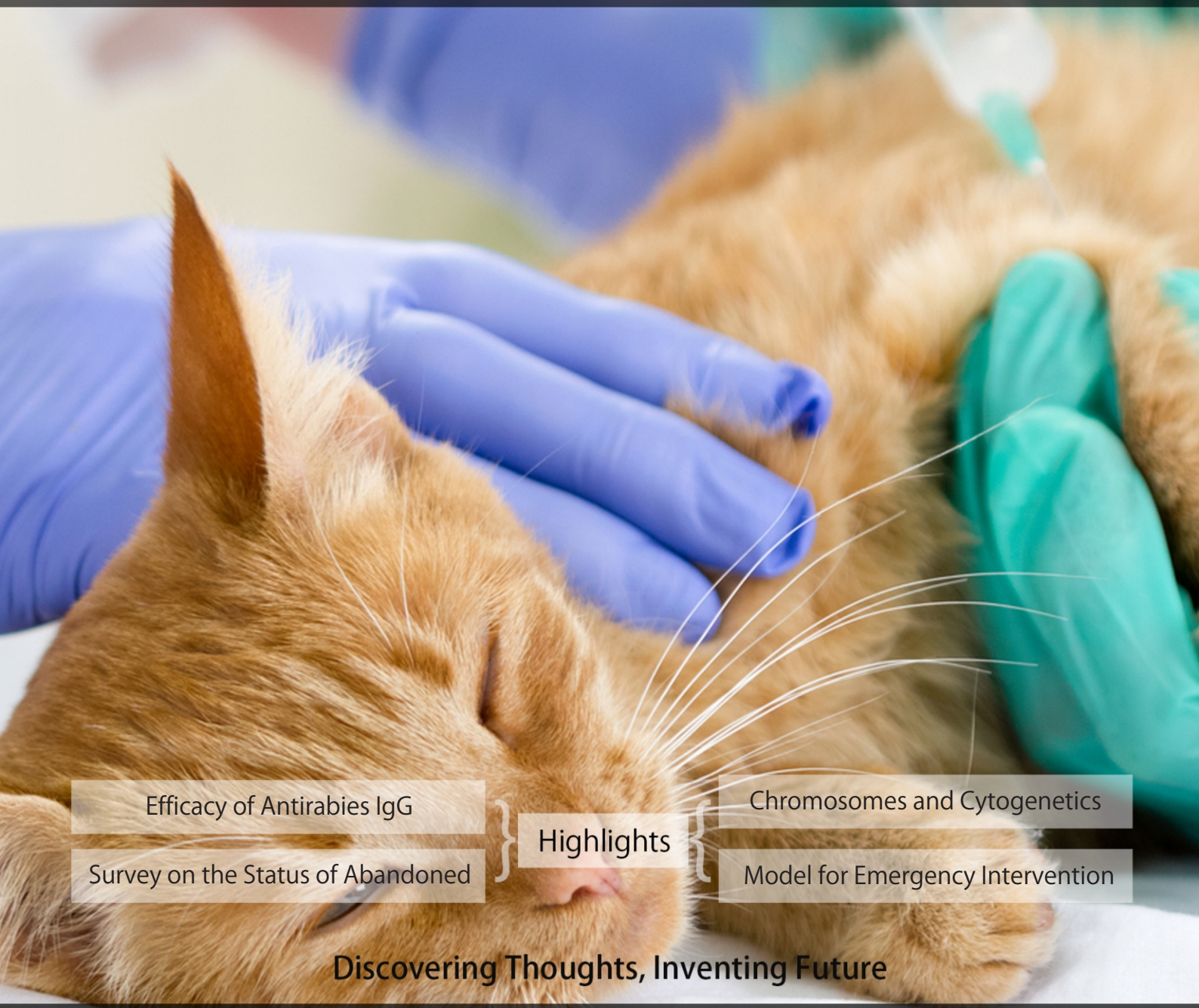
Online ISSN : 2249-4618
Print ISSN : 0975-5888
DOI : 10.17406/GJMRA

Global Journal of Medical Research: G

Veterinary Science & Veterinary Medicine



Highlights

- Efficacy of Antirabies IgG
- Survey on the Status of Abandoned
- Chromosomes and Cytogenetics
- Model for Emergency Intervention

Discovering Thoughts, Inventing Future

VOLUME 16 ISSUE 1 VERSION 1.0

© 2001-2016 by Global Journal of Medical Research, USA

Global Journal of Medical Research: G
Veterinary Science and Veterinary Medicine

Global Journal of Medical Research: G
Veterinary Science and Veterinary Medicine

Volume 16 Issue 1 (Ver. 1.0)

Open Association of Research Society

© Global Journal of Medical Research . 2016.

All rights reserved.

This is a special issue published in version 1.0 of "Global Journal of Medical Research." By Global Journals Inc.

All articles are open access articles distributed under "Global Journal of Medical Research"

Reading License, which permits restricted use. Entire contents are copyright by of "Global Journal of Medical Research" unless otherwise noted on specific articles.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage and retrieval system, without written permission.

The opinions and statements made in this book are those of the authors concerned. Ultraculture has not verified and neither confirms nor denies any of the foregoing and no warranty or fitness is implied.

Engage with the contents herein at your own risk.

The use of this journal, and the terms and conditions for our providing information, is governed by our Disclaimer, Terms and Conditions and Privacy Policy given on our website http://globaljournals.us/terms-and-condition/menu-id-1463/

By referring / using / reading / any type of association / referencing this journal, this signifies and you acknowledge that you have read them and that you accept and will be bound by the terms thereof.

All information, journals, this journal, activities undertaken, materials, services and our website, terms and conditions, privacy policy, and this journal is subject to change anytime without any prior notice.

Incorporation No.: 0423089
License No.: 42125/022010/1186
Registration No.: 430374
Import-Export Code: 1109007027
Employer Identification Number (EIN):
USA Tax ID: 98-0673427

Global Journals Inc.

*(A Delaware USA Incorporation with "Good Standing"; **Reg. Number: 0423089**)*

Sponsors: Open Association of Research Society
Open Scientific Standards

Publisher's Headquarters office

Global Journals® Headquarters
945th Concord Streets,
Framingham Massachusetts Pin: 01701,
United States of America
USA Toll Free: +001-888-839-7392
USA Toll Free Fax: +001-888-839-7392

Offset Typesetting

Global Journals Incorporated
2nd, Lansdowne, Lansdowne Rd., Croydon-Surrey,
Pin: CR9 2ER, United Kingdom

Packaging & Continental Dispatching

Global Journals
E-3130 Sudama Nagar, Near Gopur Square,
Indore, M.P., Pin: 452009, India

Find a correspondence nodal officer near you

To find nodal officer of your country, please email us at *local@globaljournals.org*

eContacts

Press Inquiries: *press@globaljournals.org*
Investor Inquiries: *investors@globaljournals.org*
Technical Support: *technology@globaljournals.org*
Media & Releases: *media@globaljournals.org*

Pricing (Including by Air Parcel Charges):

For Authors:
22 USD (B/W) & 50 USD (Color)
Yearly Subscription (Personal & Institutional):
200 USD (B/W) & 250 USD (Color)

Integrated Editorial Board
(Computer Science, Engineering, Medical, Management, Natural Science, Social Science)

John A. Hamilton,"Drew" Jr.,
Ph.D., Professor, Management
Computer Science and Software Engineering
Director, Information Assurance Laboratory
Auburn University

Dr. Henry Hexmoor
IEEE senior member since 2004
Ph.D. Computer Science, University at Buffalo
Department of Computer Science
Southern Illinois University at Carbondale

Dr. Osman Balci, Professor
Department of Computer Science
Virginia Tech, Virginia University
Ph.D. and M.S. Syracuse University, Syracuse, New York
M.S. and B.S. Bogazici University, Istanbul, Turkey

Yogita Bajpai
M.Sc. (Computer Science), FICCT
U.S.A. Email:
yogita@computerresearch.org

Dr. T. David A. Forbes
Associate Professor and Range Nutritionist
Ph.D. Edinburgh University - Animal Nutrition
M.S. Aberdeen University - Animal Nutrition
B.A. University of Dublin- Zoology

Dr. Wenying Feng
Professor, Department of Computing & Information Systems
Department of Mathematics
Trent University, Peterborough,
ON Canada K9J 7B8

Dr. Thomas Wischgoll
Computer Science and Engineering,
Wright State University, Dayton, Ohio
B.S., M.S., Ph.D.
(University of Kaiserslautern)

Dr. Abdurrahman Arslanyilmaz
Computer Science & Information Systems Department
Youngstown State University
Ph.D., Texas A&M University
University of Missouri, Columbia
Gazi University, Turkey

Dr. Xiaohong He
Professor of International Business
University of Quinnipiac
BS, Jilin Institute of Technology; MA, MS, PhD,. (University of Texas-Dallas)

Burcin Becerik-Gerber
University of Southern California
Ph.D. in Civil Engineering
DDes from Harvard University
M.S. from University of California, Berkeley & Istanbul University

Dr. Bart Lambrecht
Director of Research in Accounting and Finance Professor of Finance
Lancaster University Management School
BA (Antwerp); MPhil, MA, PhD (Cambridge)

Dr. Carlos García Pont
Associate Professor of Marketing
IESE Business School, University of Navarra
Doctor of Philosophy (Management), Massachusetts Institute of Technology (MIT)
Master in Business Administration, IESE, University of Navarra
Degree in Industrial Engineering, Universitat Politècnica de Catalunya

Dr. Fotini Labropulu
Mathematics - Luther College
University of Regina Ph.D., M.Sc. in Mathematics
B.A. (Honors) in Mathematics
University of Windso

Dr. Lynn Lim
Reader in Business and Marketing
Roehampton University, London
BCom, PGDip, MBA (Distinction), PhD, FHEA

Dr. Mihaly Mezei
ASSOCIATE PROFESSOR
Department of Structural and Chemical Biology, Mount Sinai School of Medical Center
Ph.D., Etvs Lornd University
Postdoctoral Training,
New York University

Dr. Söhnke M. Bartram
Department of Accounting and Finance Lancaster University Management School Ph.D. (WHU Koblenz)
MBA/BBA (University of Saarbrücken)

Dr. Miguel Angel Ariño
Professor of Decision Sciences
IESE Business School
Barcelona, Spain (Universidad de Navarra)
CEIBS (China Europe International Business School).
Beijing, Shanghai and Shenzhen
Ph.D. in Mathematics
University of Barcelona
BA in Mathematics (Licenciatura)
University of Barcelona

Philip G. Moscoso
Technology and Operations Management
IESE Business School, University of Navarra
Ph.D in Industrial Engineering and Management, ETH Zurich
M.Sc. in Chemical Engineering, ETH Zurich

Dr. Sanjay Dixit, M.D.
Director, EP Laboratories, Philadelphia VA Medical Center
Cardiovascular Medicine - Cardiac Arrhythmia
Univ of Penn School of Medicine

Dr. Han-Xiang Deng
MD., Ph.D
Associate Professor and Research Department Division of Neuromuscular Medicine
Davee Department of Neurology and Clinical Neuroscience Northwestern University Feinberg School of Medicine

Dr. Pina C. Sanelli
Associate Professor of Public Health
Weill Cornell Medical College
Associate Attending Radiologist
NewYork-Presbyterian Hospital
MRI, MRA, CT, and CTA
Neuroradiology and Diagnostic Radiology
M.D., State University of New York at Buffalo,School of Medicine and Biomedical Sciences

Dr. Roberto Sanchez
Associate Professor
Department of Structural and Chemical Biology
Mount Sinai School of Medicine
Ph.D., The Rockefeller University

Dr. Wen-Yih Sun
Professor of Earth and Atmospheric SciencesPurdue University Director
National Center for Typhoon and Flooding Research, Taiwan
University Chair Professor
Department of Atmospheric Sciences, National Central University, Chung-Li, TaiwanUniversity Chair Professor
Institute of Environmental Engineering, National Chiao Tung University, Hsin-chu, Taiwan.Ph.D., MS The University of Chicago, Geophysical Sciences
BS National Taiwan University, Atmospheric Sciences
Associate Professor of Radiology

Dr. Michael R. Rudnick
M.D., FACP
Associate Professor of Medicine
Chief, Renal Electrolyte and Hypertension Division (PMC)
Penn Medicine, University of Pennsylvania
Presbyterian Medical Center, Philadelphia
Nephrology and Internal Medicine
Certified by the American Board of Internal Medicine

Dr. Bassey Benjamin Esu
B.Sc. Marketing; MBA Marketing; Ph.D Marketing
Lecturer, Department of Marketing, University of Calabar
Tourism Consultant, Cross River State Tourism Development Department
Co-ordinator , Sustainable Tourism Initiative, Calabar, Nigeria

Dr. Aziz M. Barbar, Ph.D.
IEEE Senior Member
Chairperson, Department of Computer Science
AUST - American University of Science & Technology
Alfred Naccash Avenue – Ashrafieh

President Editor (HON.)

Dr. George Perry, (Neuroscientist)
Dean and Professor, College of Sciences
Denham Harman Research Award (American Aging Association)
ISI Highly Cited Researcher, Iberoamerican Molecular Biology Organization
AAAS Fellow, Correspondent Member of Spanish Royal Academy of Sciences
University of Texas at San Antonio
Postdoctoral Fellow (Department of Cell Biology)
Baylor College of Medicine
Houston, Texas, United States

Chief Author (HON.)

Dr. R.K. Dixit
M.Sc., Ph.D., FICCT
Chief Author, India
Email: authorind@computerresearch.org

Dean & Editor-in-Chief (HON.)

Vivek Dubey(HON.)
MS (Industrial Engineering),
MS (Mechanical Engineering)
University of Wisconsin, FICCT
Editor-in-Chief, USA
editorusa@computerresearch.org

Sangita Dixit
M.Sc., FICCT
Dean & Chancellor (Asia Pacific)
deanind@computerresearch.org

Suyash Dixit
(B.E., Computer Science Engineering), FICCTT
President, Web Administration and
Development , CEO at IOSRD
COO at GAOR & OSS

Er. Suyog Dixit
(M. Tech), BE (HONS. in CSE), FICCT
SAP Certified Consultant
CEO at IOSRD, GAOR & OSS
Technical Dean, Global Journals Inc. (US)
Website: www.suyogdixit.com
Email: suyog@suyogdixit.com

Pritesh Rajvaidya
(MS) Computer Science Department
California State University
BE (Computer Science), FICCT
Technical Dean, USA
Email: pritesh@computerresearch.org

Luis Galárraga
J!Research Project Leader
Saarbrücken, Germany

Contents of the Issue

i. Copyright Notice
ii. Editorial Board Members
iii. Chief Author and Dean
iv. Contents of the Issue

1. Efficacy of Antirabies IgG and IgY on Protection of Mice Against Experimental Viral Infection as a Model for Emergency Intervention. *1-5*
2. An Outbreak of *Corynebacterium Diphtheriae* Infection in Broiler Chickens in Lagos, Nigeria. *7-9*
3. Chromosomes and Cytogenetics of Trematodes. *11-39*
4. Survey on the Status of Abandoned Animals in Seoul City, 2013. *41-48*

v. Fellows
vi. Auxiliary Memberships
vii. Process of Submission of Research Paper
viii. Preferred Author Guidelines
ix. Index

Global Journal of Medical Research: G
Veterinary Science and Veterinary Medicine
Volume 16 Issue 1 Version 1.0 Year 2016
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Efficacy of Antirabies IgG and IgY on Protection of Mice Against Experimental Viral Infection as a Model for Emergency Intervention

By Ahmed Mohamed Albehwar, Abeer Atia Tammam & Amr Ismail Hassan

Veterinary Serum and Vaccine Research Institute, Egypt

Abstract- Aim: Preparation of anti-rabies IgY in chicken egg yolk in addition to preparation of anti-rabies IgG in rabbits and evaluate their efficacy in protection against rabies infection in emergency cases simulated in mice.

Materials and Methods: Fifteen, 35week-old, Rhode Island Red (RIR) hens were used for preparation of anti-rabies IgY from egg yolk. It was found that the obtained IgY had a concentration of 2.32 g/dl. In addition, ten Bosket rabbits of about 3kg bodyweight were used for preparation of anti-rabies IgG which was found to have a concentration of 3.42g/dl. Both of chicken egg yolk IgY and rabbit IgG were found to be safe when they are inoculated intraperitoneally in mice.

Results: Serum neutralization test revealed that IgY and IgG had rabies antibody titers of 64 and 128 respectively. The tow preparations were tested for determination of their potency in experimentally infected mice with rabies virus on daily intervals post infection.

Keywords: rabies, vaccine, antirabies IgY, antirabies IgG, emergency.

GJMR-G Classification : NLMC Code: QW 70

Strictly as per the compliance and regulations of:

© 2016. Ahmed Mohamed Albehwar, Abeer Atia Tammam & Amr Ismail Hassan. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/licenses/by-nc/3.0/), permitting all non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Efficacy of Antirabies IgG and IgY on Protection of Mice Against Experimental Viral Infection as a Model for Emergency Intervention

Ahmed Mohamed Albehwar [α], Abeer Atia Tammam [σ] & Amr Ismail Hassan [ρ]

Abstract- Aim: Preparation of anti-rabies IgY in chicken egg yolk in addition to preparation of anti-rabies IgG in rabbits and evaluate their efficacy in protection against rabies infection in emergency cases simulated in mice.

Materials and Methods: Fifteen, 35week-old, Rhode Island Red (RIR) hens were used for preparation of anti-rabies IgY from egg yolk. It was found that the obtained IgY had a concentration of 2.32 g/dl. In addition, ten Bosket rabbits of about 3kg bodyweight were used for preparation of anti-rabies IgG which was found to have a concentration of 3.42g/dl. Both of chicken egg yolk IgY and rabbit IgG were found to be safe when they are inoculated intraperitoneally in mice.

Results: Serum neutralization test revealed that IgY and IgG had rabies antibody titers of 64 and 128 respectively. The tow preparations were tested for determination of their potency in experimentally infected mice with rabies virus on daily intervals post infection. It was found that intraperitoneal injection of infected mice with 0.5ml containing 116 mg of IgY and 171 mg of IgG were effective to prevent and overcome the progress of rabies signs when administrated on the 0; 1st; 2nd and 3rd day post exposure to the virus infection and not after that where treated mice on the 4th to 7th day post infection were unable to withstand the virus infection. The use of IgY and IgG with inactivated rabies vaccine showed the same results using IgY and IgG alone while the use of rabies vaccine alone did not provide efficient protection for the treated mice.

Conclusion: The use of anti-rabies IgY and IgG could be recommended as post exposure intervention providing a suitable period of protection until stimulation of the active immunity induced by rabies vaccine. In addition, the preparation of chicken IgY in a non-specific host provides safe, high potent product of lower cost than that prepared in rabbits or other mammals.

Keywords: rabies, vaccine, antirabies IgY, antirabies IgG, emergency.

I. Introduction

Rabies is a zoonotic disease that affects the central nervous system (CNS), provokes acute and fatal encephalitis in its mammal hosts. The disease etiologic agent is the rabies virus which is a neurotropic, RNA virus belonging to the order Mononegavirales, family Rhabdoviridae, genus *Lyssavirus* (1). Transmission of rabies infection usually occurs when infected saliva reaches a bite wound or skin scratches, or breaches mucous membranes. A rare route of rabies infection transmission include aerosol infection as in bat caves, ingestion of an infected carrier and trans-placental infection. Transmission has occurred in man following transplants of corneas taken from infected patients. Not all animals or human bitten contract the infection. The severity, location, and multiplicity of bites inflicted on the victim, biotype of the virus and the susceptibility of the recipient influence the outcome of potential exposure to infection. Bites on the head and neck are associated with the shortest incubation period (2).

Rabies is a serious public health problem in developing countries, especially in Asia. Approximately 35000 to 50000 human deaths occur due to rabies each year (3). Administration of rabies vaccine along with anti-rabies immunoglobulin is known to prevent development of rabies; however, prompt and precise diagnosis is essential for rabies diagnosis, direct immunofluorescence detection of rabies virus antigens has been used worldwide as a rapid and reliable method.

Most rabies-specific antibodies used for diagnosis are made from sera of immunized mammals such as mice, rabbits and goats. However, producing a large amount of specific antibodies from these animals is time-consuming and labor intensive. There is a concern that handling live and large amounts of rabies virus to produce antigen may pose a potential risk of infection to laboratory personnel (4).

Recent advances in molecular biology together with newly invented methods of producing antigen-specific antibodies in egg yolk (IgY) have created new opportunities to develop a safe, convenient and inexpensive way of manufacturing various immunodiagnostics (5.6).

The IgY project which developed uncomplicated techniques for immunization of hens, isolation of egg yolk antibodies, and their applicability in various test systems creates continuous considerations in regard of changing from mammalian derived antibodies to egg yolk antibodies for both, (The National Laboratory for Immunology and diagnostics and the Internationally Orientated Service Laboratory).

Regarding commercially available polyclonal antibodies being produced worldwide it was found that

Author α σ ρ: Veterinary Serum and Vaccine Research Institute, P.O. Box: 131, Abbasia, Cairo, Egypt. e-mails: behwara@gmail.com, svri@idsc.gov.eg

© 2016 Global Journals Inc. (US)

297 from almost 16,000 which is less than 2% of polyclonal antibodies deriving from chicken, and only three from these have been prepared from egg yolk, the rest apparently from chicken sera, it may be concluded that in general, chicken are potent antibody producers. Obviously, the IgY-technology needs further propagation (7).

The present work aims to prepare anti-rabies IgY in chicken egg yolk in addition to preparation of anti-rabies IgG in rabbits and evaluate their efficacy in protection against rabies infection in emergency cases simulated in mice.

II. Materials and Methods

Ethics approval: The experiments were carried out according to the protocol of Institutional Animal Ethics Committee and the authors had a permission of the animal owners at the private farms.

a) Baby hamster kidney cell culture (BHK21)

BHK21 was supplied by DPAVR, VSVRI and used in application of SNT to estimate rabies neutralizing antibody titers in the obtained anti-rabies IgY and IgG preparations.

b) Viruses

i. *Cell culture adapted rabies virus*

Evelyn Rokitnicki Abelseth (ERA) strain of rabies virus adapted to BHK-21 cell line with a titer of 7 $\log_{10}$ $TCID_{50}$ /ml was supplied by Department of Pet Animal Vaccine Research (DPAVR), Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt. It was used in serum neutralization test to estimate the induced antibody titer in immunized chicken.

ii. *Challenge virus strain (CVS)*

Mice brain adapted rabies virus with a titer of 6.5 $\log_{10}$ MLD_{50}/ml was obtained from the DPAVR- VSVRI and used for experimental infection of mice.

c) Experimental Hosts

i. *Chickens*

Fifteen, 35 week-old Rhode Island Red (RIR) hens, were used for egg yolk IgY preparation after their immunization with local rabies vaccine in a dose of 0.5 ml/hen inoculated subcutaneously on a week intervals for 5 successive weeks according to (8). Eggs were collected weekly for 6 weeks from these hens where the anti-rabies IgY were separated and titrated.

ii. *Rabbits*

Ten Bosket rabbits of about 3kg body weight were used for preparation of anti-rabies IgG after their immunization with the local rabies vaccine in a dose of 0.5 /rabbit inoculated subcutaneously on a week intervals for 5 successive weeks according to (8).

Weekly blood samples were collected from chicken and rabbits for 6 weeks for monitoring the levels of induced rabies antibodies in their sera.

iii. *Mice*

Two hundreds and five weaned Swiss Albino mice (3-4 weeks old) were experimentally infected with 0.03 ml of CVS /mouse intramuscularly while five mice are kept without infection.

d) Rabies vaccine

Inactivated cell culture rabies vaccine (ERA strain) was obtained from DPAVR- VSVRI and used for inoculation of chicken and rabbits to prepare anti-rabies IgY from chicken egg yolk and IgG from rabbit sera.

e) Isolation and separation of chicken egg yolk IgY

IgY was separated from egg yolk with PEG-6000 as described by (9). and its protein content was measured as described by (10). Where it was 2.32 g /dl.

f) Separation of rabies IgG from rabbit serum

Rabies IgG was precipitated from rabbit serum using ammonium sulphate according to (11). And its protein content was estimated according to (10), and it was 3.42 g /dl.

g) Quality control testing of the prepared anti-rabies IgY and IgG

Quality control testing of the obtained preparations were carried out following the directions of (3). Including sterility, safety and potency tests.

h) Serum neutralization test (SNT)

SNT was carried out to estimate rabies neutralizing antibodies in test chickens and rabbits as described by (12), and the antibody titer was determined as the reciprocal of the final serum or immunoglobulin preparations dilution which neutralized and inhibited the appearance of the cytopathic effect (CPE) of 100 $TCID_{50}$ of rabies virus.

i) Experimental design

The previously mentioned infected mice were divided into seven groups where each of the first 5 groups included 40 mice, where the 1st and 2nd groups received anti-rabies IgY and IgG using a dose of 0.5 ml/mouse inoculated intraperitoneally containing 116 mg of IgY and 171 mg of IgG respectively on daily intervals started from the day of experimental infection up to 7 days post infection. The 3rd and 4th groups were treated with IgY with rabies vaccine and IgG with rabies vaccine respectively using the same mentioned doses (As in groups 1and 2) where rabies vaccine was inoculated once in a dose of 0.5ml /mouse inoculated intraperitoneally on the same day of infection. The 5th group was treated with the rabies vaccine alone with the same dose.

