvae in the treated minor water containers. Similarly the larvicide can be used to treat the ant-traps as well as the miscellaneous containers like unused tires in the areas where solid waste disposal is not easily available. Like wise both test repellents were also found to be effective with percentage protection of approximately 98% against *Aedes* mosquitoes in 90 min. In the study by M Govindarajan et al[6] *C. pulcherrima* (dose, 5mg/cm^2) and *E. coronaria* (dose, 5mg/cm^2) gave 100% protection at 90 min and at 120 min respectively under the laboratory conditions. If higher percentage protection is desired, the treated dose should be double or treble. Botanical repellents are better than mosquito coils because these coils can cause indoor air pollution and subsequent development of respiratory tract disorders especially in children and sensitive individuals due to their ingredients of synthetic chemicals and coconut husk or saw dust.Regarding public acceptance, almost all householders representing the study area were found to be interested in and accepted and demanded these new control tools.

In conclusion, the present study highlighted that new larvicides and repellents were found to be very promising to be safely and effectively used to control *Aedes* mosquitoes – vector of deadly DHF.

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Trial of Immunotherapy in HIV Patients: Our Experience with the Immuno-Modulator Dithiodinicotinic Acid (CPDS) in 34 Congolese Patients

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Abstract- Background: ART had substantially improved the quality of life of PLWHA, but cannot alone eradicate the HIV reservoirs neither fully restoring the impaired immune system functioning. Its life long duration is associated with the risk of resistant strain emerging and metabolic disorders, mainly in resources constrained settings.

Objectives: To study the impact of an Immune modulator in PLWHA.

Method: In this prospective study, we used a immune modulator, 6,6'-dithiodinicotinic acid (CPDS) in 34 Congolese PLWHA (study group) for a 2 years period opportunely compared to 60 PLWHA who underwent ARV (control group). Data were analyzed using Mixed models using IBM SPSS Statistics v. 20.

Results: Both groups were comparable at the starting point. Globally, we observed a similar evolution of weight and the CD4 counts in both groups during the first 12 months period of study with a gradual increase and a peak by the 6th month. Immunotherapy group displayed higher values of CD4, CD8 lymphocytes and the CD4/CD8 ratio. There was no significant difference between risks of dying between both groups. RR: 0.953 {0.67; 1.35} neither in the rate of hospitalization.

GJMR-K Classification: NLMC Code: QW 940

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Conclusion: This study suggests the benefit of immuno-therapy in treatment of PLWHA and its possible association to ART.

I. Introduction

In spite of the declaration that the goal of anti retroviral treatment (ART) in 2015/16 is to prolong the patient's life and maintain the best possible quality of health and life[1], only few patients in the Democratic Republic of the Congo (DRC) and other developing countries have wide-scale access to ART.

Treating AIDS in the developing countries means working in a context of limited health-care infrastructures and resources, as well as technical, financial and human resources. The few resources available are mostly concentrated in capital cities. In addition, the ART is not accessible to many patients in Sub-Saharan countries. In DRC, the ART coverage was estimated at only 12% four years ago[2] and recently, prevalence of HIV infection among general population in the DRC is at 1.2% but only 101,324 HIV patients are under ART, which is an ART coverage of 32% [3].

Additionally, when available ART stock runs out, skipping and interruptions of treatment are not uncommon. Adherence to ART is still a big challenge in our setting, with the subsequent risk of resistance and immune system impairment. In a study including Monkole's hospital, 20% of patients were found not adherent to ART mostly because of food insecurity [4].

Undernutrition also may have short- and long-term effects on HIV-positive children. The short-term effects include impaired immunity, increased risk of opportunistic infections, morbidity, and mortality. The long-term effects include poor cognitive functioning, poor achievement of developmental milestones, and poor levels of education [5].

Despite great progress globally against food insecurity, its remains a big challenge in Sub-Saharan countries, with a prevalence of 12,9% [6]. In the DRC, 43% of infants aged from 0 to 59 months display chronic malnutrition, and 14% of women display chronic energy deficiency[3]. Immune functioning is one of the affected parameters in this circumstance [7].

In Sub-Saharan Africa, HIV diagnosis and treatment are still made very late, leading to early and higher (20-50 times) mortality after the initiation of ART, compared to non-HIV related mortality. Patients who initiate ART at low CD4 counts remain at risk for opportunistic infections for a substantially longer period than patients starting ART at higher CD4 counts, increasing their risk for serious morbidity and death, with tuberculosis (TB) being the most common opportunistic illness. While information on underlying causes of death among people on ART is lacking in sub-Saharan Africa, one study found 86% of deaths in the first year following ART initiation to be HIV-related (CNS infections, TB, Kaposi's sarcoma, pneumonia, and mitochondrial toxicity), with 7% due to immune reconstitution syndrome [8].

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Although the treatment of HIV patients with antiretroviral drugs (ARV) has dramatically reduced mortality and morbidity, other studies revealed the need of new strategies with various means of enhancing and /or restoring the host's immune system [9,10,11].

Cytotoxic T lymphocytes (CTLs) and Natural Killer cells (NK cells) can eradicate virus-infected target cells via the apoptosis process through the perforin/granzyme pathway [12]. In this study an immune-modulator was used to exploit this NK ability [13], and could be indicative of the broader use of immunotherapy in HIV treatment combined with HAART [14].

Even in recent years, in addition to ART, immunomodulatory treatment strategies have been investigated. Although repeatedly discussed as an alternative or supplement, these therapies lack proof of clinical benefit. An important example is the failure of the two large IL-2 studies. Some approaches are nevertheless addressed [14].

In 1970, Grassetti used an immune-modulator, an analog of Nicotinamide, Dithiodinicotinic Acid (CPDS or Carboxy pyridine disulfyde) on Swiss mice as an alkylating agent in inoculation-induced lung tumor. CPDS treatment resulted in a significant reduction in number and volume of metastatic nodes in the mice. There were no side effects, nor were there any teratogenic or mutagenic effects [15,16].

In the period from 1977 and 1995, CPDS was used in Italy to treat patients with lung cancer, who had been previously treated by surgery, chemotherapy, or radiotherapy. The results displayed a lack of metastatis or tumor growth, a long survival compared to the control group, and unimpaired biological functions[16, 17].

CPDS also is a potent immunomodulator that significantly increases the number and activity of NK cells and increases the lymphoproliferation of T lymphocytes[15].

Given the interest of these effects through non-specific defense in viral infection, we aimed to find out whether CPDS could be useful in HIV infection. The goal of this clinical trial was not to place the immune-modulator in competition with antiretroviral drugs but; on the contrary, to evaluate the clinical and biological value of using the CPDS to People Living With HIV/AIDS (PLWHA) as a supplementary support for protecting their immune capacities. Monkole is a General Referral Hospital located in a semi rural area in Kinshasa and with a good experience in HIV management in a dedicated unit for PLWHA[18].