The 6th group of 5mice was kept infected without any treatment (Control +ve). In addition the

© 2016 Global Journals Inc. (US)

7th group of 5 mice was kept without infection as (Control -ve).

III. Results and Discussion

The present results showed that the prepared anti-rabies IgY and IgG were free from foreign contaminants (aerobic and anaerobic bacteria, fungi and mycoplasma) and safe, inducing no abnormal signs in inoculated mice either generally or at the site of inoculation as shown in Table (1). These findings come in agreement with the recommendations of (13).

Table (1) : Quality control testing of anti-rabies IgY and IgG

Tested preparation	Protein content	Sterility	Safety	Potency
Anti-rabies IgY	2.32g/dl	Sterile	Safe	Potent
Anti-rabies IgG	3.42g/dl			

In addition, estimation of the protein contents in the prepared anti-rabies chicken egg yolk IgY and rabbit IgG revealed that they had levels of 2.32g/dl and 3.42g/dl respectively Table(1). Such high levels of protein contents could be attributed to the formation of antibodies which mainly consisted of globulins as stated by (14), (15), (16) and (17).

Table (2) demonstrated that rabies serum neutralizing antibody titers began to appear in the sera of chickens and rabbits by the 1st week post immunization recorded their highest levels (64 and 128 respectively) by the 5th week. These findings showed that rabbits have higher antibody titers than that of chickens, the thing which could be attributed to the host susceptibility as rabies is a mammal host specific virus (4). It was found that the levels of rabies antibodies in chicken and rabbit sera were increased gradually started from the 1st week post immunization recording their peak by the 5th week. In this respect similar findings were obtained by (18), (19)., (20) and (21). who considered such sera as hyper- immune preparations depending on their high antibody titers where the protective rabies antibody titer is 0.5 IU (about titer of 32).

Table (2) : Rabies neutralizing antibody titers in sera of chicken and rabbits

Tested serum	Mean serum rabies neutralizing antibody titer*/WPI**					
	1WPI	2WPI	3WPI	4WPI	5WPI	6WPI
Chicken	8	16	32	32	64	64
Rabbit	8	16	32	64	128	128

**Antibody titer = the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100TCID$_{50}$ of rabies virus*

***WPI= week post immunization*

On the other hand, rabies neutralizing antibody titers were found to be 64 and 128 in chicken egg yolk IgY and rabbit serum IgG respectively Table (3) in a parallel manner confirming that such preparation could be considered as hyperimmune products against rabies virus.

Table (3) : Rabies neutralizing antibody titers in chicken egg yolk IgY and rabbit IgG preparations

Tested preparation	Mean rabies neutralizing antibody titer*/WPI**					
	1WPI	2WPI	3WPI	4WPI	5WPI	6WPI
Chicken egg yolk IgY	0	16	32	64	64	64
Rabbit IgG	4	8	16	32	64	128

**Antibody titer = the reciprocal of the final immunoglobulin preparations dilution which neutralized and inhibited the CPE of 100TCID$_{50}$ of rabies virus*

***WPI= week post immunization*

Treatment of experimentally rabies infected mice with the prepared anti-rabies IgY and IgG showed that the best time for administration of anti-rabies treatment is from 0 time to 2 days post exposure to virus infection providing 100% protection. This protection rate decreased to 40, 20 and 0% for treatment with IgY and 60, 30 and 0% for treatment with IgG on the 3, 4 and 5 days later as shown in Table (4). Non-treated infected mice showed typical rabies signs represented by paralysis of the hind limbs and tail by the 4th day post infection ended with death while non-infected non-treated mice remained healthy allover the experimental period. These findings come in complete agreement with what reported by (21)., (22). And (17). who concluded that post exposure treatment through passive immunization of the victim should be carried out as soon as possible post exposure to viral infection recommended the same present recorded times. Similar results were obtained in case of infected mice treated with either of IgY or IgG with rabies vaccine while treatment with rabies vaccine alone was unable to protect mice against rabies virus infection the thing

© 2016 Global Journals Inc. (US)

which could be attributed to the fact that stimulation of active immunity require longer time to be detectable; such time was found to be overcame by the passive immunity provided by IgY and IgG.

Table (4) : Potency of chicken egg yolk anti-rabies IgY and rabbit anti-rabies IgG in mice

Group	Received Treatment	Number of survived mice/number of treated mice = protection%							
		0 DOT*	1 DOT	2 DOT	3 DOT	4 DOT	5 DOT	6 DOT	7 DOT
1	IgY	100	100	100	40	20	0	0	0
2	IgG	100	100	100	60	30	0	0	0
3	IgY& rabies vaccine	100	100	100	40	20	0	0	0
4	IgG &rabies vaccine	100	100	100	60	30	0	0	0
5	Rabies vaccine alone	These mice were unable to withstand the virus infection where the active immunity required longer time to be effective (0%)							
6	Infected &not treated mice showed typical rabies signs stared by the 4th day post infection (control +ve) (0%)								
7	Non infected &non treated mice remained healthy allover the experimental period (control –ve)								

**DOT= day of treatment post infection*

IV. Conclusion

Depending on the obtained results through the present work, it could be concluded that both of anti-rabies chicken egg yolk IgY and rabbit serum IgG are able to withstand rabies infection when they are administrated on the optimum time post exposure as simulated in mice and further studies are in need to evaluate such preparations in farm animals. In addition, the preparation of chicken IgY in a non-specific host provides safe, high potent product of lower cost than that prepared in rabbits or other mammals where a huge amount of IgY could be obtained through the egg production life of hens with easily housing and simple management requirements.

Author's contribution

Ahmed Mohamed Albehwar immunized hens and rabbits with rabies vaccine and collected egg of hens and serum of the rabbits and prepared the IgY and IgG solutions, applied SNT on the obtained preparations to determine the antibody titers, evaluated the results and revised the data and write the research.

Abeer Atia Tammam estimated the IgY and IgG content in the obtained preparations and made the quality control testing on both of them besides sharing in application of SNT and writing the research.

Amr Ismael Hassan made experimental Infection of mice then immunized them with the obtained preparations to determine their protection rates and analyzed and tabulated the obtained data. All authors read and approved the final manuscript.

V. Acknowledgments

Authors are greatly thankful to Prof. Dr. Mohamed hasan khodeir, Chief of Researches in the DPAVR-VSVRI, Prof. Dr. Mohamed Saad Ali Saad, Chief of Researches in the Veterinary Reproduction Research Institute. And all members of Pet Animals Vaccine Research Department-VSVRI.

This work was funded by Veterinary Serum and Vaccine Research Institute, Abbasia, Cario, Egypt.

Competing interests: The authors declare that they have no competing interests.

References Références Referencias

1. *Fauquet, E. M.; Mayo, M. A.; Maniloff, J.; Desselgerger, U. and Ball, L. A. (2005):* Virus taxonomy: classification and nomenclature of viruses. Eighth report of the International Committee on the Taxonomy of viruses. Virus Taxonomy, 8th San Diego: Academic Press; p: 630-634.
2. *Green, C. E. and Rupprecht, C. E. (2006):* Rabies and other lyssavirus infections. Infec. Dis. Dog and Cat, 3rd Ed.: 167-183.
3. *Hampson, K.; Coudeville, L.; Lembo, T.; Sambo, M.; Kieffer, A.; Attlan, M.; Barrat, J.; Blanton, J.D.; Briggs, D.J.; Cleaveland, S.; Costa, P.; Freuling, C.M.; Hiby, E.; Knopf, L.; Leanes, F.; Meslin, F.X.; Metlin, A.; Miranda, M.E.; Müller, T.; Nel, L.H.; Recuenco, S.; Rupprecht, C.E.; Schumacher, C.; Taylor, L.; Vigilato, M. A.; Zinsstag, J.; Dushoff, J. and Global Alliance for Rabies Control Partners for Rabies Preventio (2015):* Estimating the global burden of endemic canine rabies. PLoS Negl Trop Dis. 16; 9 (4)
4. *Yurie Motoi, S.I.; Hajime. H; Kozue sato, K. M. and Kio, Y. (2005):* Detection of rabies-specific antigen by egg yolk antibody (IgY) to the recombinant rabies virus proteins produced in Escherichia coli Jpn. J. Infect. Dis., 58, 115-118.
5. *Vega, C. G.; Bok, M.; Vlasova, A.N.; Chattha, K.S.; Fernández, F.M.; Wigdorovitz, A.; Parreño, V.G. and Saif, L.J. (2012):* IgY antibodies protect against human Rotavirus induced diarrhea in the neonatal gnotobiotic piglet disease model. PLoS One. ; 7 (8): e42788. doi: 10.137
6. *Inoue, S.; Sato, Y.; Hasegawa, H.; Noguchi, A.; Yamada, A.; Kurata, T. and Iwasaki, T. (2003):* Cross-reactive antigenicity of nucleoproteins of lyssaviruses recognized by a monospecific

© 2016 Global Journals Inc. (US)

antirabies virus nucleoprotein antiserum on paraffin sections of formalin-fixed tissues. Pathol. Int., 53, 525-533.

7. *Tabll, A.; Abbas, A.T.; El-Kafrawy, S. and Wahid, A. (2015):* Monoclonal antibodies: Principles and applications of immmunodiagnosis and immune-therapy for hepatitis C virus. World J. Hepatol. 8; 7 (22): 2369-83
8. *Ihemelanadu, E. C.; Neduaka, O. and Ojukwa, E. M. (1985):* Hyper-immune Serum in the control of pest des petit ruminants. Trop. Anim. Heal. Prod. 17, (2): 83-88.
9. *Tong, C.; Geng, F.; He, Z.; Cai, Z. and Ma, M. (2015):* A simple method for isolating chicken egg yolk immunoglobulin using effective delipidation solution and ammonium sulfate. Poult. Sci. ; 94 (1): 104-10.
10. *Hogan, I.; Doherty, M.; Fagan, J.; Kennedy, E.; Conneely, M.; Brady, P.; Ryan, C. and Lorenz, I. (2015):* Comparison of rapid laboratory tests for failure of passive transfer in the bovine Ir. Vet. J.; 68 (1): 18.
11. *Narin, R. C. and Marrack, J. R. (1964):* Fluorescent protein tracing 2nd Ed. Livingston Edinburgh and London.
12. *Lewis, C.E.; Reising, M.M.; Fry, A.M.; Conrad, S.K.; Siev, D.; Gatewood, D.M. and Hermann, J.R. (2013):* Evaluation of a non-invasive, inhalational challenge method for rabies vaccine potency assay. J. Virol. Methods. 190 (1-2): 49-52.
13. *Hanlon C.A., Kuzmin I.V., Blanton J.D., Weldon W.C., Manangan J.S. & Rupprecht C.E. (2005).* Efficacy of rabies biologics against new lyssaviruses from Eurasia. Virus Res., 111, 44–54.
14. *Kataria, A. K. and Sharma, K. N. (1993):* Serum-electrophoretic studies on sheep vaccinated with live sheep pox virus (Rumanian strain) Ind. Vet. J. 50: 387-393.
15. *Hala, A. Fadl; Khodeir, M. H.; and Hussein, A. Z. (2003):* Preparation and evaluation of rabbit antigoat and antisheep serum conjugated with fluorescein isothiocyanate. Beni-Suef Vet. Med. J. Vol. XIII, No. (1): 125-133.
16. *Hanan, M. Ibrahim; Zeinab, M. Soror; Elham, A. El-Ibiary and Khodeir, M. H. (2003):* Preparation and evaluation of antiduck and antiturkey sera conjugated with fluorescein isothiocyanate for serological use. Beni-Suef Vet. Med. J. Vol. XIII, No. (1): 193-199.
17. *Albehwar, A. M. (2011):* The use of rabies immune globulin in rabies infection simulated in mice 29th Arab Vet. Med. Cong. 469-475.
18. *Lamprea, N.P.; Ortega, L.M.; Santamaría, G.; Sarmiento, L.; Torres-Fernández, O. (2010):* Production and evaluation of an antiserum for immunohistochemical detection of rabies virus in aldehyde fixed brain tissues. [Article in Spanish] Biomedica. 30 (1): 146-51.
19. *WHO (1992a):* Expert Committee on Rabies WHO Tech. Report Series 824, pp: 84.
20. *Edries, S. M.; Khodeir, M. H. and Habashi, Y. Z. (1999):* Production and evaluation of rabies hyperimmune serum for serological tests. 5th Sci. Cong. Egyp. Soc Cattle Dis., Assiut, Egypt; 176-179.
21. *Khodeir, M. H. and Daoud, A. M. (2008):* Preparation of anti-rabies hyperimmune serum for emergency immunization of farm animals. 4th Int. Sci. Conf. NRC, 1-9.
22. *Wilson, P.J; Oertli, E.H.; Hunt, P.R. and Sidwa, T. J. (2010):* Evaluation of a postexposure rabies prophylaxis protocol for domestic animals in Texas: 2000-2009. J Am. Vet. Med. Assoc. 15; 237 (12): 1395-401.

كفاءة إميونوجلوبيولينات السعارIgG و IgY لوقاية الفئران من عدوى الفيروس كنموذج للتدخل الاضطرارى

أحمد محمد البهوار, عبير عطية تمام, عمرو إسماعيل حسن

معهد بحوث الأمصال واللقاحات البيطرية- العباسية- القاهرة

ص.ب:131

تم خلال هذا العمل تحضير IgY وIgG مضادين لفيروس السعار فى مح بيض الدجاج ومصل الأرانب حيث وجد أن محتوى الجلوبيولين هو 2.32 جرام / ديسيلتر و 3.42 جرام/ ديسيلتر على التوالى وكان معيار أجسام السعار المناعية الخلطية (باستخدام اختبار المصل المتعادل) 64 و 128 فى IgY و IgG على التوالى موازيالمثيله فى أمصال الدجاج والأرانب المستخدمة فى تحضير هذه المستحضرات.

ولقد وجد أن كلا المستحضرين خالى من الملوثات قبل الحقن وآمن عند حقنه فى الفئران السويسرية وعند الحقن فى الفئران المعدية تجريبيا بفيروس السعار الضارى (المؤقلم على أمخاخ الفئران) بتجويف البطن على فترات يومية بعد العدوى، تبين أن أنسب وقت للتدخل بهذه المستحضرات (بتوفير مناعة سلبية) هو من وقت التعرض للإصابة وحتى اليوم الثانى معطيا نسبة حماية 100% بينما تقل هذه النسبة إلى 40، 20 ثم 0 % عند العلاج باستخدام IgY وإلى 60، 30 ثم 0% عند استخدام IgG فى الأيام 3،4، 5 بعد التعرض للعدوى.هذا وقد تم الحصول على نفس النتائج حال استخدام كل من IgY وIgG مع اللقاح فى حين لم يكفى استخدام اللقاح وحده لحماية الفئران ضد عدوى الفيروس حيث أن الأمر يتطلب وقتا أطول لاستحداث مناعة إيجابية تكفى للتغلب على الفيروس 0وعلى ذلك يمكن االتوصية باستخدام أحد المستحضرين مع اللقاح حال التعرض للعقر من حيوانات مصابة.

كما يمكن القول بأن كل من المستحضرين له القدرة على التغلب على عدوى السعار خاصة عندما يتم التدخل به فى الوقت المناسب،إلا أن تحضير IgY فى مح بيض الدجاج يستلزم جهد وتكلفة أقل وبكميات أكبر من إنتاج IgG فى الأرانب إضافة إلى كونه محضر فى عائل غير أساسى الأمر الذى يزيد من أمان تحضيره واستخدامه.

© 2016 Global Journals Inc. (US)

This page is intentionally left blank

© 2016 Global Journals Inc. (US)

Global Journal of Medical Research: G
Veterinary Science and Veterinary Medicine
Volume 16 Issue 1 Version 1.0 Year 2016
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

An Outbreak of *Corynebacterium Diphtheriae* Infection in Broiler Chickens in Lagos, Nigeria

By Enurah L.U, Olubade, T, Nwamo, A.C & Sadiku, R.T.

National Veterinary Research Institute Laboratory, Nigeria

Abstract- The outbreak involved 1,200 15 weeks old white leghorn growers in a poultry farm in Lagos out of which 163 died without any premonitory signs. Postmortem examination revealed congested lungs, haemorrhagic inflammation of the upper respiratory tract, crop, proventriculus and petechial haemorrhage of the cardiac muscles. *Corynebacterium diphtheria* was isolated from the intestine, heart blood, lung, upper respiratory tract and liver of all that died. The pathogenicity of the isolates was conducted on 8 15-weeks old white leghorn with two as controls using 0.5ml overnight broth culture administered orally. They were observed for up to 13 days with 100% mortality. The organism was re-isolated from the heart blood, liver, lung, trachea, proventiclus and crop of infected chickens. The control showed no sign of illness during the period of observation. This study revealed the zoonotic nature of *Corynebacerium diphtheriae* which association with poultry in Nigeria has not been reported as far as the authors knew.

Keywords: outbreak, corynebacterium diphtheriae, broiler chickens.

GJMR-G Classification : NLMC Code: WA 360

Strictly as per the compliance and regulations of:

© 2016. Enurah L.U, Olubade, T, Nwamo, A.C & Sadiku, R.T. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/licenses/by-nc/3.0/), permitting all non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

An Outbreak of *Corynebacterium Diphtheriae* Infection in Broiler Chickens in Lagos, Nigeria

Enurah L.U [α], Olubade, T [σ], Nwamo, A.C [ρ] & Sadiku, R.T. [ω]

Abstract- The outbreak involved 1,200 15 weeks old white leghorn growers in a poultry farm in Lagos out of which 163 died without any premonitory signs. Postmortem examination revealed congested lungs, haemorrhagic inflammation of the upper respiratory tract, crop, proventriculus and petechial haemorrhage of the cardiac muscles. *Corynebacterium diphtheria* was isolated from the intestine, heart blood, lung, upper respiratory tract and liver of all that died. The pathogenicity of the isolates was conducted on 8 15-weeks old white leghorn with two as controls using 0.5ml overnight broth culture administered orally. They were observed for up to 13 days with 100% mortality. The organism was re-isolated from the heart blood, liver, lung, trachea, proventiclus and crop of infected chickens. The control showed no sign of illness during the period of observation. This study revealed the zoonotic nature of *Corynebacerium diphtheriae* which association with poultry in Nigeria has not been reported as far as the authors knew.

Keywords: outbreak, corynebacterium diphtheriae, broiler chickens.

I. Introduction

Corynebacteria are gram-positive, catalase-positive, aerobic or facultative anaerobic, generally non motile rods. The genus contains the species *Corynebacterium diphtheria* and the non-diphtherial corynebacteria, collectively referred to as diphtheroids (Burkovski, 2013). Nondiphtherial corynebacteria, originally thought to be mainly contaminants, have increasingly over the past two decades been recognized as pathogenic, especially in immune-compromised hosts (Ott and Burkovski 2013). Today, the more common scenario is nondiphtherial corynebacterial bacteremia associated with diverse infections as well as meningitis, septic arthritis, and urinary tract infections (Bonmarin et al., 2009). Nondiphtherial corynebacteria also cause chronic and subclinical diseases in domestic animals and can lead to significant economic losses for farmers (Bonmarin et al., 2009). Examples of widespread and difficult- to- control infections include *Corynebacterium pseudotuberculosis* caseous lymphadenitis in sheep, and goats; *C. pseudotuberculosis* ulcerative dermatitis in cattle; and uninary tract infections and mastitis in cattle due to infection with *Corynebacterium renale, C. cystidis, C. pilosum and C. bovis.* (Yassin et al., 2003). *C. diphtheria* infection is typically characterized by a local inflammation, usually in the upper respiratory tract, associated with toxin-mediated cardiac and neural disease. Three strains of *C. diphtheria* are recognized in decreasing order of virulence: gravis, intermedius and mitis. These strains produce an identical toxin, but gravis strain is potentially more virulent because it grows faster and depletes the local iron supply, allowing for earlier and greater toxin production. Toxin production is encoded on the *tox* gene, which in turn, is carried on a lysogenic beta phage. When DNA of the phage integrates into the host bacteria's genetic material, the bacteria develop the capacity to produce this polypeptide toxin. The *tox* gene is regulated by a corynebacterial iron-binding repressor (DtxR). Binding of ferrous iron to the DtxR molecule forms a complex that binds to the tox gene operator and inhibits transcription. Depletion of iron from the system removes the repression and allows the toxin to be produced. The toxin is a single polypeptide with an active (A) domain, a binding (B) domain and a hydrophobic segment known as the T domain, which helps release the active part of the polypeptide into the cytoplasm. In the cytosol, the A domain catalyzes the transfer of an adenosine diphosphate-ribose molecule to one of the elongation factors (eg elongation factor 2 EF2) responsible for protein synthesis. This transfer inactivates the factor, thereby inhibiting cellular protein synthesis. Inhibiting all the protein synthesis in the cell causes cell death. In this manner, the toxin is responsible for many of the clinical manifestations of the disease. As little as 0.1μg can cause death in guinea pigs. In 1890, von Behring and Kitasato demonstrated that sublethal doses of the toxin induced neutralizatiing antibodies against the toxin in horses. In turn, this antiserum passively protected the animals against death following infection. By the early 1900s, treating the toxin with heat and formalin was discovered to render it nontoxic. When injected into recipients, the treated toxin induced neutralizing antibodies. By the 1930s, many Western countries began immunization programs using this toxoid. The disease occurs mainly in temperate zones and is endemic in certain regions of the world. Humans are the known reservoir for the disease. The primary modes of dissemination are by airborne respiratory droplets, direct contact with droplets or infected skin lesions. Asymptomatic respiratory carrier states are believed to be important in perpetuating both endemic and epidemic disease (Collins and Cummins, 1986).

Author α σ ρ ω: National Veterinary Research Institute Laboratory, Lagos, Nigeria. e-mail: enurahleonard@yahoo.com

© 2016 Global Journals Inc. (US)

The toxin induced manifestations involve mainly the heart, kidneys and peripheral nerves. Cardiac enlargement due to myocarditis is common. The kidneys become edematous and develop interstitial changes. Both the motor and sensory fibers of the peripheral nerves demonstrate fatty degenerative changes and disintegration of the medullary sheaths. The anterior horn cells and posterior columns of the spinal canal can be involved and the CNS may develop signs of haemorrhage, meningitis and encephalitis. Death is mainly due to respiratory obstruction by the membrane or toxic effects in the heart or nervous system. The epidemiology of *C. diphtheria* infection has been changing. Increasing number of skin, pharyngeal and bacteremic infections with nontoxigenic bacteria have been reported. Among 828 cultures of nontoxigenic *C.diphtheriae* isolated from different regions of Russia from 1994-2002, 14% carried the gene for the toxin (Burkovski, 2013). Molecular characterizations based on polymerase chain reaction (PCR) of some of these nontoxigenic strains have demonstrated that the bacteria often contain functional DtxR proteins, which could potentially produce toxin (Pitcher, 1983). No documented reports of an outbreak of *Corynebacterium diphtheria* infection in chicken in Nigeria have so far been made. This study describes a peculiar case of an outbreak of *C. diphtheria* infection in a private poultry farm in Lagos, Nigeria.

II. Materials and Methods

a) Collection of samples

The outbreak involved 1,200, 15-week-old white leghorn broiler chickens kept in battery cages. Out of this number 163 died without any premonitory signs. As a result they did not receive any veterinary attention. At post mortem samples of the heart blood, liver, lung, and intestine were aseptically collected for possible isolation of the causative agents.

b) Processing of samples

Samples of intestine, lung, liver and heart blood were aseptically placed in sterile universal bottles containing 9ml of nutrient broth and were subsequently incubated for 24h at 37^{0}C. After 24h incubation, the broth was plated using sterile wire loop on Tinsdale selective medium (containing Tinsdale selective agar base and Tinsdale supplement) (Oxoid, UK) and incubated at 37^{0}C for 24h. The resultant colonies were characterized by Gram stain and biochemical tests.

c) Pathogenicity test

Colonies from Tinsdale medium were inoculated into nutrient broth and incubated for 24h at 37^{0}C to obtain pure culture. This was used to challenge eight 15 week old white leghorn at 0.5ml each orally while two served as control. They were kept in separate cages, fed and given clean water and were observed daily for 14days.