Results found comparable clinical and biological indicators between the group of individuals under CPDS and the other under ART. We therefore suggest a trial with a combined use of ART and CPDS in order to augment therapy while patients are receiving HAART and to contain residual virus [19, 20, 21] .

This study has received the local ethic committee agreement (Approval reference N/Réf: 002/ CEFA-MONKOLE/CE/2002).

II. Patients and Methods

This study, a prospective case-control trial, was conducted during a two-year period (October 2002-October 2004) on 34 PLWHA regularly followed at Monkole Hospital, a semi-urban hospital located in a suburb area of Kinshasa, DRC. During the same period, 60 others PLWHA were under ART and were then opportunely checked as a control group.

a) Study design

All the individuals enrolled in this study group were PLWHA who met the following inclusion criteria: having a diagnosis of HIV infection confirmed by Elisa test and irrespectively of WHO clinical stage, providing an informed consent for the treatment. The immune-modulator was proposed only to patients who were unable to access ART (ART were to be bought by patients themselves, as this study occurred before the startup of Global Funds Program in our Hospital). Patients could leave the study when they wanted or when they could access ART. All patients received detailed information on the tested drug and on the protocol, and gave a verbal or written consent prior to participating in the study.

The control group included individuals eligible to ART with a WHO clinical stage of 3 or 4 and CD4 count less than 350/μl. Pregnant women and young children (aged less than 2-years-old) were excluded from this trial.

We monitored the following parameters: body weight, blood cells count, lymphocytes phenotyping, morbidity and mortality rate. Body weight was assessed at each hospital visit with a medical balance. Morbidity was collected from patient-reported or hospital-documented illness episodes in the medical history. Mortality was collected from the patient medical history.

To monitor the clinical course, we first collected the previous medical history of the participants, focusing on opportunistic diseases encountered at the start of the study and their occurrence over the duration of the study. Diseases encountered were: shingles (zona), tuberculosis, other lung infections, enteritis, prurigo, meningitis, anemia requiring blood replacement, abscess and weight loss.

Phenotyping of lymphocytes were determined on a Cytometer FACS Calibur série (#E5139) (Becton Dickinson) at National Laboratory of Fight against HIV-LNLS and CD4 and CD8 T cells were assessed at the patients' entering in the study, and at three-months intervals thereafter. Viral loads were not available. Blood cell counts were performed with a cytometer "Micro CT 8" (ABX, Horiba) on total blood collected in a EDTA tube.

As said above, the immuno-modulator used was a synthetic analog of Nicotinamide Dithiodinicotinic Acid (CPDS). Capsules containing 240 mg of CPDS were administered at doses of 9-12mg/kg/day, two times a day after meal. The ART consisted of a tritherapy combining 1 NNRTI (NVP or EFV) and 2 NRTIs (d4T-3TC or AZT-3TC). No other drugs neither herbal medicines were used during the study period, excepted those related to the HIV complications.

Clinical and biological evaluations were conducted every three months and at any other time, if required by the participant health state.

III. Statistical Analysis

Data were analyzed with IBM SPSS Statistics version 20 To analyze the evolution of parameters within each group over time and to compare the mean values between the two groups, we used a Mixed models allowing random intercept after adjustment of age and gender in order to neutralize the confusion effect. Frequency was evaluated in a 2X2 dichotomic table and comparison made by using the Fischer's exact test. The results were statistically significant at p-value less than 0.05. Given the lack of advantage of one group to another at the starting point, statistic decision making was made bilaterally.

IV. Results

a) Socio demographic data on population

Globally, 94 individuals were registered in this study: 34 who underwent CPDS (Immunotherapy group or Group 1) and 60 ART (Control group or Group 2). The Pillai test allowed a multivariate comparison of the two groups at the beginning of the study (p=0.09) as none of them displayed any kind of advantage apart the weight: 46.13kgs in CPDS versus 53.52 in ARV (p=0.024).

There was no difference in mean age: 35.53 yrs in CPDS versus 37.47 yrs in ARV group.

The gender distribution was globally comparable within the two groups, and showed a high prevalence of female individuals: 61.76% (21/34 cases) and 66.67% (40/60 cases) in Group 1 and Group 2, respectively. There was no significant difference in mean age between females and males in both groups: In Group 1, 36.33 yrs old versus 34.23 (p=0.71) and in Group 2: 36.25 yrs old versus 39.9 yrs (p=0.66).

According to the marital status in Group 1 vs Group 2, 38% vs 34% were unmarried and 32% vs 26% were widows; 30% vs 37% were married and 0% vs 3% were separated. Professionally, Group 1 and Group 2, respectively, displayed 56% and 35% housewives, 20% and 12% jobless, 15% and 10 % of schoolchildren, 8% and 15% employed, and 0% and 20% managerial.

b) Previous medical and biological data

The main medical data collected in patients at the beginning of the trial are summarized in Table I. Weight loss was one of the main indicators observed in a same proportion in both groups (78.6 % versus 84.2%) followed by tuberculosis and zona infections. Histories of other form of pneumopathies, prurigo and skin abscesses were more frequent in Group 1 patients at the start of the trial.

The mean weight at the entry in the trials was 47.54 kgs (SD: 14.5) and 55.67 (SD: 15.5) in Group 1 and Group 2, respectively. (p=0.137)

No significant differences were noticed in CD4 count: 393.42 in Group 1 versus 175.70 in Group 2 (p=0.055); CD8: 1114.08 in Group 1 versus 882.7 in Group 2 (p=0.439).

c) Clinical and biological evolution

Globally, we observed a similar evolution of weight and the CD4 counts in both groups during the first 12 months period of study with a gradual increase and a peak by the 6th month and a slope down by the 12th month. Immunotherapy group displayed higher values of CD4, CD8 lymphocytes and the CD4/CD8 ratio. (Figure 1).

We also observed a similar increase in lymphocytes and platelets count and Hb level (Figure 2). Morphological study of the slides showed large platelets in many of the HIV patients in both groups.

12 months after the beginning, no significant difference in mean weight between groups (54.07 kgs in the ARV group and 53.21 kgs in CPDS (p = 0.891).

11 patients under CPDS were hospitalized versus 0 in the ARV group in 24 months.

We observed no significant difference in death rate: 8/34 patients (23.5%) and 14/60 patients (23.3%) died in the immunotherapy and ARV group, respectively. There was no significant difference between risks of dying between both groups. RR: 0.991 and OR: 0,989 (p =0.983).

In course of this study, 6 patients (17.6%) from Group 1 were submitted to ARV and then joined the group 2 in the second year of the study.

Administration of CPDS was well tolerated and no side effects were reported.

V. Discussion

In this study, we examined the effect of an immunomodulator on the clinical course (weight gain, morbidity and mortality rate) and biological (CD4 and CD8 lymphocytes, Blood cell count) parameters in HIV patients.

The baseline characteristics were identical between the two groups at the entry in the study (Tab I) except the WBC and lymphocyte count that was higher in the Group 1. (Tab II)

Globally, our results showed some curative effects of the immunomodulator in inducing and maintaining immune response and increasing weight.

The evolution of patient weights during the study period showed no statistical difference between the two groups, although the mean weight was less in immunomodulator group (52,7 kg)_comparing to the ART group (62,1 kg). In fact, most of the Group 1 patients belonged to a low socio-economic stratum as shown in professional occupation and had developed more tuberculosis infections and pneumonia (Tab II).

We observed an increase of the mean weight at 3-6 months interval in course of the therapy concomitantly with the increase of CD4 cell counts. Despite of fluctuations of mean weight observed in course of the study, there was no statistical difference in the weight evolution between the two groups. (Figure A).

Weight gain could be considered as an indirect indicator of immunity rescue.[22] Additionally, weight gain or stable weight is considered as one of the positive effects of treatment and care of PLWHA, and has an important impact on the psychological status of the patients and the drug compliance.

One of the striking results we observed in the Group 1 was the increase of CD4 and CD8 cell counts, and total lymphocytes numbers, especially in the first 6 months of administration of CPDS. The successive fluctuations we observed could be due to the various clinical events and the immunoresponse of each patient.

It's known that the improvement in immune function in HIV patients is biphasic: there is an initial increase in B lymphocytes, and CD4 and CD8 cells, followed by a second phase of increased thymic cell turnover and production. Furthermore, the restoration of cell immunity in patients submitted to ART depends on the phase disease [23]

The impact of CPDS on mortality is obvious as we observed no difference between the two groups (21% and 23% in Groups 1 and 2 respectively) in a two-year interval of follow-up. Other studies on mortality rate in Africa showed data varying from 10-15% but in a short period of follow-up (6-12 months)[8].

Immunotherapy is actually considered as a complementary strategy in HIV patient management[23, 24]. Different forms of immunotherapy have been proposed, including cytokines, growth factors and virus-specific therapeutic vaccines.[1] Most of these approaches have been aimed at correcting defective elements of adaptive immunity and in recovering virus-specific responses. However, it is known that HIV-1 infection also causes functional defects in natural killer (NK) cells and in monocytes/macrophages.

Although ART can improve limited functions of certain sub-populations of NK cells and antigen-presenting cells (APCs), some authors think that cells of the innate immune system act as ARV drug –resistant virus reservoirs, contribute to virus dissemination and are believed to be the origin of defective HIV-specific lymphocyte responses in infected patients. It's then necessary to correct innate immune dysfunctions in order to restore global immunity and more efficacious long-term control of HIV-1. Murabutide, a synthetic immunomodulator, has displayed such a capacity[24] .

CPDS is a powerful immune modulator which effects are targeted in increasing the number of NK cells, inducing the lymphoproliferative function of T lymphocytes[15]. The mechanism of the CPDS in increasing the CD4 and lymphocites count is not clearly known but it probably involves cytokines production and chemokines. Moreover, the increasing number of platelets could be explained by the releasing of cytokines, including IL 6, with potential implication in megakariopoiesis[25].

Limits of our study

The lack of viral load out of our indicators deprives the result with one of the most valuable information on the impact of CPDS to the immune system in case of HIV infection. Viral load should be one of main indicator for any prospective study for this purpose.

After the first six-twelve months of the study, clinical and biological indicators were analyzed only globally at the end of the study, without indicating the values over the time. A better systematic comparison of data between the two groups should be made quarterly, as planned in the protocol. Comprehensive collection of data over the time should be a key point in a prospective study.

We used a single immunomodulator in this study even if hypothesis suggest the combination of multiple immune-based intervention strategies in order to achieve effective immune-mediated antiviral effects[14, 24, 25, 26, 27].

VI. Conclusion

This study suggests the benefit of immunotherapy in treatment of PLWHA. The immunomodulator CPDS used alone in 34 patients resulted in an increase of mean body weight and mean CD4 mostly during the first six months of treatment. Weight gain and CD4 increase indicates a recovering immune system.

In addition, our result showed no difference between the CPDS group vs ART group in mortality rate during the same 2-year period of follow up.

Despite the positive impact of ART in PLWHA in terms of quality of health and life, and given the limit of ART to overcome the immune system impairment lead by HIV, we suggest the simultaneous use of CPDS with ART.

Since many efforts are made to facilitate the adherence to treatment of PLWHA by the use of daily single dose pill, the adjunction of a CPDS pill would not

further burden the tolerance of treatment, given the lack of side effect noticed.

The most critical challenge –as for ART coverage- should be the access to this treatment tool for all individuals in need, mostly in Sub-Saharan Africa.

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Legends

Table 1: Pathologies, Cell counts and phenotyping lymphocytes at baseline

	GROUPS	
	CPDS (n34)	ARV (n60)
	n (%)	n (%)
Meningitis	1 (2.94)	1 (1.67)
Zona Infection	2 (5.89)	2 (3.33)
Tuberculosis	4 (11.76)	5 (8.33)
Other pneumopathies	2 (5.89)	1 (1.67)
Enteritis	1 (2.94)	3 (5.00)
Prurigo	4 (11.76)	3 (5.00)
Weight loss	11 (32.35)	16 (26.67)
Blood transfusion	2 (5.89)	1 (1.67)
Skin abscess	4 (11.76)	0
	Mean (SD)	Mean (SD)
Weight (kg)	47.54 (14.5)	55.67 (15.5)
CD4 (cells/mm3)	393.42 (344.5)	175.7 (109.1)
CD8 (cells/mm3)	1114.08 (946.8)	882.7 (415.2)
CD3 (cells/mm3)	1724.40 (1264	1059.24 (499.9)
CD4/CD8	0.45 (0.32)	0.32 (0.21)
WBC (cells/mm3)	4918.18 (1672.6)	3400 (1112.0)
Lymphocytes (cells/mm3)	2169.75 (780.4)	1323.9 (428.9)
Hemoglobin (g/%ml)	11.03 (0.97)	10.93 (2.2)
Platelets (cells/mm3)	2.08E5 (4.2E4)	2.2E5 (4.2E4)
VS (mm/h)	8 9.33 (40.2)	82.5(50.9)