III. Result/Discussion

The original carcasses had lesions suggestive of acute gastroenteritis, pneumonia and septicemia. The postmortem picture was characterized by haemorrhagic inflammation and oedema of the gastro-intestinal tract, fibrinous pneumonia and petechial haemorrhages of the myocardium. Pure culture of *Corynebacterium diphtheria* was isolated from the heart blood, intestine, liver and lung. Positive *Corynebacterium diphtheriae i*dentification was based on the presence of gram-positive pleomorphic rods with deeply metachromatic granules in smears. On Tinsdale medium grayish black colonies were obtained. The results were interpreted according to Barrow and Feltham (1995).

The isolate proved lethal for chicken killing all the inoculated eight birds: 6 in 11days and the rest in 13 days. Necropsy findings in the infected chickens were the same as the naturally infected chickens but in addition, the epithelial wall of the proventriculus was swollen with necrotic foci and heavily infiltrated with purulent exudate. The causal agent was re-isolated from all the infected chickens.

The isolation of *Corynebacterium diphtheria*, a primary pathogen of human diphtheria infection from chicken is interesting as there appears to be no previous records of its incidence among chickens as far as the authors knew. The virtually wide host range makes *Corynebacterium diphtheria* infection a zoonotic disease of both veterinary and public health importance. It is likely that many more cases might be occurring in chickens and other species than are reported. It is advisable to ensure individual sanitation of farm attendants as they could be the major source of infection, and a general sanitation of the farm. There should be culling of infected birds to limit the spread of infection to the healthy ones. The use of broad spectrum antibiotics in poultry feeds may be an effective prophylactic measures against *Corynebacterium diphtheriae* infection.

IV. Acknowledgement

The authors are grateful to the Executive Director, National Veterinary Research Institute, Vom Plateau State, Nigeria for permission to publish this paper.

References Références Referencias

1. Barrow, G.I and Feltham, R.K.A (1995). *Corynebacterium diphtheriae* In COWAN AND STEEL'S Manual for the identification of medical bacteria. 3rd edition. Cambridge University press. Pp 69-71.
2. Bonmarin, I., Guiso, N., Le Fleche-Mateos, A., Petery, O., Patrick, A.D and Levy-Bruhl D. (2009). Diphtheria: a zoonotic disease in France? *Vaccine* **27** (31) 4196-4200.

© 2016 Global Journals Inc. (US)

3. Burkovski A (2013). Diphtheria and its etiological agents, In *Corynebacterium diphtheria* and related toxigenic species. A. Burkovski (ed) Springer, Dortrecht, pp1-14.
4. Collins, M.D and Cummins, C.S. (1986). Genus Corynebacterium In Bergey's Manual of Systematic Bacteriology,. Vol.2, pp 1266-1276.
5. Ott, L and Burkovski A (2013). Toxigenic corynebacteria adhesion, invasion and host response, In *Corynebacterium diphtheriae* and related toxigenic species. A. Burkovski (ed). Springer, Dortrecht pp 143-170.
6. Pitcher, D.G (1983). Deoxyribonucleic acid (DNA) base composition of *Corynebacterium diphtheriae* and corynebacteria with cell wall type.*FEM Microbiol. Lett.*16 (2-3) 291-295.
7. Yassin, A.F., Kroppenstedt, R.M., Ludwig. W (2003). *Corynebacterium glaucum sp.nov. Int. J. Syst. Evol. Microbiol.* 53 (pt3): 705-709.
8. Yeruham, I., Friedman, S., Perl, S., Glad, D., Berkovich, Y and Kalgard, Y (2004). A herd level analysis of a *C. pseudotuberculosis* outbreak in a dairy cattle herd. *Veterinary Dermatology.* 15, 315-320.

© 2016 Global Journals Inc. (US)

This page is intentionally left blank

© 2016 Global Journals Inc. (US)

Global Journal of Medical Research: G
Veterinary Science and Veterinary Medicine
Volume 16 Issue 1 Version 1.0 Year 2016
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Chromosomes and Cytogenetics of Trematodes

By Fayaz Ahmad, Tanveer A. Sofi, Khalid M. Fazili, Bashir A. Sheikh, Aasif Ahmad Sheikh, Tantry Tariq Gani & Omer Mohi Ud Din Sofi

University of Kashmir, India

Abstract- Here we review the literature from 1902 to 2015 and the current status of knowledge of the chromosomes and cytogenetics within the family species of trematodes. Karyological data are discussed and tabulated for 278 species of trematodes. Numerous species of trematodes points towards the continued efforts in this field of research. The present study also revealed new data on chromosome complements of diplozoid parasites, namely Diplozoon Kashmirensis Kaw, 1950 from *Schizothorax esocinus; Diplozoon aegyptensis* Fischthal *et* Kuntz, 1963 from *Schizothorax plagiostomum; Diplozoon guptai* Fayaz and Chishti, 1999 from *Schizothorax curvifrons* and one digenean i.e., *Clinostomum schizothoraxi* Kaw, 1950 from *S. curvifrons* which included one metacentric (no. 1); two submetacentric (no.s 2 and 3); three subtelocentric (no.s 4; 5 and 6) and four acrocentric (no.s 7, 8; 9 and 10) chromosome pairs. All the three species of *Diplozoon* species are characterized by the same number of chromosomes i.e., 2n=14 in which *D. kashmirensis* is characterized by seven pairs of long (up to 14.13 **µ**m) chromosomes and all chromosome pairs are acrocentric.

Keywords: diplozoon; schzothorax; karyotype; kashmir; metacentric; acrocentric; telocentric; subtelocentric.

GJMR-G Classification : NLMC Code: WC 805

CHROMOSOMESANDCYTOGENETICSOFTREMATODES

Strictly as per the compliance and regulations of:

© 2016. Fayaz Ahmad, Tanveer A. Sofi, Khalid M. Fazili, Bashir A. Sheikh, Aasif Ahmad Sheikh, Tantry Tariq Gani & Omer Mohi Ud Din Sofi. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/licenses/by-nc/3.0/), permitting all non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Chromosomes and Cytogenetics of Trematodes

Fayaz Ahmad [α], Tanveer A. Sofi [σ], Khalid M. Fazili [ρ], Bashir A. Sheikh [ω], Aasif Ahmad Sheikh [¥], Tantry Tariq Gani [§] & Omer Mohi Ud Din Sofi [χ]

Abstract- Here we review the literature from 1902 to 2015 and the current status of knowledge of the chromosomes and cytogenetics within the family species of trematodes. Karyological data are discussed and tabulated for 278 species of trematodes. Numerous species of trematodes points towards the continued efforts in this field of research. The present study also revealed new data on chromosome complements of diplozoid parasites, namely *Diplozoon Kashmirensis* Kaw, 1950 from *Schizothorax esocinus; Diplozoon aegyptensis* Fischthal *et* Kuntz, 1963 from *Schizothorax plagiostomum*; *Diplozoon guptai* Fayaz and Chishti, 1999 from *Schizothorax curvifrons* and one digenean i.e., *Clinostomum schizothoraxi* Kaw, 1950 from *S. curvifrons* which included one metacentric (no. 1); two submetacentric (no.s 2 and 3); three subtelocentric (no.s 4; 5 and 6) and four acrocentric (no.s 7, 8; 9 and 10) chromosome pairs. All the three species of *Diplozoon* species are characterized by the same number of chromosomes *i.e.*, 2n=14 in which *D. kashmirensis* is characterized by seven pairs of long (up to 14.13 µm) chromosomes and all chromosome pairs are acrocentric. Karyotype of *D. aegyptensis* also contains 14 chromosomes but with different morphology in which first six chromosomes are metacentric and last eight chromosomes are acrocentric and the is length ranging up to 9.78 µm. Chromosomes of *D. guptai* ranges from 5.39 µm to 8.02 µm in which first four chromosomes are metacentric, two chromosomes are submetacentric, two chromosomes are subtelocentric and last six chromosomes are acrocentric. The present study describes for the first time the chromosome structure and number of three monogenean and one digenean species from the host *Schizithorax* species of Dal lake of Kashmir Valley.

Keywords: diplozoon; schzothorax; karyotype; kashmir; metacentric; acrocentric; telocentric; subtelocentric.

I. Introduction

Diplozoid monogeneans are gill ectoparasites of freshwater, mainly cyprinid fish are represented by two dozens of species in Europe (Khotenovsky 1985). Systematics of the family remains problematic due to a relatively high interspecific similarity in morphological features and limited number of species included in molecular comparisons (Matejusova *et al*. 2001; 2004; Gao *et al*. 2007; Civanova *et al*. 2013; Avenant-Oldewage *et al*. 2014). Therefore, supplementary approaches would help in the assessment of species delimitation and/or phylogeny. To date, only few studies have been focused on diplozoid cytotaxonomy and none from the Kashmir valley. Koroleva (1968a,b; 1969) showed chromosome morphology of six species from various fish hosts, and Koskova *et al*. (2011) showed specified cytogenetic characteristics of four of them. Species of *Paradiplozoon bliccae*, *Paradiplozoon sapae*, *Paradiplozoon nagibinae*, *Paradiplozoon pavlovskii* and *Paradiplozoon homoion* has 14 acrocentric elements in their diploid set (2n=14), while *Diplozoon paradoxum* has 2n=8. Baer and Euzet (1961); Bovet (1967); Koroleva (1969) showed that three undetermined diplozoids showed either 14 or 10 chromosomes in diploid set and Koroleva (1968b) studied two other species *Eudiplozoon nipponicum* and *Paradiplozoon megan*, revealed n=7 on the basis of meiotic bivalents without any information on the chromosome morphology. The present study describes for the first time the chromosome structure and number of three *Diplozoon* spp. from Kashmir Valley. The three species of *Diplozoon*, parasitizing *Schizothorax* species, have been the objects of our cytogenetic study, aimed at a comparison of a structure of their chromosome sets and an analysis of hypothetic routes of karyotype evolution within the group.

The Clinostomidae Luhe, 1901 is a family of digeneans, the members of which live in the oral cavity, gills, gill covers, eye sockets, operculum, fins, and gill lamellae of fishes. Due to the high degree of morphological variability within the same species, *Clinostomum* has been subjected to several taxonomic revisions (Gustinelli *et al*., 2010). The application of a karyology in parallel to morphological study may be particularly important for the identification of *Clinostomum* species described in the past only on the basis of morphological features.

The purpose of this study is to find out the chromosome number of trematodes from different vertebrate hosts. Differentiate trematodes on the basis of the karyological characteristics and role of these studies in cytotaxonomy. Lastly to find out the general aspects such as trends of karyotypic evolution and sex mechanism of trematodes.

II. Materials and Methods

Whole living specimens were placed in physiological saline (0.65% NaCl) containing colchicine

Author α σ ω: Department of Zoology, University of Kashmir, Srinagar, Kashmir. e-mail: stanveer96@gmail.com
Author ρ: Department of Bitechnology, University of Kashmir, Srinagar, Kashmir.
Author ¥: ICAR- National Dairy Research Institute, Karnal-132001, Haryana.
Author §: Sheri i Kashmir Institute Medical Sciences, Soura.
Author χ: SK University of Agricultural and Sciences and Technology, Shuhama, Aluestang Srinagar.

© 2016 Global Journals Inc. (US)

(0.05%) for 3–4 hours at room temperature then transferred into distilled water for about one hour for hypotony and fixed in ethanol- glacial acetic acid (3:1), with two changes, 15 minutes each. Spread preparation of mitotic and meiotic chromosomes was made as described by Petkeviciute and Leshko, 1991. Small posterior mature portions of fixed worms were transferred into drop of 60 % acetic acid on a slide and torn into fine pieces with the help of tungsten needles.

The slides were then placed on a heating plate at 45^{0}C and the drop of cell suspension was slowly drawn along the slide until it evaporated. Slides were dehydrated in an ethanol series (30%, 50%, 70%, 90% and 100%, 5 minutes each) and stored at –20^{0}C until use. Slides were stained with 4% Giesma solution (pH. 6.8) in phosphate buffer for 30 minutes, rinsed in tap water and allowed to dry. The best chromosome plates were photographed and used for morphological studies.

For karyotyping, chromosomes were cut out of the photomicrographs and paired on the basis of size and centromere position. Relative lengths of chromosomes were calculated by the division of the individual chromosome length by the total haploid length and centromeric indices (ci) were determined by division of the length, *i.e;*

$$Ci = \frac{\textit{Length of short arm} \times 100}{\textit{Total length of chromosome}}$$

Measurements are based on all chromosomes from 10 best metaphase spreads of parasites. The terminology relating to centromere position follows that of Levan *et al.,* 1964. A chromosome is metacentric (m) if the ci falls in the range of 37.5–50.0, submetacentric (sm) if 25.0–37.5, subtelocentric (st) if 12.5–25.0 and acrocentric (a) if < 12.5. When the centromere position was on the borderline between two categories, both are listed.

III. Results and Discussion

a) Chromosomes of Trematodes

A number of workers on trematode cytology, especially Jones and his co-workers, have pointed out the possible taxonomic value of the study of the numbers, volume, and/or size and shape of chromosomes, and much of our information along these lines is based upon their investigations. Among the Monogenea only the Polystomidae have been investigated for the purpose of determining the chromosome numbers. *Polystoma integerrimum* [Frolich, 1791] has the haploid number of 4 chromosomes and *Gyrodactylus elegans* has 6. This could point to a dalyelliid ancestry (n = 2 in all species studied) although more primitive ancestors of the dalyelliids may have had a larger basic number of chromosomes (Table 1). Among the Digenea, the paramphistomatids have been regarded as the most primitive group, but from a cytological standpoint the basic chromosome number is quite variable and is therefore of little guidance in determining possible relationships. For example, Gigantocotyle shows a basic number of 6, *Gastrothylax* and *Zygocotyle* have 7, *Cotylophoron* and *Diplodiscus* have 8, while *Heronimus* (chelydrae) has 10. No one family of the rhabdocoel group could be regarded as being directly ancestral based on such divergent records. As much as the exact phylogenetic relationships of the remaining families of the Digenea are not as yet fully determined, even on morphological grounds, and the various authorities disagree as to their proper taxonomic positions, no attempt will be made to discuss our knowledge of chromosome numbers in any significant succession. The diploid chromosome numbers vary among studied digenean taxa, from 12 to 28 (Bariene, 1993); chromosome sets with 20 or 22 elements predominate. But 56 chromosomes were found in diploid sets of *Clonorchis sinensis* [Cobbold, 1875] (Park *et al*., 2000). Allocreadiid species possess comparatively large chromosomes, up to 13–14 mm, but low haploid numbers of six, seven or eight were recorded in most species (for a review see Petkeviciute & Staneviciute, 2008). The chromosome complement of *Cercariaeum crassum* [Wesenberg-Lund, 1934] is unusual among digeneans due to the low number, 2n=10. The karyotype is composed of large and exclusively bi-armed chromosomes. Such a karyotype presumably results from a decrease in chromosome number through centromere–centromere Robertsonian fusions that have affected mono-armed chromosomes leading to the formation of large metacentric elements. Comparative analysis of chromosomes of related trematode species indicated that the reduction of chromosome numbers resulted from centromeric fusion rather than elimination of chromosomes (Grossman *et al*., 1981). Acrocentric mono-armed chromosomes prevail in the karyotypes of larval *B. luciopercae*, 2n = 14, and larval *A. isoporum sensu* Wisniewski, 1959, 2n = 14 (Petkeviciute & Staneviciute, 2008). It is notable that the mean total length of haploid complements (TCL) of these two species does not exceed the TCL of *C. crassum*, despite different chromosome numbers.

It may be noted, however, that all of the heterophyids (*Cryptocotyle* and *Acetodextra*), bucephalids (*Bucephalus* and *Rhipidocotyle*), fasciolids (*Fasciola*), and zoogonids (*Zoogonus*) examined (7 species), as well as 1 species of a gorgoderid (*Probolitrema*) and 1 of a paramphistomatid (*Gigantocotyle*) have a basic number of 6 [perhaps thus indicating some relationship to the more primitive paramphistomatids (n = 7)]. The notocotylids (*Notocotylus*) have 7 chromosomes, but too few examples have been studied to determine the value of such counts. One species of an allocreadid (*Bunodera*), 1 gorgoderid (*Gorgoderina*), and 1 schistosomatid

© 2016 Global Journals Inc. (US)

(*Schistosomatium*) have 7 chromosomes, in addition to the 2 amphistomids (*Zygocotyle* and *Gastrothylax*). All of the *Schistosoma* species studied show n = 8, as do 2 species of gorgoderids (*Gorgodera* and *Phyllodistomum*), 1 troglotrematid (*Paragonimus*), 3 species of allocreadids (*Bunodera*, *Crepidostomum* and *Allocreadium*), 1 species of a rhopaliid (*Rhopalias*), and 2 species of the reniferids (*Staphylodora* and *Telorchis*), in addition to the 2 species of paramphistomatids (*Cotylophoron* and *Diplodiscus*). Two species of the azygiids (*Azygia* and *Proterometra*), 2 species of the plagiorchids (*Eustomas* and *Glypthelmins*), 1 species of a spirorchid (*Spirorchis*), 1 pronocephalid (*Macrovestibulum*), 1 lecithodendriid (*Brandesia*), 1 monorchid (*Asymphylodora*), 1 hemiurid (*Halepegus*), and 1 reniferid (*Auridistomum*), in addition to 1 race of the paramphistomatid *Diplodiscus* (*temperatus*) have 9 chromosomes. Those having a basic number of 10 chromosomes are 1 species of a cyclocoelid (*Cyclocoelum*), 2 species of dicrocoelids (*Brachycoelium* and *Dicrocoelium*), 1 clinostomatid (*Clinostomum*), and 1 hemiurid (*Isoparorchis*). Only *Heronimus* of the paramphistomatids falls in this category. Four species of plagiorchiids (3 *Pneumonoeces* and 1 *Plagitura*), 1 echinostomid (*Parorchis*), 2 lecithodendriids (*Acanthatrium* and *Loxogenes*), and 15 species of reniferids (1 *Dasymetra*, 1 *Lechriorchis*, 1 *Natriodora*, 6 *Neorenifer*, 2 *Pneumatophilus*, 1 *Renifer*, and 3 *Telorchis*) have 11 chromosomes. In these cases again no direct relationship to the paramphistomatids can be noted in terms of chromosome number, although in terms of the presence of an increased number of chromosomes as indicating a possible primitive condition, these forms might be regarded as less specialized than others of the Digenea. It should be pointed out, however, that in many cases it is not only in the matter of actual number of chromosomes that similarities (relationships) may be indicated: total volume of chromatic material, shapes and sizes of the chromosomes, point of spindle attachment to individual chromosomes, and behavior during division may afford evidence of equal importance. It is also definite that sufficient differentiation occurs to facilitate identification of species. One species of *Cephalogonimus* has 14 chromosomes which may be a case of doubling of the usual number of 7 in this genus. If one follows the belief of Ciordia (1949), who doubts the existence of polyploidy among the Trematodes, this would be an example of extreme aneuploidy-duplication of individual chromosomes and not of the set as a unit. The matter of the presence or absence of the so-called heterochromosome ("sex chromosome") has not been determined in most of the trematode species examined, but in a few cases the recognition of two types of sex cells differing from each other in terms of the number, size, shape, volume or behavior of the chromosomal elements would seem to indicate that such sexual differentiation does occur. As examples we may mention the studies on *Schistosoma* and *Schistosomatium*. The earlier observations on *Schistosoma haematobium*, *S. mansoni*, and *S. japonicum* [Katsurada, 1904]; seemed to indicate that two types of sperm could be identified and that adult males possessed 15 and adult females possessed 16 somatic chromosomes. This would seem to mean that an X-O condition obtained in these forms. Other studies reported the numbers as 14 and 16, respectively, and were interpreted as showing the presence of a 2X + 12 and a 4X + 12 chromosome complex. Niyamasena (1940) in his studies on *S. mansoni* found the somatic number of chromosomes to be 16 in each sex, and could not find any evidence of the presence of recognizable sex chromosomes although the possibility of an X-Y condition could not be ruled out. Most recent studies support the finding of 16 chromosomes as the diploid number in all adults of all three of the above *Schistosoma* species and the inability to recognize sex chromosomes as being present [16 chromosomes are also present in each sex of *S. mansoni* carcariae [Bilharz, 1852]. Recent studies on *Schistosomatium douthitti* [Bilharz, 1852]; have presented 2 different interpretations of what is undoubtedly an example of the presence of distinguishable sex chromosomes. One study (Woodhead, 1957) indicates the presence of a single "X" chromosome in the male, while the other (Short, 1957) presents evidence of the heterogametic condition as prevailing in the female. Studies in this laboratory seem to substantiate this second interpretation. The somatic (diploid) number of chromosomes in each sex is 14, with the male showing a pair of large V-shaped chromosomes that are not matched in the female. In the latter case there is a single large V-shaped chromosome apparently paired with a single rod-shaped body. This rod-shaped chromosome does not appear in any of the male cells. It is interpreted as indicative of a ZZAA condition in the male and a ZWAA condition in the female. Short & Menzel (1957) report a similar condition in the cercariae of *Ornithobilharzia canaliculata*, where 2n = 16. No other records of the presence of recognizable sex chromosomes among the trematodes seem to have been substantiated by recent investigations. No records of the "diminution" phenomenon have been brought to our attention. Le Roux (1958) in a study of mammalian blood flukes, has suggested a division of the genus *Schistosonmai* into several groups: *Schistosoma* (*S. haematobium*, type), *Afrobilharzia* (*A. mansoni*, type), *Sinobilharzia* (*S. japonicum* type), *Rhodobilharzia* (*R. margrebovici*, type), and *Eurobilharzia* (*E. bomfordi*, type). These genera are in addition to the already recognized genera of *Bivitellobilharzia*, *Heterobilharzia* and *Schistosomatium* as members of this group of flukes. Restudy of the chromosomes of these species might help to evaluate such a separation. The same

© 2016 Global Journals Inc. (US)

flukes. Restudy of the chromosomes of these species might help to evaluate such a separation. The same technique might also help solve the complexities of the taxonomy of avian *Schistosomes*.