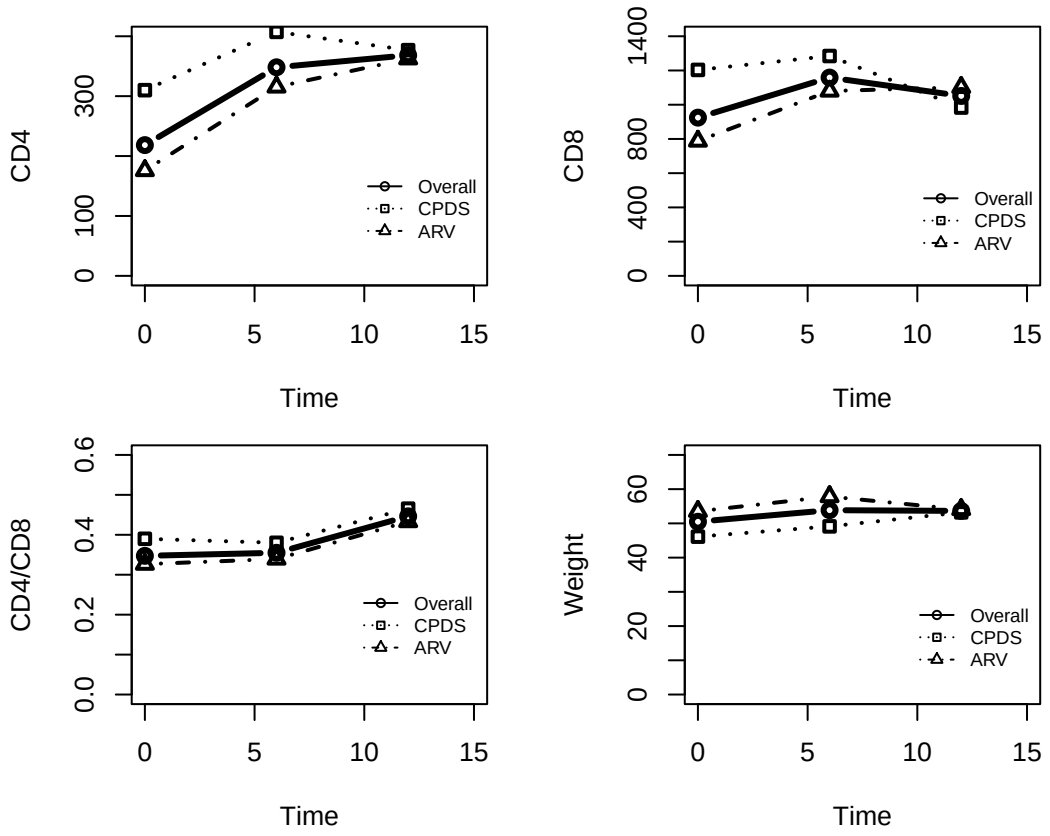

Figure 1: Evolution of CD4, CD8, CD4/CD8 count and Weight in patients submitted to CPDS and ARV.

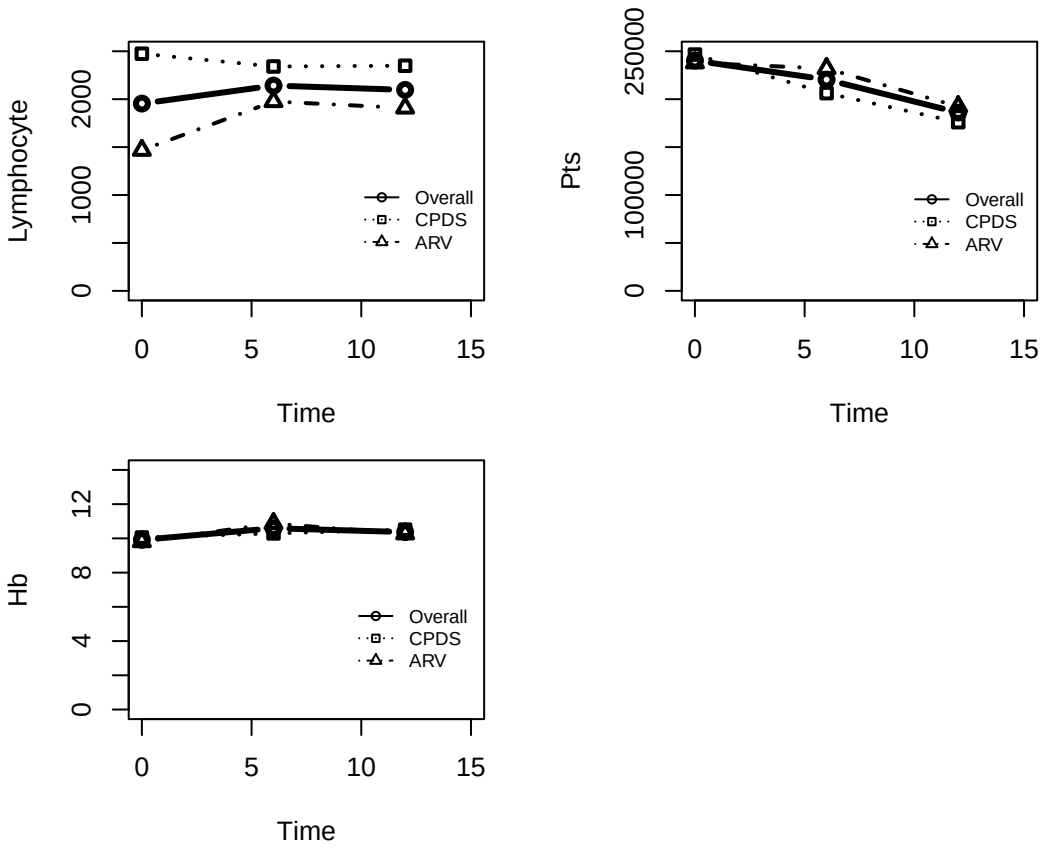

Figure 2: Evolution of lymphocytes, platelets (Pls) count and Hemoglobin level (Hb).

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Prevalence of Indigestible Foreign Bodies in the Rumen and Reticulum of Sheep Slaughtered at Jimma Municipal Abattoir, Southwestern Ethiopia

By Nejash Abdela, Feyissa Begna Deressa, Abdi Hassan & Endale Teshome
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Abstract- *Background:* Indigestible foreign bodies ingestion predisposed by environmental pollution is becoming a major global problem in ruminants. Even though, the impacts on cattle have gained some attention, shoats are neglected.

Methods: Cross-sectional study was conducted from September 2016 to December 2016 on 200 sheep slaughtered at Jimma municipal abattoir with the objective to determine the prevalence of indigestible foreign body in rumen and reticulum of sheep. The study population was sheep coming for slaughter from different districts of Jimma zone. Slaughtered sheep (study unites) were followed to collect their stomach and foreign body (indigestible materials) were assessed in the rumen and reticulum. Questionnaire was used to collect some hypothetical risk factors and data were recorded during stomach investigation. Logistic regression was used to determine the association of risk factors with occurrence of for foreign body.

Keywords: *indigestible foreign bodies, jimma municipal abattoir, sheep.*

GJMR-K Classification: *NLMC Code: QW 70, WC 900*

Strictly as per the compliance and regulations of:

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Nejash Abdela [α], Feyissa Begna Deressa [σ], Abdi Hassan [ρ] & Endale Teshome [ω]

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Results: From total of 200 sheep examined for the presence of indigestible foreign bodies, 22(11%) were found to be positive for indigestible foreign bodies in their rumen and/or reticulum. The types of different indigestible foreign bodies recovered were plastics (6.5%), cloth (2%), wire (1%) and leather (0.5) with plastics being 59.0% of the case. Prevalence of indigestible foreign body in thin, medium and good body conditioned sheep was 35.7%, 11.2 and 3.2%, respectively. Furthermore, the prevalence recorded in sheep > 3 years, 2-3 years and < 2 years was 24.3%, 8.8% and 5.2%, respectively. Age and body condition difference were significantly associated with indigestible foreign bodies ingestion. The prevalence was significantly higher in sheep of age >3 years compared to that of <2 years (OR) = 5.160, (CI: 1.234 - 21.573, $P \leq 0.031$), and the prevalence in thin animals was significantly higher than good body conditioned animals (OR= 24.165, CI: 4.787-121.989, $P= 0.000$). Out of 22 sheep positive for indigestible foreign materials higher proportion was found in rumen (86.36%) than in reticulum (13.63%). This variation is also significantly different statistically (OR= 6.893, CI: 2.0062 to 23.6843, $P=0.0022$).

Conclusion: This study revealed that indigestible foreign body ingestion by sheep in the study area is prevalent which may indicate poor environmental protection and pollution with plastics and other indigestible foreign bodies. This may pose serious health problem for extensively reared animals and negatively affect their overall productivity and production. Thus, to prevent animals from accessing indigestible foreign bodies strict regulation regarding the proper waste disposal practices and good husbandry methods are required.

Keywords: indigestible foreign bodies, jimma municipal abattoir, sheep.

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I. Introduction

Ethiopia has the largest livestock population in Africa with sheep and goat populations exceeding 58 million, which is one of the largest populations of small ruminants in Africa (CSA, 2016). Sheep and goat are integral to the livestock production systems in crop-livestock mixed agriculture in the highlands and in the pastoral and agro-pastoral livestock production. They are particularly important resources of the country as they provide more than 30% of the local meat consumption and form a vital source of income for small-scale farmers (ILCA, 2007).

There is also a growing export market for live sheep and meat in the Middle Eastern Gulf states and some African countries. At optimum off take rates, Ethiopia can export 700,000 sheep annually, and at the same time supply 1,078,000 sheep for the domestic market (Alemu and Marke, 2008). However, the benefits obtained from sheep to date do not match their tremendous potential and significant losses result each year from the death of animals as a result of lack of appropriate veterinary services, lack of attention from government, wide spread endemic disease and recurrent drought which are considered as bottleneck for development of this sector in the country (Abdela and Jilo, 2016; Jilo et al., 2016). Indigestible foreign bodies are reported to be a common cause of surgical emergency in Veterinary Medicine and have been implicated as among common causes of sudden death (Radostitis et al., 2007; Anwar et al., 2013).

Indigestible foreign bodies in the rumen and reticulum predisposed by environmental pollution are fast becoming a major global problem in ruminants worldwide (Kumar and Dhar, 2013). Furthermore, Industrialization and mechanization of agriculture have

increased the incidence of foreign body ingestion (Semieka, 2010). When ingested by animals foreign bodies get lodged in the rumen thereby compromising ruminal space and interfering with normal physiological functions of the rumen leading to weight loss with or without an enlarged abdomen or death (Anwar et al., 2013; Kumar and Dhar, 2013; Bwala et al., 2016).

Extensive plastic materials disposal is an increasing phenomenon (Arash et al., 2012), and a concern in view of the possible damage to the animals' wellbeing, particularly around urban settings in Ethiopia. The foreign bodies, especially large plastic, influence the digestion process by occupying space and blocking ingesta movement, which ultimately impair the health and productivity of animals. Plastics and other materials that are not able to decompose have no only direct effect on the animals, but also can remain in the environment for a long time which ultimately affects the soil fertility and thus may reduce the quality and quantity of pasture in the environment (Sheferaw et al., 2014).

In cattle indigestible foreign bodies was reported to be condition of great economic importance and causes severe loss of production and high mortality rates (Radostitis et al., 2007). However, Ingestion of large quantities of indigestible materials occurs in small ruminant during periods of drought, food scarcity, nutritional deficiency, pica and massive environmental pollution (Igbokweet al., 2003; Ghurashi et al., 2009; Otsyina et al., 2015). This condition is common especially in developing countries where the standard of animal management is unsatisfactory (Fasil, 2016).

Sheep are the second most important livestock species next to cattle in Ethiopia (Gizaw *et al.,* 2007) and the ingestion and lodgment of foreign bodies are common in the sheep than goats primarily due to indiscriminate feeding habits of sheep and selective nature of goats while grazing (Semieka, 2010;Fromsa and Mohammed, 2011).It has been indicated that, sheep reared in urban and peri-urban areas are more prone to indigestible foreign bodies than those reared in rural areas (Remi-Adewunmi et al., 2004).In Ethiopia small ruminants are left to roam and seek their own feed as the raising system is mainly extensive type. The areas available for grazing particularly in the case for animals reared in the urban and sub-urban areas are polluted with plastics, ropes, hair, wool and metals. This pollution may be predicated as a growing problem for grazing animals because of the poor waste management system and inadequate availability of feed during the dry season (Fromsa and Mohammed, 2011; Fasil, 2016).

Several investigation were conducted on indigestible foreign bodies in cattle in Ethiopia (Dawit et al. 2012; Tesfaye and Chanie, 2012; Nugusu et al. 2013; Sheferaw et al., 2014; Negash et al., 2015). However, there are limited studies on sheep despite free grazing system of animals in contaminated environments. Thus, there is scarcity of information on indigestible foreign bodies in sheep. Therefore, the main objectives of this study were to estimate the prevalence of foreign body in rumen and reticulum of sheep slaughtered at Jimma municipal abattoir and to assess the possible risk factors associated with the ingestion of different foreign bodies.

II. Materials and Methods

a) *Study area*

The study was conducted from September, 2016 to December, 2016 in Jimma municipal abattoir. Jimma municipal abattoir is located in Jimma town of Jimma zone. The town is located in the south western part of the Ethiopia in Oromia Regional State (figure 1). It is found at distance of about 352 km from Addis Ababa, the capital city of Ethiopia. Geographically, it is located at 7^0 13' and 8^0 56' N latitude and 35^0 52' and 37^0 E longitude. The area has an altitude ranging between 880 and 3358 meter above sea level. The annual rainfall is ranging between 1200 mm to 2000 mm; and the annual temperature of the area ranges 7 ^{0}C to 30 ^{0}C. Jimma zoone has about 2,212,962 cattle, 866,561sheep, 457,311 goats, 96,782 horses, 17,644 mules, 77,767 donkeys, 1,951,129 poultry and 546,722 beehives (CSA, 2016).