Table 1 : Chromosome number and morphology of Trematodes from 1902 till date

Family Species	No. and Morphology of Chromosomes	Reference
	SCHISTOSOMIDAE	
Schistosum japonicum Katsurada, 1904	2n = 16 (6t +2at)	Short & Menzal (1960)
Austrobilharzia variglandis	2n=16	Short & Menzal (1960)
Gigantobilharzia huronensis	2n=16(+B)	Short & Menzal (1960); LoVerde & Kuntz (1981)
Heterobilharzia americana	2n=20(ZW); ZZ/ZW, m/st; 6m+4sm+2sm-st+ 8st	Short & Grossman (1986)
Texas (Male)	2n=20(ZZ); ZZ, m; 4m + 2m-s m+4sm-st+2sm+2st-1+6st	Short *et al*. (1987)
Louisiana (Female)	2n=20(ZWA); WA, m; 3m+2m-sm +4sm-st+2sm+2st-t+6st	Short *et al*. (1987); Britt (1947)
Ornithobilharzia huronensis	2n=16 (XY)	Short & Menzel (1960)
Schistosoma bovis, Schistosoma haematobium, Schistosoma intercalatum, Schistosoma mattheei	2n=16	Short & Menzel (1960); Grossman *et al.* (1981a)
Schistosoma japinicum	2n=16; ZZ/ZW, m/sm	Grossman *et al.* (1981b)
Schistosoma mekongi	2n=16; ZZ/ZW, sm/sm; 4sm+8 a + 4t	Grossman *et al.* (1981b)
Schistosoma mansoni	2n=16 (ZW No. 1)	Short & Menzel (1960); Short *et al*. (1979); Grossman *et al.* (1980)
Schistosoma mansoni	2n=16; ZZ/ZW; st/st-sm; 2m + 4 m-sm + 4 s t-sm + 4st + 2t	Atkinson (1980); Short & Grossman *et al*. (1981a)
Schistosomatium douthitti	2n=16 (ZW No. 2); m/st; 4m + 2sm-m + 2m-sm + 2 s m-st+ 2st + 2t-st	Atkinson (1980); Grossman *et al.* (1981); Grossman (1981)
	2n=14 (ZW No. 1); m/st; 4m + 2sm-m + 2m-sm + 2 s m-st+ 2st + 2t-st	Short & Menzel (1960); Short & Grossman (1981); Puente & Short (1985); Short (1957); Short & Menzel (1959)
Trichobilharzia physellae,	2n=16	Short & Menzel (1960); Short (1983)
Trichobilharzia stagnicola	2n=16	Short & Menzel (1960); Short (1983)
Trichobilharzia szidati	2n=16 (5m+2sm+1sm-st)	Spakulova *et al*. (1996)
Trichobilharzia regent Horak, Kolarova *et* Dvorak, 1998	2n=16 ((5m+1sm+1sm-m+1sm-m(Z)+1m(W)+1sm (Supernumerary B)	Spakulova *et al*. (2001)
Schistosoma rodhaini	2n=16; ZZ/ZW, s t /st; 2m+2sm-m+4sm-st +4st +2t-st	Atkinson (1980); Grossman *et al.* (1981a); Short & Grossman (1981)
Schistosoma haematobium	2n=16; ZZ/ZW, s t/st;8m +8st	Short (1983)
Schistosoma bovis	2n=16;	LoVerde & Kuntz (1981); Short (1983)
Schistosoma matthei	2n=16;	LoVerde & Kuntz (1981); Short (1983)
Schistosoma intercalatum	2n=16;	Short (1983)
Schistosoma margrebowiei	2n=16;	Grossman *et al*. (1981a); Short (1983)
Schistosoma japonicum	2n=16; ZZ/ZW, sm/sm	Gao Longsheng *et al*. (1985)
Schistosomatium sp.	2n=14; ZZ/ZW, m/a; 12m+2m-sm	Barsiene *et al*. (1989)
Bilharziella polonica	2n=16; ZZ/ZW, st-sm (Male); 4m 4sm-m+2m-sm+4sm+2st	Barsiene & Stanyavichyute (1993)
Ornithobilharzia caniculata	2n=16; ZZ/ZW	Short (1983)
Austrobilharzia variglandis	2n=16; ZZ/ZW, sm-m/a; 12m+2sm +2a	Barsiene *et al*. (1989)
Triehoilharzia physellae	2n=16;	
Triehoilharzia szidati	2n=16; 6m+2sm-m+6sm+ 2st-sm	Barsiene & Stanyavichyute (1993)

© 2016 Global Journals Inc. (US)

Trichobilharzia sp. 1	2n=18; 14m +4sm-m	Barsiene *et al*. (1989)
Trichobilharzia sp. 2	2n=16; 12m +2sm-m+2sm	Britt (1947)
Gigantobilharzia huronesis	2n=16;	Britt (1947)
PRONOCEPHALIDAE		
Macrovestibulum kepneri	2n = 20	Jones *et al*. (1945)
PARAMPHISTOMIDAE		
Ceylonocotyle dicranocoelium	2n = 18	Subramanyam & Venkat Reddy (1977)
Cotylophoron cotylophorum	2n=16	Subramanyam & Venkat Reddy (1977)
Cotylophoron sp.	2n=16	Subramanyam & Venkat Reddy (1977)
Fischoederius elongates	2n=16	Subramanyam & Venkat Reddy (1977)
Gastrothylax crumenifer	2n=18; 4m +6sm+2a+6st	Romanenko (1974); Subramanyam & Venkat Reddy (1977)
Gigantocotyle explanatum	2n=18	Subramanyam & Venkat Reddy(1977)
Liorchis scotiae	2n=18	Romanenko (1974)
Megalodiscus (Diplodiscus) temperatus	2n=20	Grossman & Cain (1981)
Paramphistomum cervi	2n=14	Venkat Reddy & Subramanyam (1975)
Paramphistomum epiclitum	2n=18	Subramanyam & Venkat Reddy (1977)
Paramphistomum ichikawal	2n=18	Romanenko (1974)
Paramphistomum microbothrium	2n=18	Mutafova (1983a)
Stichorchis subtriquetrus	2n=18	Romanenko (1974)
Diplodiscus temporatus	2n = 16	Cray (1909)
Paramphistomum microbothrium	2n=14	Sey (1971)
	2n=18, 2 s m + 10m + 6st	Mutafova (1983a)
Paramphistomum explanatum	2n=18, 2sm+16a	Sharma & Lal (1984)
Paramphistomum hiberniae	2n=12	Willmott (1950)
Paramphistomum ichikawai	2n=18; 2 m + 4 s m + 12st	Romanenko (1974)
Paramphistomum epiclitum	2n=18; 2 m + 14sm + 2st	Subramanyam & Venkat Reddy (1977)
Paramphistomum crassum	2n= 14;	Srivastava & Iha (1964a)
Paramphistomum cervi	2n= 14; 4m +l0sm	Subramanyam &Venkat Reddy (1977)
	2n=18; 6m + 4sm + 8 st	Rhee *et al*. (1987a)
Paramphistomum elongatum	2n=16; 6m+6sm +4st	Dhingra (1955a)
Paramphistomum sp.(*Planorbarius corneus*)	2n=18; 2m + 2m-sm+6sm + 8st	Barsiene (1991)
Paramphistomum sp. (*Planorbis planorbis*)	2n=18; 2m + 10sm + 6st	Barsiene (1991b)
Gigantocotyle bothycotyle	2n= 12;	Willmott (1950)
Gigantocotyle explanatum	2n=18; 4m+l0sm + 4a	Venkat Reddy & Subramanyam (1975b); Subramanyam & Vekat Reddy (1977)
Liorchis scotiae	2n=18; 4m + 14a	Romanenko (1972)
	2n=18; 2m + 8 sm + 8a	Romanenko (1974)
Gastrothylax cruminifer	2n=18; m + sm	Britt (1947)
	2n=14;	Dhingra (1955a)
Fischoederium cobboldi	2n=18; 8 m + l0sm	Rhee *et al*. (1988)
Zygocotyle lunata	2n=14;	Willey & Godman (1951)
Cotylophoron elongatum	2n=16;	Dhingra (1955b)
Cyclonocotyle dicranocoelium	2n=18; 4m + 10sm + 4st	Britt (1947)
Cyclonocotyle orthocoelium	2n=18;	Sharma *et al*. (1968)
Cyclonocotyle dawesi	2n=20;	Britt (1947)
Cyclonocotyle scoliocoelium	2n=22;	Britt (1947)
Stuncardia dilymphosa	2n=18;	Sharma & Nakhasi (1974)
Megalodiscus temperatus	2n=18;	Van der Woude (1954); Saksena (1969)
Diplodiscus amphichrus magnus	2n=18; 6m + 6sm+6a	Saksena (1962)
Diplodiscus subclavatum	2n=20; 2sm -m + 8sm + 2st-sm + 6 s t + 2a	Petkeviciute *et al.*(1989b)
Notocotylus noyeri Joyeux, 1922	2n=20; 2 m + 12 + sm + 4st + 2a	Britt (1947)

© 2016 Global Journals Inc. (US)

	2n=21-30;	Britt (1947)
	2n=20; 2sm-m+12sm+4st+2a	Barsiene & Grabda-Kazubska (1991b)
Gastrodiscoides hominis	2n=20;	Romenenko (1974)
Stichorchis subtriguentrus	2n=20;	Britt (1947)
Heronimus chelydrae	2n=18; 4sm 14a	Guilford (1955)
ZOOGONIDAE		
Zoogonus mirus	2n = 10	Goldschmidt (1905)
	2n = 12	Schreiner (1908); Gregoire (1909); Wassermann (1913)
DICROCOELLIDAE		
Dicrocoelium lanceolatum	2n = 20	Goldschmidt (1908); Dingler (1910)
Dicrocoelium lanceolatum	2n=24	Romanenko (1979)
Eurytremum pancreuticum	2n=26	Romanenko (1979)
Paradistomoides orientalis	2n=28	Scharma & Nakahasi (1974)
Dicrocoelium lanceatum	2n=24; 22m + 2sm	Sharma & Nakhasi (1974)
Eurytrema coelomaticum	2n=26; 1 0 m + 2 sm+12st + 2t	Moriyama (1982a, 1982b)
Eurytrema pancreaticum	2n=26; 10m+4sm + 8st + 4t	Britt (1947)
Paradistomoides orientalis	2n=28; 1 4 s t + 10st + 4t	Dhar &Sharma (1984)
BRACHYCOELIDAE		
Brachycoelium salamandrae	2n = 20	Von Kemnitz (1913)
HETEROPHYIDAE		
Cryptocotyle lingua	2n = 12	Cable (1931); Britt (1947)
Apophallus miiehlingii	2n = 14 (3m+4sm)	Barsiene *et al.* (1995)
ECHINOSTOMIDAE		
Parorchis acanthus	2n = 22	Rees (1939)
Episthmium bursicola	2n=18; 12m+4sm+2st	Barsiene &Kiseliene (1990a)
Echinochasmus baleocephallus	2n=14; 6m+2m-sm+4sm+2a	Britt (1947)
Parorchis acanthus	2n=22;	Rees (1939)
Echinostoma reuolutum	2n=22;	Churchill (1950)
	2n=22; 2m +20st	Mutafova & Kanev (1986)
Echinostoma revolutum (L. stagnalis)	2n=22; 2m+4sm+2 st-sm +10st+4a	Barsiene & Kiseliene (1991)
Echinostoma revolutum (L. ovata)	2n=22; 2m 2sm-m+4sm+14st	Britt (1947)
	2n=24; 2 B	Britt (1947)
Echinostoma jurini	2n=22; 6m+8sm+4st+4a	Britt (1947)
Echinostoma miyagawai	2n=22; 2m+2sm+2st-sm+16st	Barsiene & Kiseliene, 1991
Echinostoma echinatum	2n=22; 2m + 20st	Mutafova & Kanev (1986)
	2n=22; 2m+2sm-m+2sm+12st+4a	Barsiene & Kiseliene (1991)
	2n=22; 2m + 2sm-m +2sm+16st	Britt (1947)
Echinostoma barbosai; Echinostoma echinatum	2n=22; 2sm + 20a	Mutafova & Kanev (1983)
Echinostoma hortense	2n=20; 2m+2m-sm+8sm-st + 8st-t	Terasaki *et al.* (1982)
Echinostoma tinetorchis	2n=22; 2m + 2sm-st+12st+4st + 2t	Britt (1947)
Echinostoma caproni	2n=22; 4sm-st, t	Richard & Voltz (1987)
Neoacanthoparyphium echinatoides	2n=20; 2m + 4 s t + 1 4a	Barsiene & Kiseliene (1990b)
Moliniella anceps	2n=20; 2m + 8 s t + 1 0a	Barsiene *et al.* (1990b)
Isthmiophora metis (из Lymnaea stagnalis)	2n=20; 4m+4sm+2st+10asm	Britt (1947)
Pegosomum asperum	2n=20;	Aleksandrova & Podgornova (1978)
Lymnaea saginatum	2n=20;	Britt (1947)
Echinoparyphium recurvatum	2n=20; 2m + 2sm + 16t	Mutafova *et al.* (1987)
Echinoparyphium recurvatum A (Lymnaea auricularia, Lymnaea ovata)	2n=20; 2m-+4sm + 14st	Barsiene (1991a)
Echinoparyphium recurvatum B (Lymnaea coruus, Lymnaea palustris)	2n=20; 4m + 6sm+10a	Britt (1947)

© 2016 Global Journals Inc. (US)

Echinoparyphium pseudorecurvatum A (Pl. planorbis	2n=20; 2sm -m+4 sm+4st +10a	Britt (1947)
Echinoparyphium pseudorecurvatum B (A. acronicus)	2n=20; 2sm-m + 2st-sm + 8st + 8a	Britt (1947)
Echinoparyphium bacutus (Vatvata piscinatis)	2n=20; 2 m+12 s t+2a-st+4a	Britt (1947)
Echinoparyphium aconiatum	2n=20; 2m-sm + 2sm + 16t	Mutafova *et al.*(1987); Mutafova & Kanev (1984)
	2n=20; 2m + 2st-sm+ 4st+4st-a +8a	Barsiene & Kiseliene (1990b)
Hypoderaeum conoideum	2n=20; 4 m + 1 6 s t	Mutafova *et al.,*1986
	2n=20; 2 m + 4 s m + 12st	Barsiene & Kiseliene, 1990b
Cathamaesia hians (Lymnaea stagnalis ПНР)	2n=20; 8m 4sm-m +4sm+4st	Barsiene (1990)
Cathamaesia hians (Planorbis planorbis. ПНР)	2n=20; 4m + 4sm-m+8sm +2st-s m + 2 s t	Barsiene (1991b)
BUCEPHALIDAE		
Bucephalus elagans	2n = 12	Woodhead (1931)
Bucephalus pusillus	2n = 12	Woodhead (1931); Britt (1947)
Rhipidocotyle papillosum	2n=12	Ciordia (1956)
TROGLOTREMATIDAE (PARAGONIMIDAE)		
Paragonimus kellicotti	2n = 16	Chen (1937)
Paragonimus kellicotti	2n = 22 (2a + 9sm)	Loverde (1979)
Paragonimus ohirai	2n = 22 (2a + 9sm)	Loverde (1979)
Paragonimus miyazakii	2n=22	Sakaguchi & Tada (1975, 1976); Terasaki (1977); Hirai *et al.* (1985)
Paragonimus ohirai; 2 geographical races *(Paragonimus iloktsuemensis; Paragonimus sadoensis)*	2n=22	Sakaguchi & Tada (1975, 1976); Terasaki (1977); Hirai *et al.* (1985)
Paragonimus westermani	2n=22	LoVerde (1979); Hirai *et al.* (1985); Sugiyama *et al*. (1985)
Paragonimus westermani, (Paragonimus pulmonalis)	2n=22	Sakaguchi & Tada (1976b); Terasaki (1977); Agatsuma & Habe (1985); Hirai *et al.* (1985)
Paragonimus westermani	2n=22	Blair (2000)
Paragonimus heterotremus	2n=22 (1m+4st+3m/sm+3sm/st)	Komalamisra (2005)
Paragonimus kellicotti	2n=16	Benazzi &Benazzi Lentati (1976)
Paragonimus ohirai	2n=22;	Britt (1947)
	2n=22; 4m + 18sm	Britt (1947)
	2n=22; 2 m + 1 0 sm + 10st	Hirai *et al.* (1985)
Paragonimus westermani	2n=22; 4 m + 10sm + 8st	Sakaguchi & Tada (1976a); Terasaki (1977)
	3 n = 33;	Sakaguchi & Tada (1976a); Miyazaki (1978)
	2n=22; 6m+8sm+8st	Pengpeng *et al*. (1986)
	2n=33; 2m + 8 st + 12m, sm, st	He Lian-Yin *et al*. (1982)
	2n=22; m + 6 st + 2 sm + 12m, sm, st	Britt (1947)
Paragonimus westermani Shoawu, Fujian	2n=22; 2m + 4st + 4sm + 12m, sm, st	Britt (1947)
Paragonimus westermani filipinus	2n=22; 2m + 6m-sm + 6sm-st + 8st	Terasaki (1983)
Paragonimus westermani westermani	2n=22; 8m + 6sm-st + 8st	Britt (1947)
Paragonimus skrjabini	2n=22; 6m+6sm-m + 2sm +8st	Li and Zheng (1983)
Paragonimus pulmonalis	3n=33; 2m+6m- sm+6sm-st+ 8st	Terasaki (1980); Sakaguchi & Tada (1976b)
	2n=22; 2m + 6m-sm + 6sm- st + 8st	Terasaki (1977)
	3n=33; 3 m + 12sm + 6 st +12a	Hirai *et al.* (1985)
Paragonimus iloktsuenensis	2n=22; 2m + 6m-sm+6sm-st + 8st	Terasaki (1977); Sakaguchi & Tada (1980)

© 2016 Global Journals Inc. (US)

	2n=22; 2m + 10sm+10st	Hirai *et al.* (1985)
Paragonimus sadoensis	2n=22;	Terasaki (1977); Sakaguchi & Tada (1980)
	2n=22; 2m + 10sm+ l0st	Hirai *et al.* (1985)
Paragonimus peruvian us	2n=22; 2m+6m-sm + 6sm-st + 8st	Terasaki (1978)
Paragonimus hueitungensis	2n=22; 2m + 8 st + 12m, sm, st	He Lian-Yin *et al.* (1982)
Euparagonimus cenocopiosus	2n=22; 6 m + 8 s m + 8 s t	Lei Changqui *et al.* (1985)
AZYGIIDAE		
Proterometra macrostoma	2n =18	Anderson (1935)
Azygia acuminata	2n = 18	Britt (1947)
Azygia lucii	2n=20; 10m + 6 a + 4 st	Barsiene (1991b)
ALLORCEADIIDAE		
Allocreadium isoporum	2n =16	Britt (1947)
Crepidostomum serpentinum	2n = 16	Britt (1947)
Bunodera saculata	2n = 16	Britt (1947)
Bunodera luciopercae	2n = 14	Britt (1947)
Cercariaeum crassum Wesenberg-Lund, 1934	2n=10 (1m+1sm+sm-m+1m-sm)	Petkeviciute *et al.* (2011)
Allocreadium fasciatusi	3n=21; 3 m + 12sm + 6st	Ramanjaneyulu &Madhavi (1983)
Bunodera sacculata	3n=23;	Cannon (1971)
Allocreadium fasciatusi	3n=21	Ramanjaneyulu & Madhavi (1984)
Allocreadium handiai	2n=14	Ramanjaneyulu & Madhavi (1984)
Bunodera luciopercae	2n=14 (2m+1sm/st+4a)	Petkeviciute & Staneviciute (2008)
Allocredium isoporum	2n=14 (2m+5a)	Petkeviciute & Staneviciute (2008)
Crepidostomum serpentinum	2n=14 (1m+5a)	Petkeviciute & Staneviciute (2008)
Cercariaeum crassum Wesenberg-Lund, 1934	2n=10 (1m+1sm+2sm-m+1m-sm)	Petkeviciute *et al.* (2011)
CLINOSTOMIDAE		
Clinostomum marginatum	2n = 20	Britt (1947)
Clinostomum schizothoraxi Kaw, 1950	2n = 20, 1m+2sm+3t+4a	Present study
LECITHODENDRIIDAE		
Loxogenes bicolor	2n = 22	Britt (1947)
Acanthatrium pipistrella	2n = 22	Britt (1947)
Ganeo kumaonensis	2n=20;	Saksena (1969)
Acanthatrium pipistrella	2n=22;	Britt (1947)
Mahroarchis ranarum	2n=22;	Saksena (1969)
Pleurogenoides medians	2n=22; 16m + 4st + 2a	Barsiene, Grabda-Kazubska (1991c)
Pleurogens claviger	2n=22; 1 2 m + 6 s t + 4a	Barsiene, Grabda-Kazubska (1991c)
Pleurogonidum orientalis	2n=18;	Saksena (1969)
Prosotocus kashabia	2n=12;	Britt (1947)
Ganeo tigrinum	2n=22	Subramanyam & Venkat Reddy (1977)
CEPHALOGONIMIDAE		
Cephalogonimus americanus	2n = 28	Britt (1947)
GORGODERIDAE		
Probolitrema californiense	2n = 12	Markell (1943)
Gorgoderina attenuate	2n = 14	Britt (1947)
Gorgodera amplicava	2n = 16	Britt (1947)
Phyllodistomum folium	2n=18	Petkeviciute *et al.* (2003)
Gorgodera amplicava	2n=16;	Britt (1947)
Gorgodera pagenstecheri	2n=18; 2m +2sm +2st-a+12a	Barsiene (1991)
Gorgoderina attenuata	2n=14;	Willey & Koulish (1950)
Probilotrema californiense	2n=12;	Britt (1947)
Phyllodistomum spatula	2n=16;	Dhingra (1954a)
Phyllodistomum pungitti	2n=18; 2 m + 12st +4a	Britt (1947)
PLAGIORCHIDAE		
Eustomas chelydrae	2n = 18	Britt (1947)
Glypthelmins quieta	2n = 18	Britt (1947)
Plagitura salamandra	2n = 22	Britt (1947)
Pneumobites breviplexus	2n = 22	Britt (1947)
Pleumocoeces medioplexus	2n = 22	Pennypacker (1936)

© 2016 Global Journals Inc. (US)

Pleumocoeces similiplexus	2n = 22	Pennypacker (1940)
Pleumocoeces similiplexus	2n = 22	Britt (1947)
Trematorchis ranarum	2n=18	Subramanyam & Venkat Reddy (1977)
Glypthelmins gulieta	2n=18;	Britt (1947)
Plagitura salamandrae	2n=22;	Britt (1947)
Haematoloechus mediplexus	2n=22;	Burton (1960)
Haematoloechus parviplexus	2n=22;	Pennypacker (1936)
Haematoloechus semiplexus	2n=22;	Britt (1947)
Haematoloechus similis	2n=22; 12m+6sm + 2sm-m+2st	Barsiene, Grabda-Kazubska (1988b)
Haematoloechus asper	2n=22; 14m+4sm + 2sm-m +2st	Britt (1947)
Skrjabinoeces sp.	2n=22; 10m + 2sm-m + 4sm +2sm-st + 4st	Petkeviciute *et al.* (1990)
Monodistomum salamandra	2n=20;	Britt (1947)
Encylometra colubrimurorum	2n=12;	Saksena (1969)
Staphylodora bascaniensis	2n=16;	Britt (1947)
Haplometra cylindracea	2n=20;	Sanderson (1959)
	2n=22; 4m + 8 s m-m + 4 sm +6st	Barsiene, Grabda-Kazubska (1988a)
Plagiorchis sp. *(L. stagnalis, ПНР)*	2n=22; 2m + 8sm-m + 4sm + + 8st	Barsiene, Grabda-Kazubska (1988b)
Opisthioglyphe ranae	2n=22; 2m + 6sm-m+6sm+ 4st + 4a	Barsiene, Grabda-Kazubska (1988a)
Opisthioglyphe ranae (L. stagnalis)	2n=22; 6m +6sm + 2sm-st +2st +4a-st+2a	Petkeviciute *et al*. (1990)
Leptophallus nigrouenosus	2n=20; 12m + 2sm-m + 2sm +2st + 2a	Barsiene, Grabda-Kaka (1988a)
Paralepoderma progeneticum	2n=20; 14m + 2sm + 2st-a + 2a	Barsiene, Grabda-Kaka (1991a)
Paralepoderma brumpti	2n=20; 10m+2m-sm + 2sm+ 2st + 4a	Petkeviciute *et al*. (1990)
Omphalometra flexuosum	2n=20; 4sm-m + 4 sm + 4st +8a	Barsiene, Grabda-Kazubska (1991a)
RENIFERIDAE		
Natriodera verlatum	2n = 22	Britt (1947)
Dasymetra villicoeca	2n = 22	Britt (1947)
Pneumatophilus leidyi	2n = 22	Britt (1947)
Pneumatophilus variabilis	2n = 22	Britt (1947)
Lechriorchis abduscens	2n = 22	Britt (1947)
Renifer ellipticus	2n = 22	Britt (1947)
Neorenifer wardi	2n = 22	Britt (1947)
Neorenifer georgianus	2n = 22	Britt (1947)
Neorenifer aniarum	2n = 22	Britt (1947)
Neorenifer orula	2n = 22	Britt (1947)
Neorenifer drymarchon	2n = 22	Britt (1947)
Neorenifer elongates	2n = 22	Britt (1947)
Staphylodora bascaniensis	2n = 16	Britt (1947)
Auridistomum chelydrae	2n = 18	Britt (1947)
Telorchis robustus	2n = 16	Britt (1947)
Telorchis lobusus	2n = 22	Britt (1947)
Telorchis medius	2n = 22	Britt (1947)
Telorchis corti	2n = 22	Britt (1947)
RHOPALIADIDAE		
Rhopalias macracanthus Chandler, 1932	2n = 16	Ciordia (1949)
DICLYBOTHRIIDAE		
Diclybothrium hamulatum (Simer, 1929) Price, 1942	2n = 12	Pickle &Jones (1967)
SPIRORCHIIDAE		
Spirorchis magnitestis	2n = 18 (4a/t + 1a + 3sm)	Teehan & Short (1989)
Spirorchis parvus	2n = 18 (4a/t + 1a + 3sm)	Teehan & Short (1989)

© 2016 Global Journals Inc. (US)