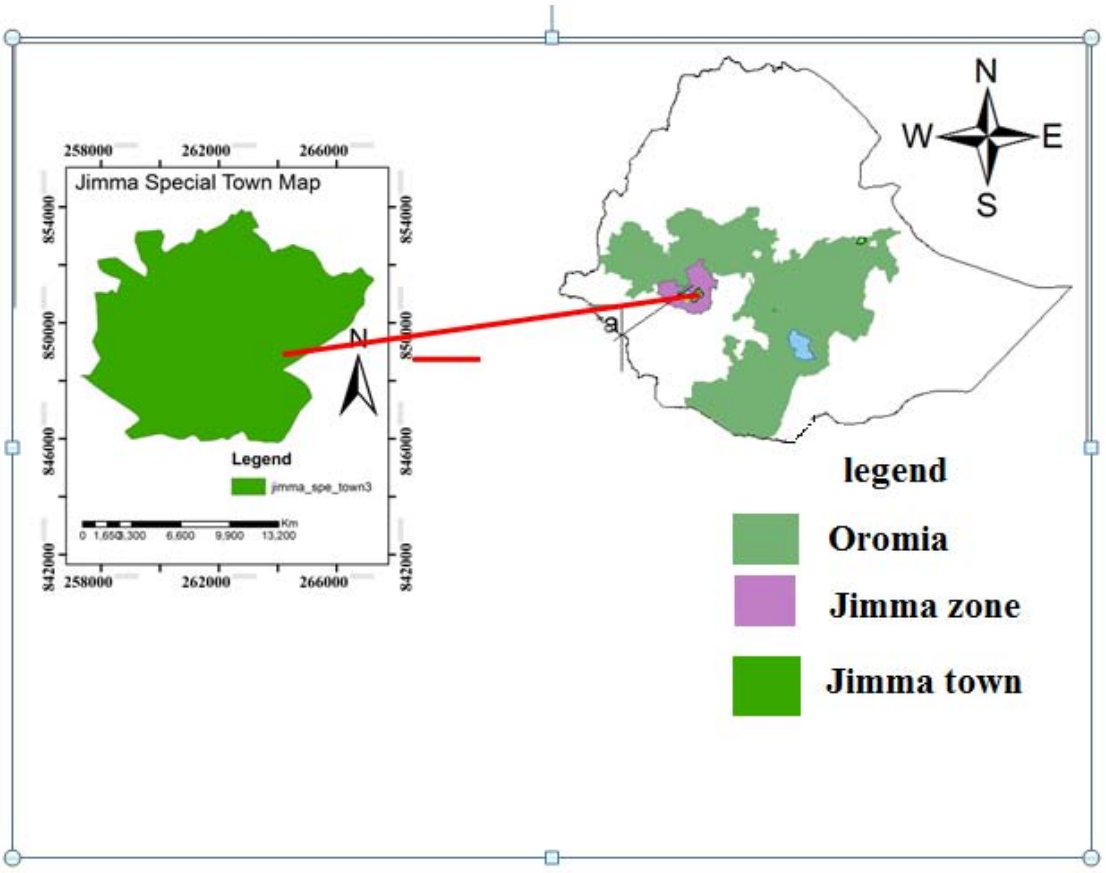

Figure 1: Map of the study area

b) Study Population and Study Design

A cross-sectional study design was employed for estimating the prevalence of indigestible foreign body types in the rumen and reticulum of sheep and to assess the possible risk factors associated with different indigestible foreign body. The study was conducted on local breed sheep with different age groups slaughtered at Jimma municipal abattoir. All animals considered in this study were males as female were not slaughtered in Jimma municipal abattoir during study period. The animals were brought from different areas including Dedo, Asandabo, Serbo, Mana, Seka, and Bilida. Most of these animals are managed under an extensive management system.

c) Sampling Techniques and Sample size determination

A simple random sampling technique was used for sampling sheep brought for slaughter from various localities to Jimma municipal abattoir. On average about 6 sheep were selected pre-slaughter and followed to collect their stomach and diagnose the indigestible foreign bodies. The abattoir was visited twice weekly and averagely the numbers of daily sheep slaughtered in the abattoir were around eight sheep. The required samples size for the study were determined by the formula given by Thrusfield (2005) based on the expected prevalence (9.7%) (Tesfaye et al., 2012) of sheep indigestible foreign bodies and the 5% desired absolute precision and 95% confidence interval (CI). Accordingly, the required number of animals was 134. However, to increase precision the sample size was increased to 200.

The following formula was used to determine sample size

$$N = \frac{1.96^2 \times P_{exp}(1 - P_{exp})}{d^2}$$

Where, N= required sample size

P_{exp} = expected prevalence=9.7%

d = Desired absolute precision = 5%

1.96 = the value of z at 95% confidence interval

d) Ante mortem and Post-mortem examination

During ante mortem examination, each study units selected randomly was given temporary identification number and data like body condition score and age of each study animals were recorded. The age grouping was based on eruption patterns as described by Steel (1996) and the sheep were grouped to <2 years, 2-3 years and > 3 years. The body condition was recorded as thin, medium and good based on the appearance of the animal and manual palpation of the spinus and transverse processes of the lumbar vertebrae as described by Thompson and Meyer (1994). After slaughtering, the stomach was removed carefully from the abdominal cavity and the rumen and reticulum

were incised to examine their contents. Rumen and reticulum of each study animals were thoroughly examined by visual inspection and palpation for the presence of indigestible foreign bodies during post-mortem examination. When the positive animas encountered, the location and type of the foreign bodies was recorded on format prepared for this purpose.

e) Data Management and Analysis

The data was entered and managed in a Microsoft Excel spread sheet and analysed using Statistical Package for Social Sciences version 20. Descriptive statistics was used to determine frequencies and over all prevalence. The prevalence of indigestible foreign bodies was determined as a proportion of affected animals out of the total animal examined. The differences or association between risk factors were analysed by binary logistic regression and OR and p-values were used to describe statistical significance associations and p-value of <0.05 was considered as statistically significant.

III. Results

A total of 200 sheep were examined for presence of indigestible foreign bodies in their rumen and reticulum. Out of these, 22 (11%) were found to have various types of indigestible foreign bodies in the rumen and/or reticulum. The types of foreign bodies detected were plastic, cloth, leather, wire, and rope (Figure 2). The most commonly observed foreign bodies were plastics 13(59.0%) followed by cloth 4(18.1%); cloth, plastic and rope 2(9.0%), wire 2(9.0%) and leather 1(4.54) in order of occurrence.

a) Prevalence of indigestible foreign body types in the rumen and reticulum

From 22 positive cases of foreign body, 19(86.36%) were occurred in rumen while only 3(13.63%) in reticulum. The types of foreign bodies encountered and their frequency of occurrence was summarized in table 1. Significantly higher prevalence (p= 000) of indigestible foreign bodies was found in rumen (9.5%) than reticulum (1.5%). The odd of occurrences of indigestible foreign bodies in rumen was 6.893 times more likely than reticulum (Table 1).