Spirorchis magnitestis	2n=18; 2m + 16a	Jones & Mayer (1953)
Spirorchis parvus	2n=18; 2m + 16st	Grossman *et al*. (1981b)
Spirorchis sp.	2n=18; 2 sm + 6 st-sm +8t-st + 2t	Teehan & Short (1989)
CONVOLUTIDAE		
Convolute convolute	2n = 16 (7m-sm + 1st)	Birstein (1990)
Baltalimania agile	2n = 14	Birstein (1990)
MIROPHALLIDAE		
Microphallus pygmaeus	2n = 18	Birstein & Mikhailova (1990)
Microphallus piriformis	2n = 18; 8m + 2sm + 4st + 4?	Birstein & Mikhailova (1990)
Microphallus triangulatus	2n = 18	Birstein & Mikhailova (1990)
Microphallus pygmaeus	2n=18; 8m + 2sm + 4st +4?	Britt (1947)
Microphallus triangulatus	2n=18; 8m + 6 s m + 4?	Britt (1947)
MONORCHIIDAE		
Asymphylodora sp.	2n=18;	Dhingra (1955a, 1955b)
	2n=20; 6m+2m -sm + 2sm+ 4st + 6a	Dhingra (1955a)
	2n=20; 12m + 6sm-m + 2 s t-sm	Dhingra (1955a)
	2n=22; 8m + 8sm-m+2sm-st + 2st	Dhingra (1955a)
Palaeorchis sp.	2n=14; 2m + 2sm-m+6sm+ 2st + 2a	Dhingra (1955a)
Asymphylodora spp.	2n = 20 (5m+4sm+1st)	Barsiene *et al.* (1995)
NOTOCOTYLIDAE		
Notocotylus attenuates, Notocotylus imbricatus,	2n=20	Petkeviciute & Barsiene (1988)
Notocotylus ephemera	2n=20,21	Petkeviciute & Barsiene (1988)
Notocotylus filamentis	2n=14;	Ciordia (1950)
Notocotylus ephemera	2n=20; 2m + 6sm-m+ 4sm+ 2st + 6a	Petkeviciute & Barsiene (1988)
	2n=21;	Britt (1947)
	2n=22;	Britt (1947)
Notocotylus attenuatus	2n=22; 4m + 4sm-m+4sm+ 8a	Britt (1947)
	2n=20; 4sm + ?	Rao & Venkat Reddy (1982)
Notocotylus imbricatus	2n=20; 2 m + 10sm +2st+6a	Petkeviciute & Barsiene (1988)
Notocotylus noyeri	2n=20; 2m + 2m-sm + 4sm+ 4st +8a	Petkeviciute *et al*. (1989a)
	2n=21;	Britt (1947)
	2n=20; 4m + 4sm +4st +8t	Barsiene & Grabda-Kazubska (1991b)
Notocotylus sp. *(Anisus acronicus,)*	2n=20; 2m +6sm+4 st+8a	Barsiene *et al*. (1990)
	2n=21;	Britt (1947)
	2n=21-30;	Britt (1947)
CYCLOCOELIDAE		
Cyclocoelium oculeum	2n=20;4m + 6sm + 8st + 2a	Taft & LeGrande (1979)
Cyclocoelum bivesiculatum	2n=20;	Dhingra (1954a)
FASCIOLIDAE		
Fasciola gigantica	2n=20 (2sm+1t+7st)	Romanenko & Pleshanova (1975); Subramanyam & Venkat Reddy (1977)
Fasciola gigantica	2n=20 (6sm+1m-sm+3st)	Venkat Reddy & Subramanyam (1973); Subramanyam & Venkat Reddy (1977)
Fasciola hepatica	2n=20 (1sm-m+5st+2sm+1m+1sm)	Romanenko & Pleshanova (1975)
Fasciola hepatica	2n=20 (5sm+4st+1t)	Li *et al.* (1988)
Fasciola hepatica	2n=20 (1sm-m+4st+5sm)	Spakulova & Kralova (1991)
Fasciola hepatica	2n=20 (1m+5sm+4st)	Reblanova *et al*. (2011)
Fasciola sp.	2n=20	Sakaguchi & Wakako (1976); Sakaguchi (1980); Moriyama *et al*. (1979); Rhee *et al*. (1987); Yin & Ye (1990)
Fasciola sp.	3n=30 (8sm+2st)	Sakaguchi and Nakayama (1975); Sakaguchi & Wakako (1976);

© 2016 Global Journals Inc. (US)

Fasciola gigantica	2n=20	Sakaguchi (1980)
Fasciola hepatica	2n=12 2n=22 (1sm-m+1sm+9st)	Srimuzipo *et al*. (2000); Henneguy (1902); Schubmann (1905); Schellenburg (1911)
Fascioloides magna	2n=22 (9st+1sm-m+1sm)	Reblanova *et al*. (2010)
Fasciolopsis buski	2n = 14 (6m+1t)	Gao (1985)
Fasciolopsis buski	2n = 14 (4m+2sm+1t)	Dai (1990)
Parafasciolopsis fasciolaemorpha	2n=20 (1m+1t+6st+2sm)	Barsiene (1990)
Fasciola sp.	3n=30	Britt (1947)
	2n=20/30;	Britt (1947)
	2n=20; 2m + 10sm + 8st	Rhee *et al*. (1987b)
	2n=20/30;	Sakaguchi, Yoneda (1976)
	2n=20;	Britt (1947)
	3n=30; 3m + 12sm+ 15st	Rhee *et al*. (1987b)
Fasciola hepatica	2n=20	Sanderson (1953, 1959)
	2n=20; 6 m + 2 sm + 1 2 st	Li *et al*. (1988)
Fasciola gigantica	2n=20; 4sm+4sm-s t + 12st	Moriyama *et al*. (1979)
	2n=20; 2m + 12sm + 6st	Subramanyam & Venkat Reddy (1977)
	3n=30;	Sakaguchi (1980)
	2n=20/30;	Mariyama *et al*. (1979)
PHILOPHTHALMIDAE		
Philophthalmus gralli	2n=20	Grossman & Cain (1981)
Philophthalmus sp.	2n=20	Venkat Reddy & Subramanyam (1971)
Philophthalmus sp. (Georgia, USSR; Bulgaria)	2n=20	Mutafova *et al*. (1986)
Philophthalmus megalurus	2n=20;	Kahlil & Cable (1968)
Philophthalmus indieus	2n=20; 2sm + 2 a + 1 6t	Subramanyam & Venkat Reddy (1977)
Philophthalmus hegeneri	2n=20;	Fried (1975)
Philophthalmus sp.	2n=20; 2sm + 18a	Mutafova (1983b)
Philophthalmus gralli	2n=20; 8sm + 12a	LoVerde (1978)
HEMIURIDAE		
Isoparorchis euritremum; Isoparorchis hypselobargi	2n=20 (XY)	Chattopadhyay & Manna (1987)
Halipegus occidualis	2n=18;	Jones (1956); Guilford1(1961)
Halipegus eccentricus	2n=22;	Guilford (1961)
Isoparorchis eurytreum	2n=18;	Srivastava & Iha (1964b)
Isoparorchis hypselobagri	2n=20; 10sm + 8a + 2XY (X = sm; Y = a)	Chattopadhyay & Manna (1987); Dhingra (1954b)
	2n=18; 4m + 14a	Srivastava & Iha (1964b); Iha (1975)
DIPLOSTOMATIDAE		
Diplostomum indisticum; Diplostomum mergi; Diplostomum pseudospathaceum; Diplostomum spathaceum	2n=20	Romanenko & Shigin (1977); Mutafova & Niewiadomska (1988)
Tylodelphys clavata	2n=20	Romanenko & Shigin (1977)
Diplostomum sp. 1	2n=20; 6m + 4sm-m + 2st-sm + 8 s t	Barsiene & Staneviciiite (1991)
Diplostomum sp. 2	2n=20; 6m+2sm-m + 4sm + 4st + 2st- a + 2a	Britt (1947)
Diplostomum baeri	2n=20; 2m + 2sm-m + 6sm + 6st + 4a	Barsiene *et al*. (1990a); Barsiene & Staneviciute (1991)
Diplostomum mergi	2n=20; 10m + l0t	Romenenko & Shigin (1977)
Diplostomum pseudospathaceum	2n=20; 6m + 4sm + 6st + 4a	Barsiene & Staneviciute (1991) Barsiene *et al*. (1991)
Diplostomum paracaudum	2n=20; 6m+6sm +8st	Barsiene *et al.* (1990a) Barsiene & Staneviciute (1991)
Tylodelphys clavata	2n=20; 8m + 2m-sm + 8st + 2?	Romenenko &Shigin (1977)
	2n=20; 6m + 2sm-m + 4st+ 2st-a + 6 a	Barsiene (1991c)

© 2016 Global Journals Inc. (US)

Proalorioides tropidonotis	2n=16;	Saksena (1969)
Posthodiplostomum cuticola	2n=20; 4 sm + 6 st + l0t	Barsiene (1991c)
STRIGEIDAE		
Gogatea serpentium indica	2n=16	Subramanyam & Venkat Reddy (1977)
Ichthyocotylurus erraticus (Rudolphi, 1809)	2n=20 (4m+2sm+1sm-st+3st-a)	Bell *et al.* (1998)
Ichthyocotylurus variegates (Creplin, 1825)	2n=20 (4m+1sm+1m-sm+4st)	Bell *et al.* (1998)
Apatemon gracilis (Rudolphi, 1819)	2n=20 (3m+1m-sm+3sm-st+1sm+1a+1st-a)	Bell *et al.* (1998)
Apatemon gracilis	2n=20 (3m+3sm-st+1sm+2a+1st-a)	Petkeviciute & Staneviciute (1999)
Cotylurus cornutus (Lymnaea zazuriensis)	2n=20; 2m + 6st + 2a-s t + 1 0a	Barsiene *et al*. (1990)
Apatemon gracilis (Lymnaea ovata)	2n=20; 6m + 4sm + 4sm-st +2st + 4a	Petkeviciute (1991)
Apatemon minor (Planorbarius planorbis)	2n=20; 2 m + 6 s m + 4st + 2a-st + 6a	Barsiene (1992)
	2n=21;	
Apatemon fuligulae	2n=20; 4 m + 8 s m + 4 s t + 6a	Barsiene *et al*. (1990)
OPISTHORCHIIDAE		
Opisthorchis felineus	2n=14	Romanenko (1973)
Opisthorchis felineus	2n=14 (4sm+3m)	Polyakov *et al*. (2010)
Clonorchis sinensis	2n=56 (3m+1m-sm+16sm+8st) – Korea (2m+2m/sm+16sm+8st) – China	Park *et al*. (2000)
Clonorchis sinensis	2n=56	Park & Young (2003)
Opisthorchis felineus (Rivolta, 1884)	2n=14 (2m/sm+5)	Zadesenets *et al*. (2012)
Opisthorchis viverrini (Poirier, 1886)	2n= 12 (2sm+1sm+1sm/st+1st/a +1a)	Zadesenets *et al*. (2012)
Metorchis xanthosomus (Creplin, 1846)	2n=14	Zadesenets *et al*. (2012)
Metorchis billis (Braun, 1893)	2n=14	Zadesenets *et al*. (2012)
Clonorchis sinensis (Cobbold, 1875)	2n=14, 2n=56	Zadesenets *et al*. (2012)
Opisthorchis viverrini	2n=12 (4m+1sm+1a)	Kaewkong *et al.* (2012)
TRANSVERSOTREMATIDAE		
Transversotrema patialense	2n=20	Madhavi & Ramanjaneyulu (1986)
OMPHALOMETRIDAE		
Rubenstrema exasperatum	2n=16 (3m+4sm-m+1sm(X_1)+1st(X)	Mutafova &Kanev (1996)
NEODIPLOSTOMATIDAE		
Neodiplostomum seoulense	2n=20 (2m+5sm/st+3t)	Park *et al*. (1998)
ASPIDOGASTREA		
Aspidogaster conchicola	2n=10 (1st+4a)	Petkeviciute (2001b)
Cotylogaster occidentalis	2n=12 (2m+2sm+2a)	Loverde & Fredericksen (1978)
Cotylapis insignis	2n=22	Loverde & Fredericksen (1978)
DIPLOZOIDAE		
Diplozoon paradoxum	2n=8 (3m+1a)	Koskova *et al*. (2011)
Paradiplozoon bliccae	2n=14 (7a)	Koskova *et al*. (2011)
Paradiplozoon sapae	2n=14 (7a)	Koskova *et al*. (2011)
Paradiplozoon nagibinae	2n=14 (7a)	Koskova *et al*. (2011)
Eudiplozoon nipponicum	2n=7	Koroleva (1968b)
Paradiplozoon megan	2n=7	Koroleva (1968b)
Diplozoon paradoxum	2n=8, (3m+1a)	Koroleva (1968a,b)
Paradiplozoon bliccae (syn. *Diplozoon gussevi)*	2n=14, (7a)	Koroleva (1968a,b)
Paradiplozoon bliccae (syn. *Diplozoon markevitchi)*	2n=14, (7a)	Koroleva (1968b, 1969)
Paradiplozoon sapae	2n=14, (7a)	Koroleva (1969)
Paradiplozoon nagibinae	2n=14, (7a)	Koroleva (1969)
Paradiplozoon pavlovskii	2n=14, (7a)	Koroleva (1968a,b)

© 2016 Global Journals Inc. (US)

Paradiplozoon homoion	2n=14, (7a)	Koroleva (1968a,b)
Diplozoidae sp.	2n=14, (7a)	Bovet (1967)
Diplozoidae sp. (sp. n.)	2n=10, (2m+3a)	Koroleva (1969)
Diplozoidae sp.	2n=7	Baer & Euzet (1961)
Diplozoidae sp.	2n=7	Bovet (1967) Incorrect data according to Koroleva (1968b)
Diplozoon kashmirensis **Kaw, 1950**	**2n=14 (7a)**	**Present study**
Diplozoon aegyptensis **Fischthal *et* Kuntz, 1963**	**2n=14 (3m+4a)**	**Present study**
Diplozoon guptai **Fayaz and Chishti, 1999**	**2n=14 (2m+1sm+1st+3a)**	**Present study**
	PSILOSTOMIDAE	
Psilotrema sp.	2n=16; 4m + 2sm-m + 2sm+ 8st	Britt (1947)
Sphaeridiotrema globulus	2n=14; 4m + 4sm-m + 4s m+ 2a	Britt (1947)
	SANGUINICOLIDAE	
Sanguinicola sp.	2n=22; 1 6 m + 2 sm + 4st	Britt (1947)
	BRACHYLAEMIDAE	
Leucochloridiomorpha constantiae	2n=16;	Filippone & Fried (1974)
	CYATHOCOTYLIDAE	
Gogotea serpentium	2n=16;	Saksena, 1969, Subramanyam, Venkat Reddy (1977)
	CRYPTOGONIMIDAE	
Acetodexira amiuri	2n=12;	Perkins (1956)
Atrophecaecum bur minis	2n=14; 14sm	Madhavi & Ramanjaneyulu (1988)
	OPECOELIDAE	
Sphaerostoma bramae	2n=24;	Gresson (1958)
	EUCOTILIDAE	
Cercaria pectinata	2n=12; 6m + 2st+4m-sm	Ieyama & Ozaki (1987)
Cercaria tapidis	2n=16; 8m + 2st-sm + 2st +2sm-st +2sm-m	Britt (1947)

During the present study three monogeneans and one digenean trematodes were investigated for cytological investigation.

b) Monogeneans

i. *Diplozoon kashmirensis Kaw, 1950*

Analysis of mitotic metaphase spreads from ten specimens of *Diplozoon kashmirensis* showed that the karyotype of *D. kashmirensis* comprised 14 acrocentric chromosomes (2n=14; Fig. 1a). The karyotype formula can be summarized as 2n=14a (Fig. 1b). The longest pair is 14.13 μm and the shortest pair is 6.21 μm long (Table 2), the fundamental arm number (NF) = 7 and total chromosome length (TCL) is 72.57 μm. Arm ratio of the complement ranges between 7.67-14.15 and the centromeric index ranges 6.60 to 12.28. Chromosome pair no. 2, 3 and 4 are nearly similar in their size and are very difficult to identify on the basis of chromosome morphology (Students T- test; P-value = 0.002; $P<0.05$), precise identification of second, third and fourth chromosome pairs is rather difficult because of the low degree of significance of length differences, but there is significant difference between chromosome pairs of 5, 6 and 7 (P-Value = 0.000; $P<0.001$; Students T- test). Whereas statistical processing of the established relative lengths of chromosome pairs of 1 & 2 ($P>0.05$) and 4, 5, 6 and 7 ($P<0.005$) are significant due to length difference between them. So, on the basis of absolute length and centromeric position, the chromosomes have been arranged in order of decreasing length in an ideogram (Fig. 2a,b)

© 2016 Global Journals Inc. (US)

Karyotype Formula: (K) 2n=14= 14a

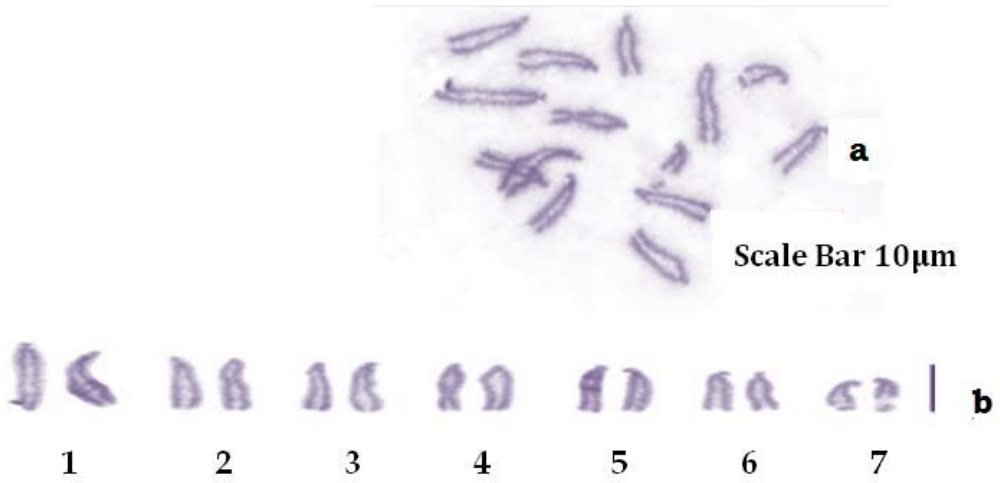
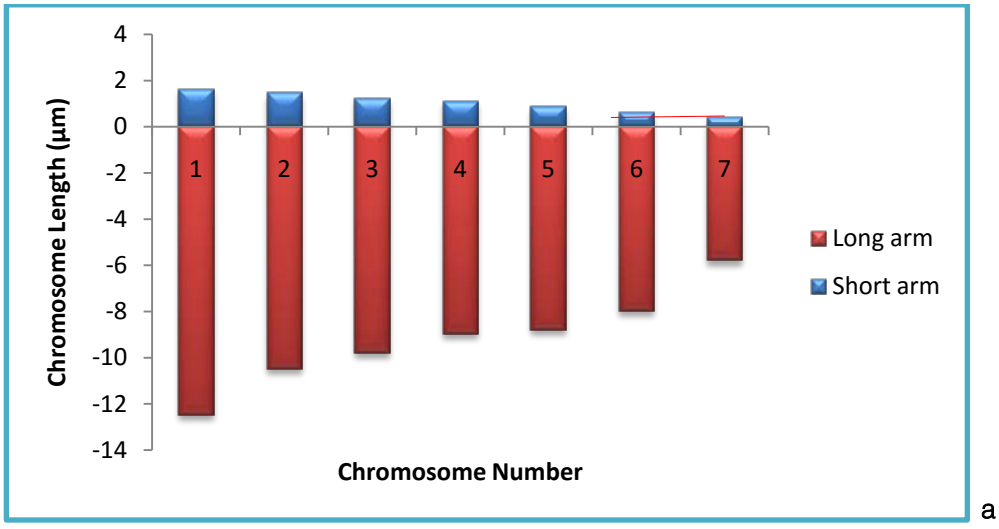
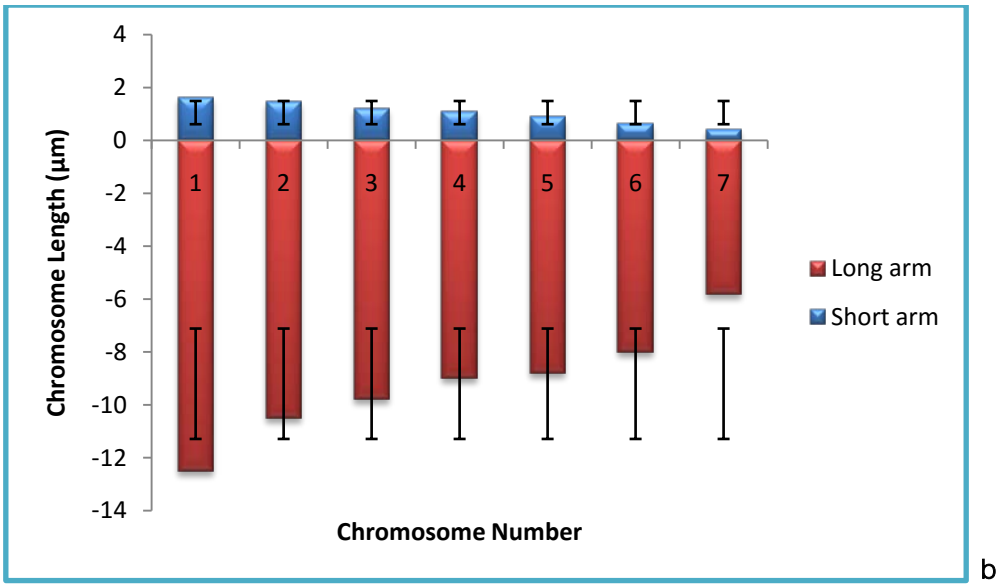


Figure 1 : (a)Chromosome preparation of *Diplozoon kashmirensis* (b) Ideogram constructed from mitotic cells of *Diplozoon kashmirensis* stained with Giemsa(a= acrocentric)



a



b

Figure 2 : (a) Idiogram of *Diplozoon kashmirensis* (b) Error Bars with Standard Deviation of *Diplozoon kashmirensis*

© 2016 Global Journals Inc. (US)

Table 2 : Measurements and classification of chromosomes of *Diplozoon kashmirensis* Kaw, 1950

Chromosome pair number	Length of short arm (μm) 'S'	Length of long arm (μm) 'L'	Total Length/Absolute Length (μm) L+S	Arm Ratio (L/S)	Relative Length (%)	Centromeric Index (ci)	Classification	
1	1.63	12.5	14.13	7.67	19.47	11.54	Acrocentric	T-Value = -25.39 P-Value = 0.002 P<0.05
2	1.47	10.5	11.97	7.14	16.49	12.28	Acrocentric	
3	1.22	9.80	11.02	8.03	15.19	11.07	Acrocentric	
4	1.12	9.00	10.92	8.03	15.05	10.26	Acrocentric	
5	0.89	8.80	9.69	9.89	13.35	9.19	Acrocentric	T-Value = -10.88 P-Value = 0.000 P<0.001
6	0.63	8.00	8.63	12.60	11.89	7.30	Acrocentric	
7	0.41	5.80	6.21	14.15	8.56	6.60	Acrocentric	

ii. *Diplozoon aegyptensis Fischthal et Kuntz, 1963*

The somatic complement of *Diplozoon aegyptensis* species revealed a diploid number of 2n = 14 (Fig. 3a) comprising first three pairs of chromosomes as metacentric and last four pairs of chromosomes as acrocentric in which a fundamental arm number (FN) equals 10 (Fig. 3b). The chromosomes range in length between 7.11 μm to 8.08 μm. The total length of the haploid complement equals 55.78 μm. Arm ratio of the complement ranges between 1.09–17.23 and the centromeric index ranges between 5.49–47.90 (Table. 3). On the basis of total length of chromosomes and relative length there is less significant difference between first three pairs of metacentric chromosomes (P=0.002 ; P<0.05 ; Students T-test) and significant difference between last four pairs of acrocentric chromosome pairs (P=0.001 ; P<0.001 ; Students T-test). The absolute length and centromeric position of the chromosomes have been arranged in order of decreasing length in an ideogram (Fig.4a,b).

Karyotype Formula : (K) 2n=14= 6m+8a

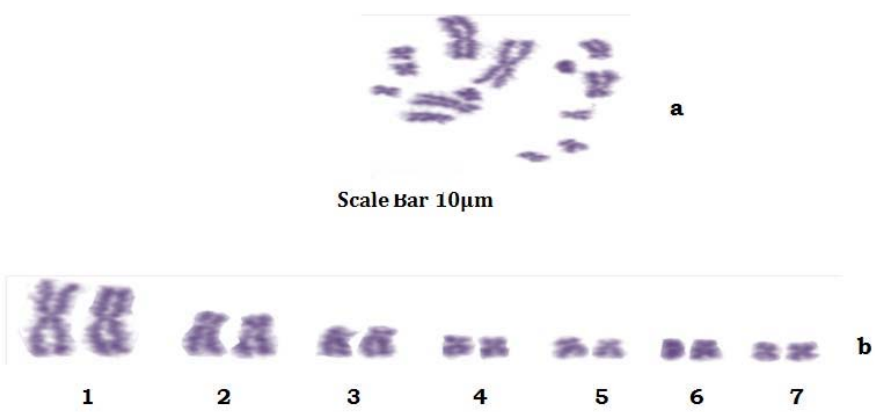


Figure 3 : (a) Chromosome preparation of *Diplozoon aegyptensis* (b) Karyotype constructed from mitotic cells of *Diplozoon aegyptensis* stained with Giemsa (m= metacentric; a= acrocentric;)

© 2016 Global Journals Inc. (US)

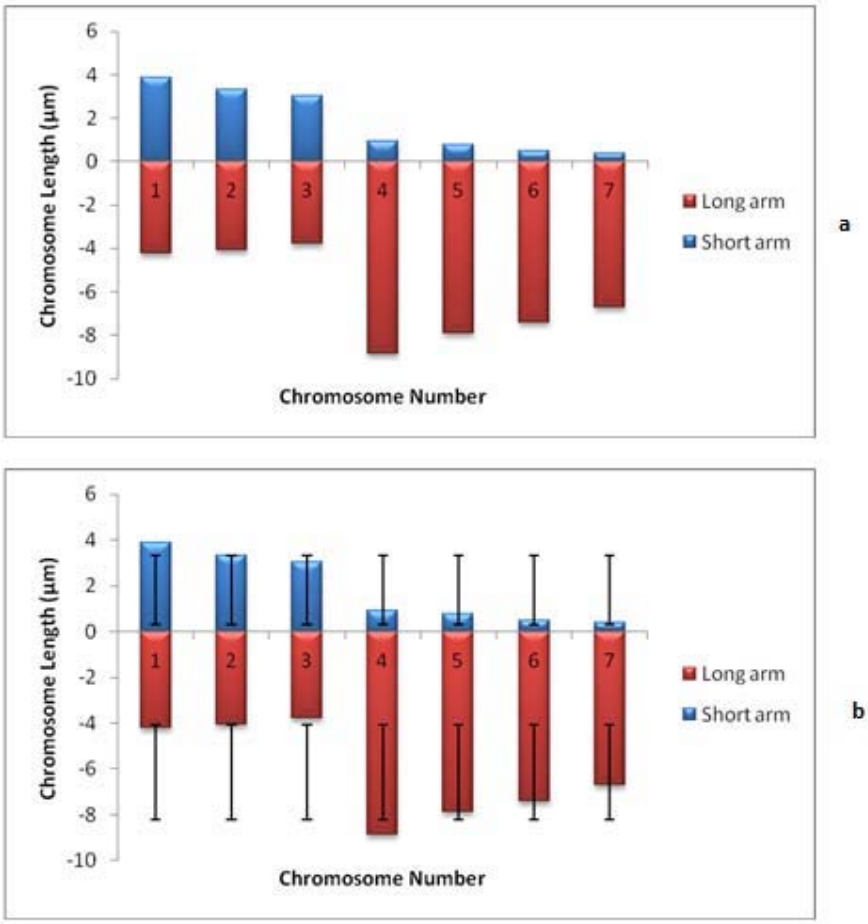


Figure 4 : (a) Ideogram of *Diplozoon aegyptensis* (b) Error Bars with Standard Deviation of *Diplozoon aegyptensis*

Table 3 : Measurements and classification of chromosomes of *Diplozoon aegyptensis* Fischthal et Kuntz, 1963

Chromosome pair number	Length of short arm (µm) 'S'	Length of long arm (µm) 'L'	Total Length/Absolute Length (µm) L+S	Arm Ratio (L/S)	Relative Length (%)	Centromeric Index (ci)	Classification	
1	3.87	4.21	8.08	1.09	14.49	47.90	Metacentric	T-Value = -20.56 P-Value = 0.002 P<0.05
2	3.31	4.07	7.38	1.23	13.23	44.85	Metacentric	
3	3.03	3.81	6.84	1.26	12.26	44.30	Metacentric	
4	0.93	8.85	9.78	9.52	17.53	9.51	Acrocentric	T-Value = -14.82 P-Value = 0.001 P<0.001
5	0.77	7.89	8.66	10.25	15.53	8.89	Acrocentric	
6	0.51	7.42	7.93	14.55	14.22	6.43	Acrocentric	
7	0.39	6.72	7.11	17.23	12.75	5.49	Acrocentric	

iii. *Diplozoon guptai Fayaz and Chishti, 1999*

Diploid chromosome number of *D. guptai* is 2n=14 as revealed after examination of mitotic metaphase spreads from 13 specimens (Fig. 5a). Karyotype (Fig. 5b) included two metacentric (nos. 1 and 2); one submetacentric (no. 3); one subtelocentric (no. 4) and three acrocentric (no.s 5, 6 and 7) chromosome pair; the karyotype formula may be summarized as 2n=4m+2sm+2st+6a. The chromosomes are comparatively large; the smallest and the largest chromosomes measured 5.39 µm and 8.02 µm, respectively (for chromosome measurements, see Table 4). The number of chromosome arms (NF) is 20 and total chromosome length (TCL) is 47.25 µm. Arm ratio of the complement ranges between 1.04–34.53 and the centromeric index ranges between 2.81–49.00 (Table. 3). Length difference between first three pairs are much less and there is less significant difference between them (P=0.002; P<0.05; Students T-test), and these are classified metacentric and submetacentric

© 2016 Global Journals Inc. (US)

pairs, but there are significant length difference between four pairs of chromosomes (P=0.001 ; P<0.001 ; Students T-test). On the basis of absolute length and centromeric position, the chromosomes have been arranged in order of decreasing length in an ideogram (Fig.6a,b).

Karyotype Formula : (K) 2n=14=4m+2sm+2st+6a

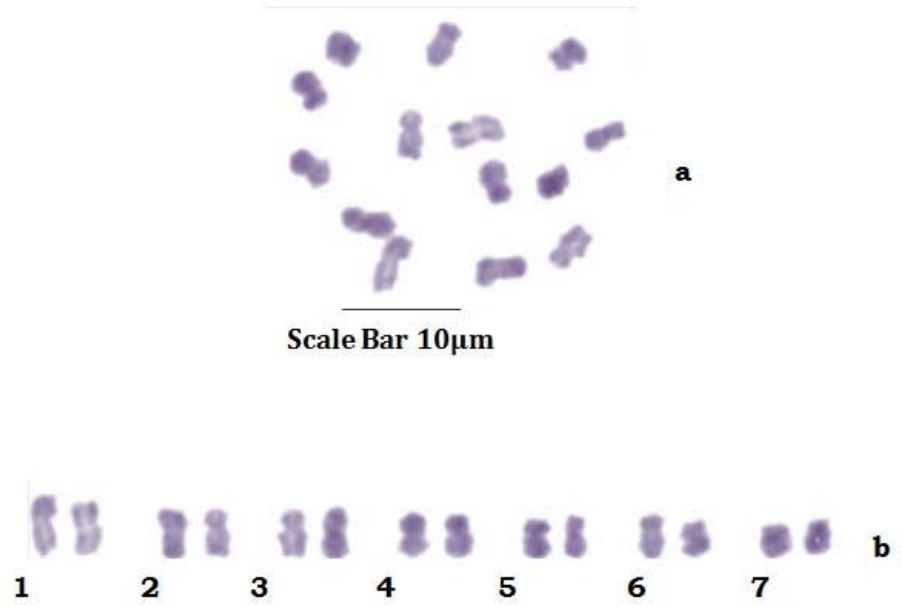


Figure 5 : (a) Chromosome preparation of *Diplozoon guptai* (b) Karyotype constructed from mitotic cells of *Diplozoon guptai* stained with Giemsa (m= metacentric; sm= Submetacentric; st= Subtelocentric; a= acrocentric;)

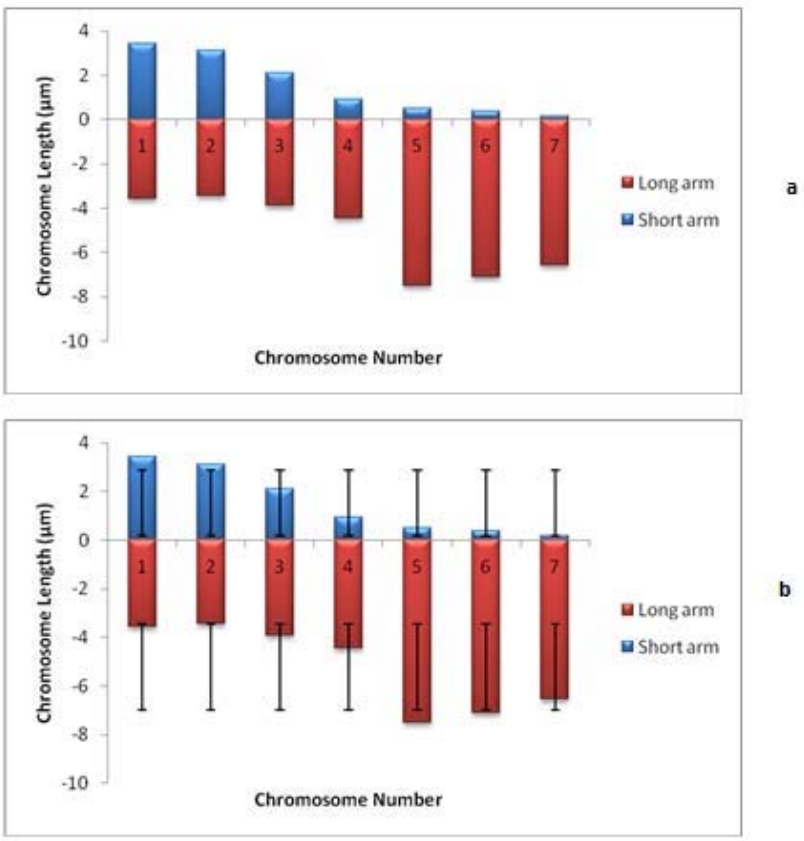


Figure 6 : (a) Ideogram of *Diplozoon guptai* (b) Error Bars with Standard Deviation *Diplozoon guptai*

© 2016 Global Journals Inc. (US)

IV. Discussion

Karyological characterization of Monogenea species is a neglected subject. However, the study of karyotype of *Diplozoon* spp. was the first study in Kashmir Valley. Regarding diplozoids, 17 species have been studied cytogenetically to date (13 identified and 4 unclassified taxa, Table 4); karyotypes of only 9 additional monogeneans have been published (Benazzi and Benazzi Lentati 1976; Harris 1985; Rohde 1994; Cable and Harris 2002). As summarized in Table 4, all diplozoid species have chromosome sets comprising 14 acrocentric elements except two species which comprising 3 metacentric and 1 acrocentric elements. Taking into account a hypothesis that less advanced species of a group often have non-symmetric karyotypes (White 1973), this karyotype seems to represent an ancestral type (Koroleva 1969). Thus, Koroleva (1969) suggested that species with chromosome numbers lower than 14 might originate *via* Robertsonian centric-fusion translocations during evolution.

Four analyzed karyotypes of *D. paradoxum, P. bliccae, P. nagibinae,* and *P. sapae* were previously studied by Koroleva (1968a, b, 1969), and the data on number and classification of chromosomes fit well with the present results. However, our study has revealed new information on chromosome measurements of *Diplozoon* species of the Kashmir Valley. Koroleva (1968a, 1969) showed no interspecific differences among species with 2n=14. She reported maximum chromosome length from 3 to 5 µm in *P. bliccae* (syn. *Diplozoon gussevi*) and from 4 to 13 µm in *D. paradoxum* (Koroleva 1968a**).** Our analysis revealed lower chromosome length, but such differences are likely related to different methodology used; it is known that air-dry and spreading techniques produce longer chromosomes than formerly used squashes (Reblanova *et al*. 2010). The most related congeners *P. bliccae, P. nagibinae, and P. sapae* (Matejusova *et al.* 2001, 2004; Gao *et al.* 2007) have equal number of 14 chromosomes of very similar morphology, all being acrocentric. However, our study showed that all the three species of *Diplozoon* examined contain 14 chromosomes with varying length of short and long arm and having different chromosome morphology. *D. kashmirensis* contains 14 chromosomes of which all are acrocentric (2n=14=14a) and have chromosome length ranging between 6.21-14.13 µm where as *D. aegyptensis* also contains 14 chromosomes but with different chromosome morphology in which the first three pairs are metacentric and rest of four pairs are acrocentric (2n=14=6m+8a) and have smallest and largest chromosome length between 6.84 and 9.78 µm. The third species *D. guptai* differs markedly in chromosome morphology (2n=14=4m+2sm+2st+6a) but it has nearly the same chromosome length as of *D. aegyptensis i.e*., having a short and long arm between 5.39 and 8.02 µm. These data correspond well with the above-mentioned hypothesis of Koroleva regarding an evolution of the *D. paradoxum* karyotype from an ancestral type with seven one-armed pairs.

Regarding interspecific differences of different species of *Diplozoon* spp. they show variation of their relative lengths (Table 5). Thus, when comparing the relative length of *Diplozoon kashmirensis* with those of *Diplozoon aegyptensis*, the differences are not significant (T-value =-0.00, P-value = 0.999 and Pearson correlation =0.190; P-value = 0.683; P>0.05). Those of *Diplozoon kashmirensis* with *Diplozoon guptai* (T-Value =0.00, P-Value = 1.000 and Pearson correlation= -0.202, P-value = 0.664; P>0.05) again the differences are not significant and in *Diplozoon aegyptensis* compared to *Diplozoon guptai* (T-value =0.00, P-Value = 0.999 and Pearson correlation= -0.127 P-Value =0.787; P>0.05, here we again see that differences are not significant statically. Therefore, the noted differences in the relative chromosome lengths between the individual *Diplozoon* species cannot be used as a reliable criterion for establishing identification of the *Diplozoon* species. So, on the basis of centromeric index *Diplozoon* species can be used for the identification of different species. Thus, pericentromeric heterochromatin, occurring in acrocentric chromosomes of any of studied species with 2n=14, might be lost in the process of centric fusions. It is evident that further detailed cytogenetic study of subsequent diplozoid monogeneans will better reveal general routs of chromosome evolution within the relatively narrow group of interesting fish parasites.

a) Digenean Trematode

i. *Clinostomum schizothoraxi Kaw, 1950*

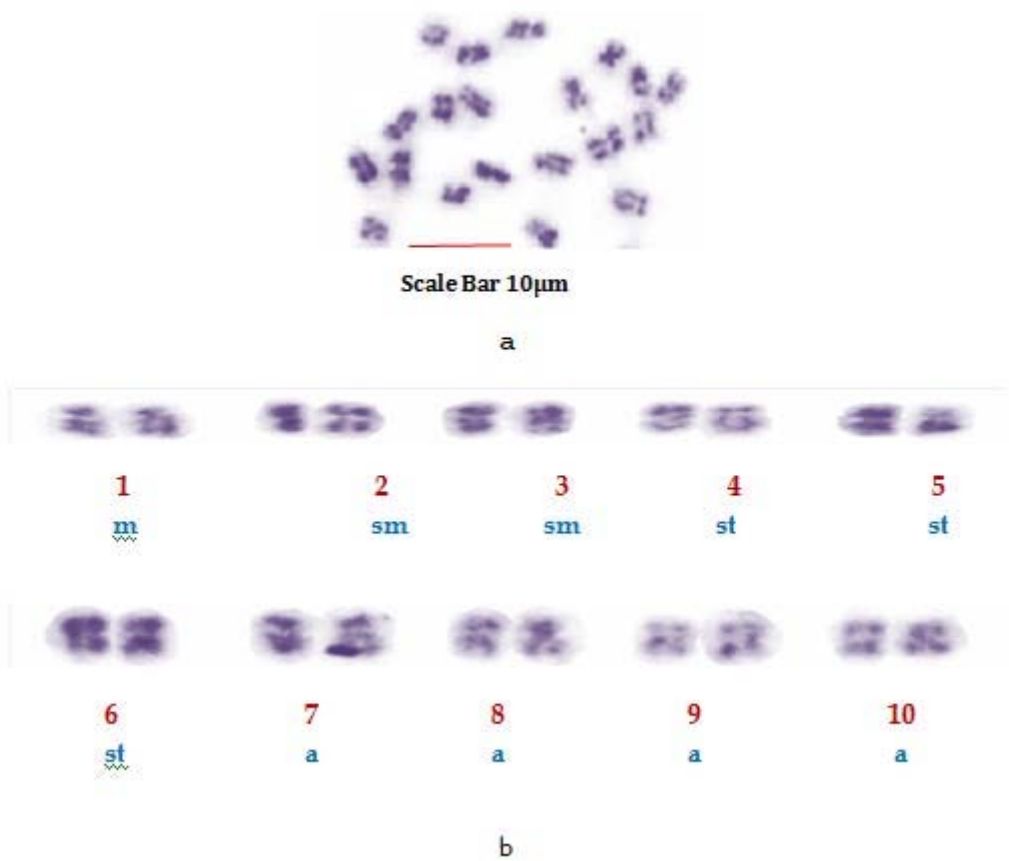
Colchicine treatment allowed us to examine enough number of metaphase plates with well-contracted mitotic chromosomes. In this way, the number and morphology was determined with high accuracy. A diploid complement of 2n=20 was found in 43 dividing cells of *Clinostomum schizothoraxi* (Fig. 7 &8) collected from *Schizothorax* and *Carassius* spp. The chromosomes are large; the smallest measured 5.88 µm and the largest 9.15µm (Table 5 & 6). Karyotype (Pmg. 4.37) included one metacentric (no. 1); two submetacentric (no.s 2 and 3); three subtelocentric (no.s 4; 5 and 6) and four acrocentric (no.s 7, 8; 9 and 10) chromosome pair; the karyotype formula may be summarized as 2n=20=1m+2sm+3t+4a. Number of chromosome arms (NF) was 26; total haploid complement length (TCL) was 77.12 µm. Arm ratio of the complement ranges between 1.51–18.31 and the centromeric index ranges from 5.18–39.88 (Table 4.29). There is less chromosome length differece between 2 & 3; 4 & 5, 6, 7 & 8 and 9 &10 which are statistically less significant (P<0.05 ; Students T-test). On the basis of

© 2016 Global Journals Inc. (US)

absolute length and centromeric position the chromosomes have been arranged in order of decreasing length in an ideogram and the karyotype formula is; **Karyotype Formula : (K) 2n=20=1m+ 2sm+3t+4a.**

Table 5 : Measurements and classification of chromosomes of *Clinostomum schizothoraxi* Kaw, 1950

Chromosome pair number	Length of short arm (µm) 'S'	Length of long arm (µm) 'L'	Total Length/Absolute Length (µm) L+S	Arm Ratio (L/S)	Relative Length (%)	Centromeric Index (ci)	Classification	
1	3.21	4.84	8.05	1.51	10.44	39.88	Metacentric	
2	2.13	4.11	6.24	1.93	8.09	34.13	Submetacentric	P-Value = 0.020 P<0.05
3	2.00	3.88	5.88	1.94	7.62	34.01	Submetacentric	
4	1.54	6.63	8.17	4.31	10.59	18.85	Subtelocentric	P-Value = 0.012; P<0.05
5	1.33	6.52	7.85	4.90	10.18	16.94	Subtelocentric	
6	1.00	5.43	6.43	5.43	8.34	15.55	Subtelocentric	P-Value = 0.011 P<0.05
7	0.84	8.31	9.15	9.89	11.86	9.18	Acrocentric	
8	0.71	8.13	8.84	11.45	11.46	8.03	Acrocentric	
9	0.56	7.84	8.40	14.00	10.89	6.67	Acrocentric	P-Value = 0.010 P<0.001
10	0.42	7.69	8.11	18.31	10.52	5.18	Acrocentric	



Pmg. 7 : **(a)** Chromosome preparation of *Clinostomum schizothoraxi*. **(b)** Karyotype constructed from mitotic cells of *Clinostomum schizothoraxi* stained with Giemsa (m= Metacentric; sm= Submetacentric; st= Subtelocentric; a= Acrocentric)

© 2016 Global Journals Inc. (US)

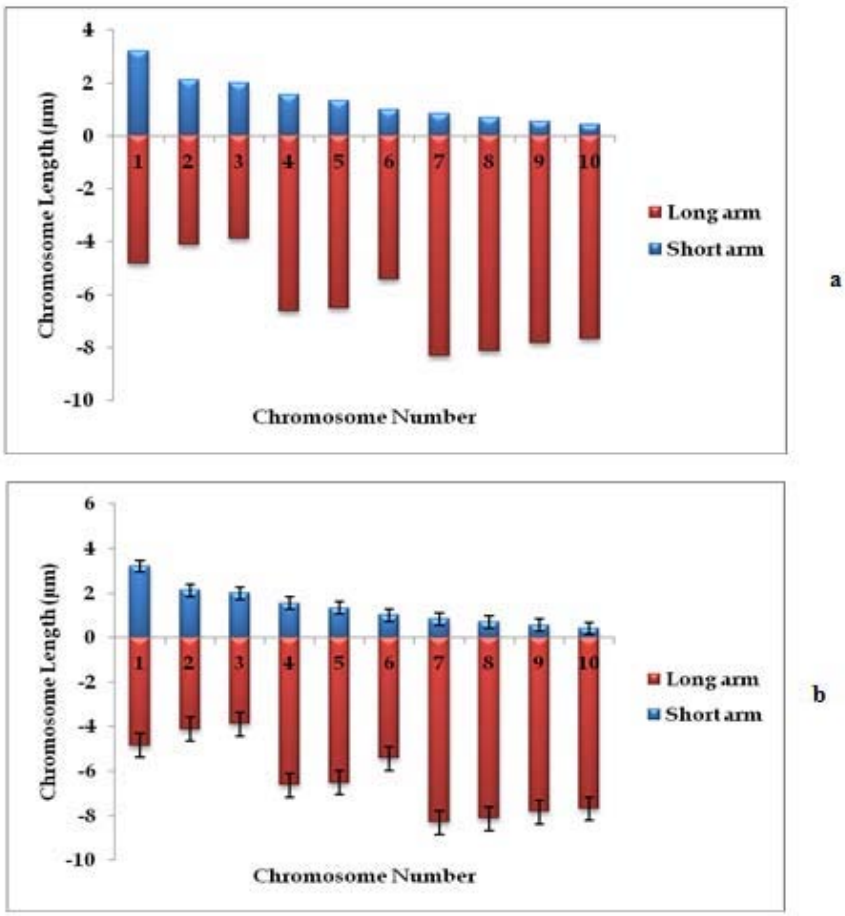


Figure 8 : (a) Haploid idiogram, (b) Error bars with standard error of *Clinostomum schizothoraxi* Kaw, 1950

Table 6 : Comparative Relative Lengths (%)of Trematodes

Chromosome Pair No.	*Diplozoon kashmirensis* Kaw, 1950	*Diplozoon aegyptensis* Fischthal et Kuntz, 1963	*Diplozoon guptai* Fayaz and Chishti, 1999	*Clinostomum schizothoraxi* Kaw, 1950
1	19.47	14.49	14.81	10.44
2	16.49	13.23	13.93	8.09
3	15.19	12.26	12.76	7.62
4	15.05	17.53	11.41	10.59
5	13.35	15.53	16.97	10.18
6	11.89	14.22	15.83	8.34
7	8.56	12.75	14.29	11.86
8				11.46
9				10.89
10				10.52

V. Discussion

Digenean Trematodes are karyotypically conservative, and their karyotypes tend to have the same number and closely related gross chromosome morphology of the genus and family (or even higher) taxonomic level. Most of the karyologically studied members of Digenean families are Troglotrematidae, Plagiorchiidae, Telorchiidae, Prosthogonimidae, and Lecithodendriidae. Information on the cytogenetics of Clinostomidae trematodes is limited largely to the description of the chromosome numbers. *Clinostomum schizothoraxi* possess 10 haploid chromosomes in which first pair is metacentric; pairs 2 & 3 are submetacentric while pairs 4; 5 & 6 are subtelocentric and pairs 7; 8; 9 and 10 are acrocentric which were observed during the present investigation. Britt (1947) also reported 10 haploid chromosomes in various species of the family Clinostomidae. According to Britt (1947), the largest chromosome were observed in the

© 2016 Global Journals Inc. (US)

family Allocreadiidae, where some pairs measures up to 8 μm in length and smallest in the families Clinostomidae and Plagiorchiidae, in case of the present study the chromosomes are medium sized; the smallest measured 5.88 μm and the largest 9.15 μm. His work was mainly concerned with collecting cytological evidences to find an evolutionary mechanism in Platyhelminthes. However, Short and Menzel (1960) concluded that morphological changes accompanying separation of the genera seem to have been the results of translocations, inversions, deletions, and changes in the number of smaller chromosomes of Digeneans. The present results on karyotype of *Clinostomum schizothoraxi*, 2n=20, are in agreement with Britt (1947) who described 20 chromosomes of *Clinostomum marginatum*. Despite limitations of the method used, Britt (1947) established that small chromosomes are characteristic of Clinostomidae species and our data confirm these findings. Mitotic chromosomes of *Clinostomum schizothoraxi* are medium sized, up to 9.15 μm. Notable workers like Cable (1931, 1974); Anderson (1935); Chen (1937); Rees (1937); Pennypacker (1936, 1940); Markell (1943); Ciordia (1949; 1950, 1956); Willmott (1950); Willey and Godman (1950); John (1956); Dhingra (1954a-c,1955a-c); Guildford (1955, 1961); Short, (1955, 1957) and Short and Menzel (1957, 1959, 1960); Sanderson (1959); Barton (1960); Greson (1958, 1964); Sharma *et al.* (1968a-b); Saksena (1969); Reddy and Subramanyam (1973, 1975); Sharma *et al* (1974) and Jha (1975) have observed interesting cytological variations regarding the chromosome number and morphology in the order Digeneans.