Table 1: Proportion of indigestible foreign body types in the rumen and reticulum

Organs	Frequency of indigestible foreign body						Association			
	Plastic (%)	Cloth (%)	Wire (%)	Leather (%)	Cloth, plastic and rope (%)	Overall (%)	Prevalence (%)	OR	(95% CI)	P-Value
Rumen	12(54.5)	4(18.1)	-	1(4.5)	2(9.0)	19(86.3)	9.5	6.8	2.0062 -	
Reticulum	1(4.5)	-	2(9.0)	-	-	3(13.6)	1.5	93	23.6843	0.0022
Total	13(59.0)	4(18.1)	2(9.0)	1(4.5)	2(9.0)	22 (100)				

b) Risk factor associated with foreign body ingestion

From total of 200 sheep examined, higher foreign body prevalence was observed in the older animals (> 3 years) 10(24.3%) followed by 2-3 years 9(8.8%) and lower prevalence was observed in young age groups (<2 years) 3 (5.2%) (table2). The odd of foreign body occurrence in sheep > 3 years was 5.160 times more likely than sheep under 2 years. This variation in the foreign body prevalence was found statistically significant (p<0.05) (table 2).

Thin body conditioned sheep were found to have highest prevalence of harbouring indigestible foreign body (10(35.7 %)) and in contrary good body conditioned sheep were found to have lowest indigestible foreign body prevalence 3(3.2%). The most prevalent foreign body were a plastic (6.5%) which was the most frequently recovered foreign body type in thin and medium body conditioned sheep. Cloth was encountered in medium and good body condition sheep. Leather was encountered only in single thin sheep and good body conditioned sheep were found to have only plastic and cloth. The odd of foreign body occurrence in thin sheep was 24.165 more likely than good body condition sheep (Table 2). There was significant statistical difference (p = 0.000) between different body condition categories.

Table 2: Prevalence of different foreign body and multivariable logistic regression analysis of factors associated foreign body ingestion

Risk factors		No. examined	Frequency and prevalence of occurrence						Association		
			Plastic (%)	Cloth (%)	Wire (%)	Leather (%)	Cloth, Plastic, rope (%)	Overall (%)	OR	(95% CI)	P-value
Age	<2 years	57	2(3.5)	1(1.7)	-	-	-	3(5.2)			
	2-3 years	102	5(4.9)	1(0.9)	1(0.9)		2(0.9%)	9(8.8)	1.578	(0.376,6.627)	0.031
	>3 years	41	6(14.6)	2(4.8)	1(2.4)	1(2.4)	-	10(24.3)	5.160	1.234, 21.573	
Body Condition	Thin	28	6(21.4)	-	2(7.1)	1(3.5)	1(3.5)	10(35.7)	24.165	(4.787, 121.989)	
	Medium	80	6(7.5)	2(2.5)	-	-	1 (1.2%)	9(11.2%)	5.320	(1.091, 25. 940)	0.000
	Good	92	1(1.0)	2(2.1)	-	-	-	3(3.2)			
	Total	200	13(6.5)	4(2)	2(1%)	1(0.5)	2(1)	22(11)			

IV. Discussions

Ingestion of indigestible foreign materials by ruminants is a common worldwide problem and has been reported from different area of Ethiopia in both cattle and small ruminant (Tiruneh and Yesuwork, 2010; Fromsa and Mohammed, 2011; Negash et al., 2015; Fasil, 2016). This study showed an overall rumen and reticulum foreign body prevalence of 11% (22/200) in sheep slaughtered at Jimma municipal abattoir. This is in agreement with the finding in Kenya by (Otsyina et al., 2015) who reported 10.1% of foreign body prevalence. This result is larger to reportby Firomsa and Nura, (2011) who reported 7.5% rumen foreign body in sheep Slaughtered at Luna Export Abattoir, East Shoa, Ethiopia and report from Jordan by Hailat et al (1996) who reported a prevalence rate of 8.9%.

This finding is relatively lower compared to 56.7% report from eastern Ethiopia at Haramaya University and Haramaya municipal abattoirs (Negash et al., 2015), 34.4 % at Jigjiga Municipal Abattoir (Fasil, 2016), 53.1% at Addis Ababa Municipality Abattoir (Tiruneh and Yesuwork, 2010) and 20.6% at Bahirdar municipality abattoir and hotels in Bahirdar town (Sheferaw et al., 2014). It also disagrees with study In Nigeria by Remi-Adewunmi et al., 2004, in South Darfur (Ghurashi et al., 2009) and Ghana (Atawalna et al., 2015) who reported 77%, 87% and 17.4%, respectively. This difference in prevalence may be due to the differences in origin of the animals slaughtered accompanied by feed availability and the type of waste management system between the study areas. Furthermore, this difference could also be due to the difference in the sex composition as all sheep slaughtered at Jimma municipal abattoir during study period are males. Higher prevalence rate of foreign body in the female animals was reported (Tiruneh and Yesuwork, 2010).If there is shortage of feed in the area this may predispose the animals to negative energy balance and force them to feed on unusual materials including plastics, clothes, ropes and even wire. On other hand, if there is no or less waste management system in the area the chance of animals to ingest foreign bodies is high.

The current study indicated as larger number of foreign bodies occurred in the rumen (86.3%) than reticulum (13.6%) of sheep. this may be due to the fact that many ingested feed goes to the rumen due to its larger size as compared to reticulum. In agreement with this finding, different scholars have reported higher frequency of foreign bodies from rumen than from the reticulum (Tiruneh and Yesuwork, 2010, Fromsa and Mohammed, 2011; Negash et al., 2015; Fasil, 2016).

This study revealed that plastics were more common (59%) indigestible foreign body in the rumen and reticulum of sheep. The wide spread use and improper disposal of plastic which is bio non degradable could be the reason for it high prevalence. Similar findings were reported in different area of Ethiopia (Tiruneh and Yesuwork, 2010; Fromsa and Mohammed, 2011; Sheferaw et al., 2014; Negash et al., 2015; Fasil, 2016) and other countries like Nigeria (Remi-Adewunmi et al., 2004) and Jordan (Hailat et al, 1996). Extensive plastic materials disposal is an increasing phenomenon (Arash et al. 2012), and a concern in view of the possible damage to the animals' wellbeing, particularly around urban settings in Ethiopia. The foreign bodies, especially large plastic, negatively influence the digestion process by occupying space and blocking ingesta movement, which ultimately impair the health and productivity of animals. Plastics and other materials that are not able to decompose have not only direct effect on the animals, but also can remain in the environment for a long time which ultimately affect the soil fertility and thus may reduce the quality and quantity of pasture in the environment (Sheferaw et al., 2014).

Older sheep (> 3 years) (24.3 %) and sheep having thin body condition (35.7%) were found to be more frequently harbouring indigestible foreign body. In agreement with this finding there are reports from different area of Ethiopia and other country that older and thin animals to be more harbouring indigestible foreign body (Hailat et al. 1996; Remi-Adewunmi et al., 2004; Fromsa and Mohammed, 2011; Tiruneh and Yesuwork, 2010; Negash et al., 2015; Fasil, 2016) and

this difference are also statistically significant. The finding of significantly more foreign bodies in older animals than the young ones may be due to the gradual ingestion of indigestible materials over the prolonged period of time. The more frequent occurrence of rumen and reticulum indigestible foreign body in thin sheep might be attributed to the interference of the foreign body with the absorption of volatile fatty acids causing reduced weight gain (Remi- Adewunmi et al., 2004).

The finding of 11% prevalence of indigestible rumen and reticulum indigestible foreign body shows the widespread distribution of plastic bags in the environment as a result of improper disposal of waste. Unless appropriate measure is taken increased ingestion of indigestible foreign bodies could pose serious health problem for free grazing sheep particularly in urban and peri-urban areas and negatively affect their overall productivity and production. Proper waste disposal practices and good husbandry methods are required to prevent animals from accessing indigestible foreign bodies. Policy makers, veterinarians and environmental health experts are expected to work conjointly in reducing its adverse effect in animals. Furthermore, in order to reduce the problems associated with plastic bag wastes, it is recommended to aware the community not to use plastic bags, and to use ecologically-friend alternative materials.

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Figure 2 and 3: Plastic, cloth and rope removed from rumen of three years old sheep

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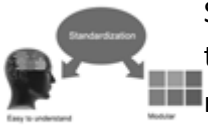

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(b) A brief Summary, "Abstract" (less than 150 words) containing the major results and conclusions.

(c) Up to ten keywords, that precisely identifies the paper's subject, purpose, and focus.

(d) An Introduction, giving necessary background excluding subheadings; objectives must be clearly declared.

(e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition; sources of information must be given and numerical methods must be specified by reference, unless non-standard.

(f) Results should be presented concisely, by well-designed tables and/or figures; the same data may not be used in both; suitable statistical data should be given. All data must be obtained with attention to numerical detail in the planning stage. As reproduced design has been recognized to be important to experiments for a considerable time, the Editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned un-refereed;

(g) Discussion should cover the implications and consequences, not just recapitulating the results; conclusions should be summarizing.

(h) Brief Acknowledgements.

(i) References in the proper form.

Authors should very cautiously consider the preparation of papers to ensure that they communicate efficiently. Papers are much more likely to be accepted, if they are cautiously designed and laid out, contain few or no errors, are summarizing, and be conventional to the approach and instructions. They will in addition, be published with much less delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and to make suggestions to improve briefness.

It is vital, that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

Format

Language: The language of publication is UK English. Authors, for whom English is a second language, must have their manuscript efficiently edited by an English-speaking person before submission to make sure that, the English is of high excellence. It is preferable, that manuscripts should be professionally edited.

Standard Usage, Abbreviations, and Units: Spelling and hyphenation should be conventional to The Concise Oxford English Dictionary. Statistics and measurements should at all times be given in figures, e.g. 16 min, except for when the number begins a sentence. When the number does not refer to a unit of measurement it should be spelt in full unless, it is 160 or greater.

Abbreviations supposed to be used carefully. The abbreviated name or expression is supposed to be cited in full at first usage, followed by the conventional abbreviation in parentheses.

Metric SI units are supposed to generally be used excluding where they conflict with current practice or are confusing. For illustration, 1.4 l rather than 1.4 × 10-3 m3, or 4 mm somewhat than 4 × 10-3 m. Chemical formula and solutions must identify the form used, e.g. anhydrous or hydrated, and the concentration must be in clearly defined units. Common species names should be followed by underlines at the first mention. For following use the generic name should be constricted to a single letter, if it is clear.

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All manuscripts submitted to Global Journals Inc. (US), ought to include:

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Abstract, used in Original Papers and Reviews:

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Many researchers searching for information online will use search engines such as Google, Yahoo or similar. By optimizing your paper for search engines, you will amplify the chance of someone finding it. This in turn will make it more likely to be viewed and/or cited in a further work. Global Journals Inc. (US) have compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Key Words

A major linchpin in research work for the writing research paper is the keyword search, which one will employ to find both library and Internet resources.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy and planning a list of possible keywords and phrases to try.

Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

Choice of key words is first tool of tips to write research paper. Research paper writing is an art.A few tips for deciding as strategically as possible about keyword search:

- One should start brainstorming lists of possible keywords before even begin searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in research paper?" Then consider synonyms for the important words.
- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

Acknowledgements: Please make these as concise as possible.

References

References follow the Harvard scheme of referencing. References in the text should cite the authors' names followed by the time of their publication, unless there are three or more authors when simply the first author's name is quoted followed by et al. unpublished work has to only be cited where necessary, and only in the text. Copies of references in press in other journals have to be supplied with submitted typescripts. It is necessary that all citations and references be carefully checked before submission, as mistakes or omissions will cause delays.

References to information on the World Wide Web can be given, but only if the information is available without charge to readers on an official site. Wikipedia and Similar websites are not allowed where anyone can change the information. Authors will be asked to make available electronic copies of the cited information for inclusion on the Global Journals Inc. (US) homepage at the judgment of the Editorial Board.

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Figures: Figures are supposed to be submitted as separate files. Always take in a citation in the text for each figure using Arabic numbers, e.g. Fig. 4. Artwork must be submitted online in electronic form by e-mailing them.

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11. Revise what you wrote: When you write anything, always read it, summarize it and then finalize it.

12. Make all efforts: Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

13. Have backups: When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

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16. Use proper verb tense: Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

17. Never use online paper: If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

18. Pick a good study spot: To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

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20. Use good quality grammar: Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

21. Arrangement of information: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

23. Multitasking in research is not good: Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. Go for seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

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33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

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Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

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- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

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· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

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- Fundamental goal
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- Significant conclusions or questions that track from the research(es)

Approach:

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The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

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- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
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- If use of a definite type of tools.
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- Report the method (not particulars of each process that engaged the same methodology)
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- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

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- Resources and methods are not a set of information.
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- Leave out information that is immaterial to a third party.

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The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

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- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
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- Present a background, such as by describing the question that was addressed by creation an exacting study.
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Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
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- In spite of position, each table must be titled, numbered one after the other and complete with heading
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- Give details all of your remarks as much as possible, focus on mechanisms.
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- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
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Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
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References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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