Changes in chromosome form in digeneans trematodes were believed most commonly from centric fusion, pericentric inversions, changes in the amount of heterochromatin and euchromatin, and through other chromosomal rearrangements (Grossman & Cain, 1981). Often a likely route for the evolution of chromosomes within a family or genus can be visualised; key indicators include: karyotypic variation between related species whose diploid complements differ (Barsiene & Grabda-Kazubska, 1988a); atypically large chromosomes (Grossman & Cain, 1981); the relative lengths of chromosomes comprising the haploid genome (White, 1973); and the chromosome arm number (Mutafova, 1994). One of the most commonly observed evolutionary pathways occurring in the digenean genome results from a Robertsonian translocation.

VI. Conclusion

Studies of trematode material have been relatively numerous, and the results point definitely toward the desirability of continued efforts in this field of research. The discovery of the presence of heterochromosomes, particularly of the fact that some forms show the female as the heterogamic sex, is of especial interest and importance. Some definite taxonomic relationships are recognizable, but since in some cases they substantiate other types of evidence used in establishing phylogenetic position and in other cases the results seem to be contradictory, further observations are in order. Perhaps some of the apparent contradictions may be eliminated as our knowledge increases. Such definitely has been the case among the Turbellaria, as mentioned in the body of this paper. In the digenetic trematodes studied till to date, most variations in the chromosome numbers within a genus are seldom greater than + 1 or 2 bivalents. Thus the mechanism for an addition or deletion of the chromosome must operate at a low level or inefficient level in this group. This suggest that the differences in the have come about by a doubling of the whole sets of chromosome but by a gradual addition or losses. Each change which represents aneuploid condition becomes stabilized. When variation in the chromosome number exceeds 1 or 2 bivalents, it probably represents successive aneuploid conditions, each change followed by a period of stability in the new chromosome number.

VII. Acknowledgement

The authors are highly thankful to the Department of Zoology for providing the Laboratory and Library facility, the first author is also thankful to Prof. Fayaz Ahmad for compiling the paper.

References Références Referencias

1. Agatsuma T, Habe S. 1985. Electrophoretic studies on enzymes of diploid and triploid *Paragonimus westermani*. Parasitology, Vol. 91, pp. 489-97.
2. Aleksandrova, O. V. and Podgornova, G. P. 1978. Taxonomic analysis of *Pegosomum asperum* and *Pegosomum saginatum* (Trematoda: Echinostomatidae). Parazitologiia, Vol. 12, pp. 413-417.
3. Anderson, M. G. 1935. Gametogenesis in the primary generation of a digenetic trematode, *Proterometra macrostoma* Horsfall, 1933. Trans. Amer. Micr. Soc., Vol. 54, pp. 271-297.
4. Atkinson, K. H. 1980. Chromosome analysis of *Schistosoma rodhaini* (Trematoda: Schistosomatidae). Can. J. Genet. Cytol., Vol. 22, pp. 143-147.
5. Avenant-Oldewage, A.; le Roux, L. E.; Mashego S. N. and Jansen van Vuuren, B. 2014. *Paradiplozoon ichthyoxanthon* n. sp. (Monogenea: Diplozoidae) from *Labeobarbus aeneus* (Cyprinidae) in the Vaal River, South Africa. Journal of Helminthology, **88**: 166–172.
6. Baer, J. G, Euzet, L. 1961. Classe des Monogenes, Monogenoidea Bychowsky. In: Grasse PP (ed) Traite de Zoologie. Anatomie, Systématique, Biologie. IV. Plathelminthes, Mesozoaires, Acantho-

© 2016 Global Journals Inc. (US)

cephales, Nemertiens. Masson et Cie Editeurs, Paris, pp 243-325.

7. Barsiene J. V. and Stanyavichyute G. J. 1993. Karyotypes of *Trichobilharzia szidati* and *Bilharzieha polvnicu* (Schistosomatidae, Trematoda). Parazitologiya, Vol. 27, pp. 41-47.
8. Barsiene, J. 1990. Chromosome sets of trematodes *Parafasciolopsis fasciolaemorpha* (Ejsmont, 1932) and *Cathemasia hians* (Rudolphi, 1809) Looss, 1899. Helminthologia, Vol. 27(3), pp. 145-152.
9. Barsiene, J. 1991a. Karyological studies of trematodes within genus *Echinoparyphium* (Echinostomatidae). Ekologija. Vol. 2.
10. Barsiene, J. 1991b. Karyotypes of *Paramphistomum* sp., *Cathaemasia hians* (Rudolphi, 1809) Looss, 1899, *Sphaeridiotrema globulus* (Rudolphi, 1819) and *Azygia lucii* (Szidat, 1932). Ekologija. Vol. 2, pp. 21-27.
11. Barsiene, J. 1991c. Chromosome sets of Tylodelphys clavata, Tylodelphys excavata and Posthodiplostomum cuticola (Diplostomatidae, Trematoda) from freshwater snails Angew. Parasitol., Vol. 32, pp. 87-92.
12. Barsiene, J. 1992. The chromosome set of *Apatemon minor* and *Apatemon* sp. (Trematoda) with description of tetraploid embryos. Angewandte Parasitologie, Vol. 32, pp. 87-92.
13. Barsiene, J. 1993. The karyotypes of trematodes. 370 pp. Vilnius, Academia (in Russian).
14. Barsiene, J. and Grabda-Kazubska, B. 1988a. A comparative study on chromosomes in plagiorchiid trematodes. I. Karyotypes of *Opisthioglyphe ranae* (Frolich, 1791), *Haplometra cylindracea* (Zeder, 1800) and *Leptophallus nigrovenosus* (Belingham, 1844). Acta Parasitol. Polonica., Vol. 33(4), pp. 249-257.
15. Barsiene, J. and Grabda-Kazubska, B. 1988b. A comparative study on chromosomes in plagiorchiid trematodes. II. Karyotypes of *Plagriorchis* sp., *Haematoloechus similis* Looss, 1899, and *Haematoloechus asper* Looss, 1899. Acta Parasitol. Polonica., Vol. 33(4), pp. 259-265.
16. Barsiene, J. and Grabda-Kazubska, B. 1991a. A comparative study on chromosomes in plagiorchiid trematodes. III. Karyotypes of *Paralepoderma progeneticum* (Buttner, 1951) and *Omphalometra flexuosum* (Rudolphi, 1809). Acta Parasitol. Polonica., Vol. 36, pp. 19-21.
17. Barsiene, J. and Grabda-Kazubska, B. 1991b. Chromosome sets of *Diplodiscus subclavatus* (Pallas, 1760) and *Notocotylus noyeri* Joyeux, 1922 (Trematoda). Acta Parasitol. Polonica., Vol. 36, pp. 1-3.
18. Barsiene, J. and Grabda-Kazubska, B. 1991c. Karyotypes of *Pleurogenes claviger* (Rudolphi, 1819) and *Pleurogenoides medians* (Olsson, 1876) (Trematoda, Pleurogenidae). Acta Parasitol. Polonica., Vol. 36, pp. 13-15.
19. Barsiene, J. and Kise1iene, V. 1990b. Karyological studies of *Echinoparyphium aconiatum* (Dietz, 1909), *Hypoderaeum conoideum* (Blanch, 1782) Dietz, 1909 and *Neoacanthoparyphium echinatoides* (Filippi, 1854) Odening, 1962 (Trematoda: Echinostomatidae). Acta Parasitol. Polonica., Vol. 35, pp. 271-276.
20. Barsiene, J. and Kiseliene, V. 1990a. Karyological studies of trematodes within the families Psilostomidae and Echinochasmidae. Helminthologia., Vol. 27, pp. 99-108.
21. Barsiene, J. and Kiseliene, V. 1991. Karyological studies of trematodes within the genus *Echinostoma*. Acta Parasitol. Polonica., Vol. 36, pp. 23-29.
22. Barsiene, J. and Staneviciute, G. 1991. A comparative karyological study of trematodes within the genus *Diplostomum*. Helminthologia., Vol. 28, pp. 31-36.
23. Barsiene, J. V.; Stanyavichyute, G. J. and Oriovskaya, O. M. 1989. Karyological investigations on trematodes of the family Schistosomatidae in North-West Chukotka. Parazitologiya, Vol. 23, pp. 496-503.
24. Barsiene, J., Kiseliene, V. and Grabda-Kazubska, B. 1990b. Karyotypes of *Isthmiophora melis* (Schrank, 1788) and *Moliniella anceps* (Molin, 1858) (Trematoda: Echinostomatidae). Acta Parasitol. Polonica., Vol. 35, pp. 265-269.
25. Barsiene, J.; Roca, V.; Tapia, G. and Martin, J. E. 1995. Chromosome Analysis of Two Digenean Species of the Families Heterophyidae and Monorchiidae (Trematoda). Research and Reviews in Parasilology, Vol. 55(3), pp. 149-154.
26. Barsiene, J.; Staneviciute, G.; Niewiadomska, K. and Kiseliene, V. 1990a. Chromosome sets of *Diplostomum paracaudum* (Lies, 1959) Shigin 1977 and *Diplostomum baeri* (Dubois, 1937). Acta Parasitol. Polonica., Vol. 35(2), pp. 107-112.
27. Barton, P. R. 1960. Gametogenesis and fertilization in the frog lung fluke *Haematoloechus medioplexus* Stafford (Trematoda: Plagiochiidae) J. Morph., **107**: 93-122.
28. Bell, A. S.; Sommerville, C. and Gibson, D. I. 1998. Karyological studies on three strigeid digeneans: *Ichthyocotylurus erraticus* (Rudolphi, 1809), *I. variegatus* (Creplin, 1825) and *Apatemon gracilis* (Rudolphi, 1819). Systematic Parasitology, Vol. 41, pp. 169-178.
29. Benazzi, M. and Benazzi-Lentati, G. 1976. Platy*helminthes. Animal cytogenetics. Ed. B. John. Berlin, Stuttgart: Gerzuder Borntraeger*. Vol. 1. 182 p.
30. Birstein, V. J. 1990. First contribution to karyology of two acoels (Turbellaria) and a dinophilid (Annelida). Biol. Zbl., Vol. 109, pp. 169-174.

© 2016 Global Journals Inc. (US)

31. Birstein, V. J. and Mikhailova, N. A. 1990. On the Karyology of trematodes of the Genus *Microphallus* and their intermediate gastropod host II. Karyological study of *Littorina saxatilis* (Gastropoda: Prosobranchia).
32. Blair, D. 2000. Genomes of *Paragonimus westermani* and related species: current state of knowledge. International Journal for Parasitology, Vol. 30, pp. 421-426.
33. Bovet, J. 1967. Contribution a la morphologie et a la biologie de *Diplozoon paradoxum* v. Nordmann, 1832. Bull Soc Neuchatel Sci., Vol. 3, pp. 64-159.
34. Britt, H. G. 1947. Chromosomes of digenetic Trematodes. Amer. Nat., Vol. 81, pp. 276-296.
35. Burton, P. R. 1960. Gametogenesis and fertilization in the frog lung fluke, *Haematoloechus medioplexus* Stafford (Trematoda: Plagiorchiidae). J. Morph., Vol. 107, pp. 93-122.
36. Cable, J. and Harris, P. D. 2002. Gyrodactylid developmental biology: historical review, current status and future trends. Int. J. Parasitol., **32**: 255-280.
37. Cable, R. M. 1931. Studies on the germ-cell cycle of Cryptocotyle linqua Creplin. I. Gametogenesis in the adult. Quarterly Journal of Microscopic Science, Vol. 74, pp. 563-589.
38. Cable, R. M. 1974. Phylogeny and taxonomy of the trematodes with reference to marine species. In W. A. Vernberg (ed.), Symbiosis in the Sea. Univ. South Carolina Press, Columbia, S.C., p. 173-193.
39. Cannon, L. R. G. 1971.The life cycles of *Bunodera sacculata* and *Bunodera luciopercae* (Trematoda: Allocreadidae) in Algonquin Park, Ontario. Can. J. Zool., Vol. 49, pp. 1417-1429.
40. Chattopadhyay, I. and Manna, B. 1987. Chromosome study of *Fsoparorchis hypselobagri* Billet, 1898 (Digenea: Hemiuridae). Journal of Helminthology, Vol. 61, pp. 346-347.
41. Chen, P. D. 1937. The germ cell cycle in the trematode, *Paragonimus kellicotti* Ward. Trans. Amer. Micros. Soc., Vol. 56, pp. 208-236.
42. Churchill, H. M. 1950. Germ cell cycle of *Echinostoma revolutum* (Froelich, 1802). J. Parasitol., Vol. 36, pp. 15-20.
43. Ciordia, H. 1949. Cytological study of *Rhopalias macracanthus* Chandler, 1932, a trematode from the opossum, *Didelphys virginiana*. J. Parasitol., Vol. 35, pp. 417-422.
44. Ciordia, H. 1950. The chromosomes of *Notocotylus filamentis* Barker, 1915, a monostome from the muskrat (*Fiber zibethicus*). Trans. Amer. micr. Soc., Vol. 69, pp. 64-65.
45. Ciordia, H. 1956. Cytological studies on the germ cell cycle of the trematode family Bucephalidae. Trans. Amer. Micr. soc., Vol. 75, pp. 103-116.
46. Civanova, K.; Koyun, M. and Koubkova, B. 2013. The molecular and morphometrical description of a new diplozoid species from the gills of the Garra rufa (Heckel, 1843) (Cyprinidae) from Turkey-including a commentary on taxonomic division of Diplozoidae. Parasitol Res., **112**: 3053-3062.
47. Dai, X. 1990. Karyotype analysis of Fasciolopsis buski. J. Chongquin Med. Univ., Vol. 4, pp. 11.
48. Dhar, V. N. and Sharma, G. P. 1984. Behaviour of chromosomes during gametogenesis and fertilization in *Paradistomoides orientalis* (Digenea: Trematoda). Caryologia, Vol. 37(3), pp. 207-218.
49. Dhingra, O. P. 1954a. Taxonomic values of chromosomes and cytoplasmic inclusions in a digenetic trematode-*Phyllodistomum spatula*. Res. Bull. Panjab. Univer. Zool., Vol. 51, pp. 101-109.
50. Dhingra, O. P. 1954b. Gametogenesis and fertilization in *Isoparorchis eurytremum*. Res. Bull. Panjab. Univ., Vol. 44, pp. 21-24.
51. Dhingra, O. P. 1955a. Spermatogenesis of a digenetic trematode *Gastrothylax cruminifer*. Res. Bull. Panjab Univ., Vol. 65, pp. 11-17.
52. Dhingra, O. P. 1955b. Spermatogenesis of a digenetic trematode *Cotylophoron elongatum*. Res. Bull. Panjab Univ., Vol. 64, pp. 1-10.
53. Dingler, M. 1910. Uber die Spermatogeneso des *Dicrocoelium lanceolatum*. Arch. f. Zellf., Vol. 4, pp. 672-712.
54. Fi1ippone, E. J. and Fried, B. 1974. The chromosome number of *Leucochloridiomorpha constantiae* (Trematoda). J. Parasitol. Vol. 60, pp. 929.
55. Fried, B. 1975. The chromosome number of *Philophthalmus hegeneri* Penner, Fried, 1963 (Trematoda). Proc. Helminthol. Soc. Wasch., Vol. 42(2), pp. 197.
56. Gao, L. 1985. Observation of meiosis of *Fasciolopsis buski*. Hereditas (Beijing), Vol. 7(2), pp. 22-23.
57. Gao, Q.; Chen, M. X.; Yao, W. J.; Gao, Y.; Song, Y.; Wang, G. T.; Wang, M. X. and Nie, P. 2007. Phylogeny of diplozoids in five genera of the subfamily Diplozoinae Palombi, 1949 as inferred from ITS-2 rDNA sequences. Parasitology, **134**: 695–703.
58. GaoLongsheng.; YouShaoyang.; ChenShanlong.; YuHaisin.; XiaoJianhua and WuMengjin. 1985. Studies on the karyotype of *Schistosoma japonicum*. J. Parasitol. and Parasitic Dis., Vol. 3(1), pp. 29-31.
59. Goldschmidt, R. 1905. Eireifung und Befruchtung und Embryonalentwicklung des *Zoogonus minis* Lss., Zool. Jahrb. Abt. f. Anat., pp. 21.
60. Goldschmidt, R. 1908. Uber das Verhalten des Chromatins bei der Eireifung und Befruchtung des Dicrocoelium lanceolatum. Arch. f. Zellf., pp. 1.
61. Gregoire, V. 1909. La reduction dans le Zoogonus mirus Lss. ct la Primartypus. La Cellule., Vol. 25, pp. 85-99.

© 2016 Global Journals Inc. (US)

62. Gresson, R. A. R. 1964. Oogenesis in the hermaphroditic digenea (Trematoda). Parasitology, **54**(3): 409-421.
63. Gresson, R. A. R. 1958. The gametogenesis of the digenetic trematode, *Sphaerostoma bramae* (Muller) Luhe. Parasitol., Vol. 48, pp. 293-302.
64. Grossman, A. I. and Cain, G. D. 1981. Karyotypes and chromosome morphologies of *Megalodiscus temperatus* and *Philophthalmus gralli*. Journal of Helminthology, Vol. 55, pp. 71-78.
65. Grossman, A. I., Mckenzie, R. and Cain, G. D. 1980. Sex chromatin in *Schistosoma mansoni*. Journal of Parasitology, Vol. 66, pp. 368-370.
66. Grossman, A. I.; Short, R. B. and Cain, G. D. 1981a. Karyotype evolution and sex chromosome differentiation in Schistosomes (Trematoda, Schistosomatidae). Chromosoma, Vol. 84, pp. 413-430.
67. Grossman, A. I.; Short, R. B. and Kuntz, R. E. 1981b. Somatic chromosomes of *Schistosoma rodhaini*, *Schistosoma matthei* and *Schistosoma intercalatum*. J. Parasitol., Vol. 67, pp. 41-44.
68. Guilford, H. G. 1955. Gametogenesis in *Heronimus chelydrae*. Trans. Amer. mic. Soc., Vol. 74, pp. 182-190.
69. Guilford, H. G. 1961. Gametogenesis, egg-capsule formation, and early miracidial development in the digenetic trematode *Halipegus eccentricus* Thomas. J. Parasitol., Vol. 47(5), pp. 757-764.
70. Gustinelli, A.; Caffara, M.; Florio, D.; Otachi, E. O.; Wathuta, E. U.; Fioravanti, M. L. 2010. First description of the adult stage of *Clinostomum cutaneum* Paperna, 1964 (Digenea: Clinostomidae) from grey herons Ardea cinerea L. and a redescription of the metacercaria from the Nile tilapia *Oreochromis niloticus niloticus* (L.) in Kenya. Syst. Parasitol., **76**: 39-51.
71. Harris, P. D. 1985. Observations on the development of the male reproductive system on *Gyrodactylus gasterostei* Glaser (1974) (Monogenea, Gyrodactylidae). Parasitology, **91**: 519–529.
72. HeLian-Yin.; Zhong Hui-lan.; Gao Pei-Zhi.; LLi Hao-hong. and Xu Zhi-biao. 1982. Preliminary studies on chromosomes of 9 species and subspecies of lung fluke in China. Chinese Medical J., Vol. 95(6), pp. 404-408.
73. Henneguy, L. F. 1902. Sur la Formation de l'CEuf, la Maturation et la Fecondation de l'Oocyte chez lo Distomum hepaticum, Paris, C. R. Acad. Sci., pp. 134
74. Hirai, H.; Sakakuchi, Y.; Habe, S. and Imai, H. T. 1985. C banding analysis of six species of lung flukes, *Paragonimus* spp. (Trematoda: Platyhelminthes), from Japan and Korea. Zeitschrift fur Parasitenkunde, Vol. 71, pp. 617-629.
75. Ieyma, H. and Ozaki, F. 1987. Chromosomes of two species in Cercaria parasitized on the clam, *Ruditapes philippinarum*. Chromosome Inform. Service, Vol. 43, pp. 27-28.
76. Iha, A. G. 1975. Cytogenetics, evolution and systematics of Digenea (Trematoda: Platyhelminthes). Egypt. J. Genet. Cytol., Vol. 4, pp. 201-233.
77. Jones, A. W. 1956. The chromosomes of a species of *Halipegus* Looss, 1899 (Digenea: Hemiuridae). J. Tenn. Acad. Sci., Vol. 31, pp. 186-187.
78. Jones, A. W. and Mayer, T. C. 1953. The chromosomes of *Spirorchis magnitestis* Byrd, 1939 (Trematoda, Digenea). J. Tenn. Acad. Sci., Vol. 28, pp. 125-134.
79. Jones, A. W.; Mounts, B. W. and Wo1cott, G. B. 1945. *Macravestibulum kepneri* n. sp.; a morphological and cytological study of a pronocephalid trematode. J. Morphol., Vol. 77, pp. 285-297.
80. Kaewkong, W.; Imtawil, K.; Maleewong, W.; Intapan, P. M.; Sri-aroon, P. and Wongkham, S. 2012. Genome size estimation of liver fluke *Opisthorchis viverrini* by real-time polymerase chain reaction based method. Parasitology International, Vol. 61(1), pp. 77-80.
81. Kahlil, G. M. and Cable, R. M. 1968. Germinal development in *Philophthalmus megalurus* (Cort, 1914) (Trematoda: Digenea). Z. Parasitenkd. Vol. 31, pp. 211-231.
82. Khotenovsky, I. A. 1985. Fauna of the USSR. Monogenea. Nauka, Leningrad.
83. Komalamisra, C. 2005. Chromosomes and Chromosome Breakage in the Lung Fluke, *Paragonimus heterotremus*. J. Trop. Med. Parasitol., Vol. 28, pp. 69-72.
84. Koroleva, Y. I. 1968a. Karyological study on several species of Monogenea of the genus *Diplozoon*. Dokl Akad Nauk USSR, Zoologia, Vol. 179, pp. 739-741.
85. Koroleva, Y. I. 1968b. New data on karyology of some species of the genus *Diplozoon*. Parazitologiya, Vol. 2, pp. 294-296.
86. Koroleva, Y. I. 1969. Karyology of some species of *Diplozoon*. Parazitologiya, Vol. 3, pp. 411-414.
87. Koskova, E.; Spakulova, M.; Koubkova, B.; Reblanova M. and Orosova, M. 2011. Comparative karyological analysis of four diplozoid species (Monogenea, Diplozoidae), gill parasites of cyprinid fishes. Parasitol. Res., Vol. 108, pp. 935-941.
88. Koskova, E.; Spakulova, M.; Koubkova, B.; Reblanova M. and Orosova, M. 2011. Comparative karyological analysis of four diplozoid species (Monogenea, Diplozoidae), gill parasites of cyprinid fishes. Parasitol. Res., **108**: 935-941.
89. Lei Changqiu.; Wang Pengpeng and Song Changeun. 1985. Preliminary studies on the

© 2016 Global Journals Inc. (US)

karyotype of *Euparagonimus cenocopiosus* Chen, 1962. J. Parasitol. Parasitic Dis., Vol. 3(1), pp. 32-34.
90. Levan A., Fredga, K. and Sandberg, A. 1964. Nomenclature for centromere position on chromosomes. Hereditas, Vol. 52, pp. 201-220.
91. Li, G. O.; Jin, J. S. and Wang, P. Y. 1988. A study on the chromosomes of *Fasciola hepatica*. Chinese J. Veter. Sci. Technol., 6: 11-16.
92. Lin, T. P. 1954. The chromosomal cycle in *Parascaris equorum*: oogenesis and diminution. Chromosoma, Vol. 6, pp. 175-198.
93. LoVerde, P. T. 1978. Chromosomes in the evolution of Platyhelminthes. Genetics of parasitic helminths: recent progress and future directions. 53rd Annual Meeting, American Society of Parasitologist. Chicago, Illinois.
94. LoVerde, P. T. 1979. Chromosomes of two species of *Paragonimus*. Trans. Am. Microsc. Soc., Vol. 98, pp. 280-5.
95. Loverde, P. T. and Frederickse, D. W. 1978. The Chromosomes of *Cotylogaster occidentalis* and *Cotylaspis insignis* (Trematoda: Aspidogastrea) with Evolutionary Considerations. Proceedings of the Helminthological Society, Vol. 45(2), pp. 158-161.
96. LoVerde, P. T. and Kuntz, R. E. 1981. Chromosome numbers of some Schistosomes. J. Parasitol.; Vol. 67, pp. 726.
97. Madhavi, R. and Ramanjaneulu, J. V. 1986. Observation on chromosomes and gametogenesis of *Transversotrema patialense* (Trematoda). Parasitology, Vol. 12, pp. 245-252.
98. Madhavi, R.and Ramanjaneyulu, J. V. 1988. Chromosomes of *Atrophecaecum burminis* (Bhalerao, 1926) (Trematoda: Acanthostomidae). Caryologia, Vol. 41(3-4), pp. 341-346.
99. Markell, E. K. 1943. Gametogenesis and egg-shell formation in *Probolitrema californiense* Stunkard, 1935 (Trematoda: Gorgoderidae). Trans. Am. Microsc. Soc., Vol. 62, pp. 27–56.
100. Matejusova, I.; Gelnar, M.; McBeath, A. J. A.; Collins, C. M. and Cunningham, C. O. 2001. Molecular markers for gyrodactylids (Gyrodactylidae: Monogenea) from five fish families (Teleostei). Int. J. Parasitol., **31**: 738–745.
101. Matejusova, I.; Koubkova, B. and Cunningham, C. O. 2004. Identification of European diplozoids (Monogenea, Diplozoinae) by restriction digestion of ribosomal RNA internal transcribed spacer. J. Parasitol., **90**: 817–822.
102. Matthieson, E. 1904. Ein Beitrag zur Embryologie der Susswasserdendrocoelen. Z wiss Zool, Vol. 77, pp. 274–361.
103. Miyazaki, I. 1978. Two types of the lung fluke which has been called *Paragonimus westermani* (Kerbert, 1878). Medical Bull of Fukuoka Univ., Vol. 5, pp. 251-269.
104. Moriyama, N. 1982a. Karyological studies of bovine pancreatic flukes (Eurytrema sp.) and their phenotypes. J. Parasitol., Vol. 68(5), pp. 898-904.
105. Moriyama, N. 1982b. Taxonomical studies of Japanese bovine pancreatic flukes (Eurytrema sp.). Jap. J. Parasitol., Vol. 31(2), pp. 67-79.
106. Moriyama, N.; Tsuji, M. and Seto, T. 1979. Three karyotypes and their phenotypes of Japanese liver flukes (Fasciola sp). Jpn. J. Parasitol; Vol. 28, pp. 23-33.
107. Mutafova, T and Kanev, I. 1983. Studies on the karyotype of the echinostome Echinostoma barbosai Lie et Basch, 1966 (Trematoda: Echinostomatidae) from Bulgaria. Helminthology (Bulg.). Vol. 16, pp. 42-46.
108. Mutafova, T and Kanev, I. 1984. Studies on the karyotype of *Echinoparyphium aconiatum* Diez, 1909 (Trematoda: Echinostomatidae). Helminthology (Bulg.). Vol. 17, pp. 37-40.
109. Mutafova, T and Kanev, I. 1986. Studies on the karyotype of *Echinostoma revolutum* (Frolich, 1802) and Echinostoma echinatum (Zeder, 1803) (Trematoda: Echinostomatidae). Helminthology (Bulg.). Vol. 22, pp. 37-41.
110. Mutafova, T. 1983a. Studies on the karyotype of *Paramphistomum microbothrium* Fischoeder, 1901. Helminthology (Bulg.), Vol. 16, pp. 37-41.
111. Mutafova, T. 1983b. Morphology and behavior of the acrocentric chromosomes in the karyotype of *Philophthalmus* sp. during mitosis. Helminthology (Bulg.). Vol. 15, pp. 57-62.
112. Mutafova, T. and Kanev, I. 1996. Cytological study of *Rubenstrema exasperatum* (Trematoda: Omphalometridae). Int. J. Parasitol., Vol. 26, pp. 1405-1406.
113. Mutafova, T. and Komandarev, S. 1976. On the Karyotype of a Laboratory Trichinella Strain from Bulgaria. Z. Parasitenk., Vol. 48, pp. 247-250.
114. Mutafova, T. and Niewiadomska, K. 1988. The morphology of chromosomes of *Diplostomum pseudospathaceum* Niewiadomska, 1984 (Diplostomidae) karyotype. Acta Parasitol. Polonica, Vol. 33, pp. 83-88.
115. Mutafova, T.; Kanev, I. and Vassilev, I. 1987. Comparative karyological investigation of Echinoparyphium aconiatum Dietz, 1909 and *Echinoparyphium recuriatum* (Linstow. 1873) Dietz, 1909 (Trematoda: Echinosomatidae). Helminthology (Bulg.). Vol. 24, pp. 32-36.
116. Mutafova, T.; Tsocheva, N.; Polyakova-Krsteva, O. and Krstev, L. 1986. Effect of diethylnitrosamin on the chromosome structure of rats infected with *Fasciola hepatica* and on that of the liver fluke. Khelmithologiya (Sofia), Vol. 22, pp. 42-50.
117. Mutafova, T. and Niewiadomska, K. 1988. The morphology of chromosomes of *Diplostomum pseudospathaceum* Niewiadomska, 1984 (Digenea,

© 2016 Global Journals Inc. (US)

Diplostomatidae) karyotype. Acta Parasitol. Polonica, Vol. 33(2), pp. 83-88.
118. Niyamasena, S. G. 1940. Chromosomen und Geschlecht bei Bilharzia mansoni. Ztschr. Parasitenk. Vol. 11, pp. 690-701.
119. Park, G. M.; Im, K.; Huh, S. and Yong, T. S. 2000. Chromosomes of the liver fluke, *Clonorchis sinensis*. Korean J. Parasitol., Vol. 38, pp. 201-206.
120. Park, G. M.; Lee, S. U.; Park, H. Y. and Huh, S. 1998. Karyotype analysis of *Neodiplostomum seoulense*. Korean J. Parasitol., Vol. 36, pp. 277-279.
121. Park, M. and Yong, S. 2003. Effects of gamma-irradiation on the infectivity and chromosome aberration of *Clonorchis sinensis*. The Korean Journal of Parasitology, Vol. 41(1), pp. 41-45.
122. Pengpeng, W.; Changqui, L. and Changeun, S. 1986. Karyotype studies on *Paragonimus westermani* from Shaoxing County, Zhejiang. J. Parasitol. and Parasitic. Dis. Vol. 4(3), pp. 203-205.
123. Pennypacker, M. I. 1936. The chromosomes in the maturation of the germ cells of the frog lung fluke, *Pneumonoeces medioplexus*., Arch. Biol. Paris., Vol. 47, pp. 309-317.
124. Pennypacker, M. I. 1940. The chromosomes and extranuclear material in the maturing germ cells of a frog lung-fluke, *Pneumonoeces similiplexus* Stafford. J. Morph., Vol. 66, pp. 481-495.
125. Perkins, K. W. 1956. Studies on morphology and biology of *Acetodextra amiuri* (Stafford) (Trematoda: Heterophyidae). Amer. Midi. Nat., Vol. 55, pp. 139-161.
126. Petkeviciute, R. 2001b. Chromosomes of *Aspidogaster conchicola*. Journal of Helminthology, Vol. 75, pp. 295-297.
127. Petkeviciute, R. 2003. Comparative karyological analysis of three species of *Bothriocephalus* Rudolphi 1808 (Cestoda: Pseudophyllidea). Parasitol. Res., Vol. 89, pp. 358-363.
128. Petkeviciute, R. and Barsiene, J. 1988. The comparative karyological analysis of three species of trematodes of genus *Notocotylus*. Parazitologiya, Vol. 22, pp. 21-28.
129. Petkeviciute, R. and Leshko, E. P. 1991. The karyotypes of *Triaenophorus nodulosus* and *T. crassus* (Cestoda: Pseudophyllidea). Int. J. Parasitol., Vol. 21, pp. 11-15.
130. Petkeviciute, R. and Staneviciute, G. 1999. Karyotypic characterization of *Apatemon gracilis*. Journal of Helminthology, Vol. 73, pp. 73-77.
131. Petkeviciute, R. and Staneviciute, G. 2008. Comparative karyological analysis of three members of Allocreadiidae (Digenea): taxonomic and phylogenetic implications. Parasitology Research, Vol. 103, pp. 1105-1110.
132. Petkeviciute, R. B.; Stanevieiute, G. J. and Stenko, R. P. 1990. Structure of the karyotypes of *Opisthioglyphe ranae*, *Paralepoderma brumpti* and *Skrjabinoeces* sp. (Trematoda: Plagiorchiidae) (in Russian). Parazitologya, Vol. 24, pp.128-134.
133. Petkeviciute, R. Stunzenas, V. and Staneviciute, G. 2011. Clarification of the systematic position of *Cercariaeum crassum* Wesenberg-Lund, 1934 (Digenea), based on karyological analysis and DNA sequences. Journal of Helminthology, pp. 1-9.
134. Petkeviciute, R.; Barsiene, J. and Mazeika, V. 1989a. Cytogenetic characteristics of *Notocotylus noyeri* Joyeux, 1922 (Trematoda, Notocotylidae). Acta Parasitologica Lituanica, Vol. 23, pp. 93–98 (in Russian).
135. Petkeviciute, R.; Kiseliene, V. and Stenko, R. P. 1989b. Cytogenetic analysis of two populations of *Diplodiscus subclavatus* (Trematoda, Diplodiscidae). Parazitologiya, Vol. 23, pp. 489–495 (in Russian).
136. Petkeviciute, R.; Staneviciute, G. and Molloy, D. P. 2003. Chromosome analysis of *Phyllodistomum folium* (Trematoda, Gorgoderidae) infecting three European populations of zebra mussels. Parasitol. Res., Vol. 90, pp. 377-382.
137. Pickel, V. M. and Jones, A. W. 1967. Chromosomes of a Monogenetic Trematode, *Diclybothriumha mulatum* (Simer, 1929) Price, 1942. Transactions of the American Microscopical Society, Vol. 86(2), pp. 212-214.
138. Puente, H. S. and Short, R. B. 1985. Redescription of chromosomes of *Schbtomatium douthitti* (Trematoda: Schistosomatidae). Journal of Parasitology, Vol. 71, pp. 345-348.
139. Ramanjaneyulu, J. V. and Madhavi, R. 1983. Occurrence of triploidy and parthenogenesis in the allocreadiid trematode, *Allocreadium fasciatusi* Kakaji, 1969. Current Sci., Vol. 52(10), pp. 502-503.
140. Ramanjaneyulu, J. V. and Madhavi, R. 1984. Cytological investigations on two species of allocreadiid trematodes with special references to the occurrence of triploidy and parthenogenesis in *Allocreadium fasciatusi*. Int. J. Parasitol., Vol. 14, pp. 309-316.
141. Rao, K. J. and Venkat Reddy, P. 1982. Karyological study in the digenetic trematode *Notocotylus attenuates*. Curr. Sci., Vol. 51, pp. 309-310.
142. Reblanova, M.; Spakulova, M.; Orosova, M.; Bazsalovicsova, E. and Rajsky, D. 2010. A description of karyotype of the giant liver fluke *Fascioloides magna* (Trematoda, Platyhelminthes) and a review of Fasciolidae cytogenetics. Helminthologia, Vol. 47, pp. 2: 69-75.
143. Reblanova, M.; Spakulova, M.; Orosova, M.; Kralova-Hromadova, I.; Bazsalovicsova, Eva and Rajsky, D. 2011. A comparative study of karyotypes and chromosomal location of rDNA genes in important liver flukes *Fasciola hepatica* and

© 2016 Global Journals Inc. (US)

Fascioloides magna (Trematoda: Fasciolidae). Parasitol. Res., DOI 10.1007/s00436-011-2339-y.
144. Reddy, P. V. and Subramanyam, S. 1973. Chromosome studies in the liver fluke, Fasciola gigantica Cobbold, 1856, from Andhra Pradesh. Curr. Sci., 42: 288- 291.
145. Reddy, P. V. and Subramanyam, S. 1975. Chromosome studies in *Paramphistomum cervi* Zeder 1970 (Trematoda: Digenea- Paramphistomidae). Caryologia, **28**: 181-186.
146. Rees, G. 1937. Studies on the germ cell cycle of the digenetic trematode *Parorchis acanthus* Nicoll. Proc. Zool. Soc., Lond., B, Part I: 65-73.
147. Reese, G. 1939. Studies on the germ cell cycle of the digenetic trematode *Parorchis acanthus* Nicoll. Part. 1. Anatomy of the genitalia and gametogenesis in the adult. Parasitol., Vol. 31, pp. 417-433.
148. Rhee, J. K.; Eun, G. S. and Lee, S. B. 1987. Karyotype of *Fasciola* sp. obtained from Korean cattle. Korean J. Parasitol., Vol. 25(1), pp. 37-44.
149. Rhee, J. K.; Youn, R. H. and Lee, H. I. 1988. The karyotype of *Fischoederius cobboldi* (Poirier, 1883) from Korean cattle. Korean J. Parasitol., Vol. 26(2), pp. 107-111.
150. Richard, I. and Vollz, A. 1987. Preliminary data on the chromosomes of *Echinostoma caproni* Richard, 1964 (Trematoda: Echinostomatidae). Syst. Parasitol., Vol. 9, pp. 169-172.
151. Rohde, K. 1994. Chromosomes of *Kuhnia scombri* and *Kuhnia sprostonae* (Monogenea, Polyopisthocotylea, Mazocraeidae). Acta Parasitol., **39**: 117–119.
152. Romanenko, L. N. and Pleshanova, N. M. 1975. Chromosome sets of *Fasciola hepatica* and *Fasciola gigantica*. Tr. VIGIS (Teoreticheskie problemy veterinarnoy gel'mintologii), Vol. 22, pp. 137-142.
153. Romanenko, L. N. and Shigin, A. A. 1977. Chromosomal apparatus of trematodes of the general *Diplostomum* and *Tylodelphys* (Strigeidida, Diplostomatidae) and its taxonomic significance. Parazitologiia., Vol. 11(6), pp. 530-536.
154. Sakaguchi, Y. 1980. Karyotype and gametogenesis of the common liver fluke, *Fasciola* sp., in Japan. Jpn. J. Parasitol., Vol. 29, pp. 507-513.
155. Sakaguchi, Y. and Nakagawa, C. 1975. A note on the chromosomes of the common liver fluke (*Fasciola* sp.) from Japan. Chrom. Inf. Serv., Vol. 19, pp. 20-21.
156. Sakaguchi, Y. and Tada, I. 1975. Chromosomes of two species of the lung fluke, *Paragonimus ohirai* and *Paragonimus miyazakii*. Chrom. Inf. Serv., Vol. 19, pp. 21-23.
157. Sakaguchi, Y. and Tada, I. 1976a. Chromosomes of a lung fluke, *Paragonimus westermani*. Chrom. Inf. Serv., Vol. 20, pp. 23-24.
158. Sakaguchi, Y. and Tada, I. 1976b. A comparative karyotype study of lung flukes, *Paragonimus ohiria* and *Paragonimus miyazakii*. Jap. J. Parasit., Vol. 25, pp. 5-7.
159. Sakaguchi, Y. and Wakako, Y. 1976. A further chromosome study of the common liver fluke (*Fasciola* sp.) in Japan. Chrom. Inf. Serv., Vol. 20, pp. 25-26.
160. Sakaguchi, Y.; Tada, I. 1980. Karyotypic studies of lung flukes, *Paragonimus iloktsuenensis*, *Paragonimus sadoensis* and *Paragonimus westermani*. Jap. J. Parasitol., Vol. 29, pp. 251-257.
161. Sakaguchi, Y.; Yoneda, W. 1976. A further chromosome study of the common liver fluke (*Fasciola* sp.) in Japan. CIS., Vol. 20, pp. 25-26.
162. Saksena, J. N. 1962. Chromosome studies in the digenetic trematodes of the family Paramphistomatidae. Proc. Nat. Acad. Sci, India. Vol. 32, pp. 177-184.
163. Saksena, J. N. 1969. Chromosome studies of fifteen species of Indian digenetic trematodes. Proc. Nat. Acad. Sci. India. Vol. 39(1-4), pp. 81-110.
164. Sanderson, A. R. 1959. Maturation and fertilization in two digenetic trematodes, *Haplometra cylindracea* (Zeder, 1800) and Fasciola hepatica (L.). Proc. Roy. Soc. Edinburg., Vol. 67, pp. 83-98.
165. Scharma, G. P. and Nakahasi, V. 1974. Studies on the chromosomes of three species of the Indian digenetic trematodes of three species of the Indian digenetic trematodes. Proc. 3rd Intern. Congr. Parasitol. Munch., August 25-31. Vol. 1, pp. 378.
166. Schellenberg, A. 1911. Oogenese, Eireifung und Befruchtung von *Fasciola hepatica* L., Arch. f. Zellf., Vol. 6, pp. 443-484.
167. Schockaert, R. 1905. La Fecondation et la Segmentation chez le *Thysanozoon brocchii*. Cellule, Vol. 22, pp. 7-37.
168. Schreiner, A. 1908. JVidenskabs. Selskabets Schrifter. I. Math-Naturw., Vol. 8, pp. 1-28.
169. Sey, O. 1971. Gametogenesis in *Paramphistomum microbothrium* Faschoeder, 1901. Acta Vet. Hung., Vol. 21, pp. 93-106.
170. Sharma, A. K. and Lal, S. S. 1984. Oogenesis and the chromosomes of *Paramphistomum explanatum* (Digenea: Trematoda). C I S. Vol. 36, pp. 14-15.
171. Sharma, G. P. and Nakhasi, V. 1974. Studies on the chromosomes of three species of the Indian Digenetic trematodes. 3rd Intern. Congr. Parasitol. Miunchen, August 25—31. Proceed. N 1. P. pp. 376.
172. Sharma, G. P.; Mittal, O. P. and Nadhubala, P. 1968. Chromosome studies in the family Paramphistomidae (Digenea-Trematoda). Proc. 55th Ind. Sci. Cong., Part II, Abstracts. pp. 472-473.
173. Short and Menzel, M. Y. 1957. Chromosomes of *Schistosoma mansoni* and *Ornithobilharzia*

© 2016 Global Journals Inc. (US)

canaliculata (Trematoda: Schistosomatidae). Assoc. South. Biol. Bull., Vol. 4, pp. 15.
174. Short, R. B. 1957. Chromosomes and sex in *Schistosomatium douthitti* (Trematoda: Schistosomatidae). J. Heredity, Vol. 48, pp. 2-6.
175. Short, R. B. 1983. Presidential address. J. Parasitol., Vol. 69(1), pp. 3-22.
176. Short, R. B. and Grossman, A. I. 1981. Conventional Giemsa and C-banded karyotypes of *Schistosoma mnonsoni* and *Schistosoma rodhaini*. Journal of Parasitology, Vol. 67, pp. 66l-671.
177. Short, R. B. and Grossman, A. I. 1986. Chromosomes of *Heterobilharzia americana* (Digenea: Schistosomatidae) from Texas. J. Parasitol., Vol. 72, pp. 807-809.
178. Short, R. B. and Menzel, M. Y. 1960. Chromosomes of nine species of *Schistosomes*. Journal of Parasitology, Vol. 4, pp. 213-287.
179. Short, R. B.; Menzel, M. V. and Pathak, S. 1979. Somatic chromosomes of *Schistosoma mansoni*. Journal of Parasitology, Vol. 65, pp. 471-473.
180. Short, R. B.; Teehan, W. H. and Liberatos, J. D. 1987. Chromosomes of *Heterobilharzia americana* (Digenea: Schgaria), with ZWA sex determination, from Louisiana. Journal of Parasitology, Vol. 73(5), pp. 941-946.
181. Short, R. B. and Menzel, M. Y. 1959. Chromosomes in parthenogenetic miracidia and embryonic cercariae of *Schistosomatium douthitti*. Exper. Parasitol., Vol. 8, pp. 249-264.
182. Short, R. B. 1957. Chromosomes and sex in *Schistosomatium douthitti*. J . Hered., Vol. 48, pp. 2-6.
183. Spakulova, M. and Kraova, I. 1991. Chromosomes of *Fasciola hepatica* (Digenea: Fasciolidae) from western Bohemia (CSFR). Helminthologia, Vol. 28(4), pp. 197-200.
184. Spakulova, M.; Horak, P. and Kralova, I. 1996. Karyotype of an avian *Schistosome Trichobilharzia* szidati (Digenea: Schistosomatidae). International Journal for Pararasitology, Vol. 26(7), pp. 783-785.
185. Srimuzipo, P.; Komalamisra, C.; Choochote, W.; Jipakdi, A.; Vanichthanakom, P.; Keha, P.; Riyong, D.; Sukontason, K.; Komalamisra, N.; Sukontason, K. and Tippawangkosol, P. 2000. Comparative morphometry, morphology of egg and adult surface topography under light and scanning electron microscopies, and metaphase karyotype among three size-races of *Fasciola gigantica* in Thailand. Southeast Asian J. Trop. Med. Publ. Health, Vol. 31(2), pp. 366-373.
186. Srivastava, M. D. L. and Iha, A. G. 1964a. Structure and behaviour of the chromosomes of *Paramphistomum crassum* Stiles and Goldberger (Trematoda, Digenea). Proc. Nat. Acad. Sci India Ann., N 126.
187. Srivastava, M. D. L. and Iha, A. G. 1964b. Structure and behaviour of the chromosomes of *Isoparorchis hypselobargi* Billet, (Trematoda, Digenea, Hemiuridae). Proc. Nat. Acad. Sci. India, N 126.
188. Subramanyam, S. and Venkat Reddy, P. 1977. The role of chromosomes in the taxonomy of some digenetic trematodes. The Nucleus, Vol. 20(1 and 2), pp. 128-138.
189. Sugiyama, H.; Okuda, M.; Matsumoto, M.; Kikuchi, T.; Odagiri, Y. and Tomimura, T. 1985. Karyotypic findings of the lung fluke, *Paragonimus westermani* (Kerbert, 1878), in the Uda Area of Nara Prefecture, Japan. Jap. J. Vet. Sci., Vol. 47, pp. 889-893.
190. Taft, S. J. and Le Grande, W. H. 1979. Chromosomes of *Cyclocoelum oculeum* Trematoda Cyclocoelidae. Journal of Parasitology, Vol. 65(4), pp. 666-667
191. Teehan, W. H. and Short, R. B. 1989. Mitotic chromosomes of a species of Spirorchis (Trematoda: Spirorchiidae). J. Parasitol. Vol. 73, pp. 474-476.
192. Terasaki, K. 1977. Studies on chromosomes of the lung fluke in Japan. Jap. J. Parasitol., Vol. 26, pp. 222-229.
193. Terasaki, K. 1978. Chromosome analysis on a South American lung fluke, *Paragonimus peruvianus*. Jap. J. Parasitol., Vol. 27(1), pp. 51—55.
194. Terasaki, K. 1980. Comparative studies on the karyotypes of *Paragonimus westermani* and *Paragonimus pulmonalis* Japan. J. Parasitol., Vol. 29, pp. 239-243.
195. Terasaki, K. 1983. Karyotype of lung fluke, *Paragonimus westermani* filipinus Miyazaki. Jap. J. of Veter. Sci., Vol. 45(1), pp. 9-14.
196. Terasaki, K.; Moriyama, N.; Tani, S. and Ishida, K. 1982. Comparative studies on the karyotypes of *Echinostoma cinetorchis* and hortense (Echinostomatidae: Trematoda). Jap. J. Parasitol., Vol. 31(6), pp. 569-574.
197. VanDer Woude, A. 1954. Germ cell cycle of *Megalodiscus temperatus* (Stafford, 1905) Harwood, 1932. (Paramphistomidae: Trematoda). Amer. Midland. Naturalist, Vol. 51, pp. 172-202.
198. Venkat Reddy, P. and Subramanyam, S. 1971. Some observation in the digenetic trematode *Philophthalmus* sp. from eagle. Curr . Sci., Vol. 40, pp. 578-580.
199. Venkat Reddy, P. and Subramanyam, S. 1973. Chromosome studies in the liver fluke, *Fasciola gigantica* Cobbold, 1856, from Andhra Pradesh. Curr. Sci., Vol. 42, pp. 288-291.
200. Venkat Reddy, P. and Subramanyam, S. 1975. Chromosomes studies in *Paramphistomum cervi* Zeder 1970 (Trematoda: Digenea – Paramphistomatidae). Caryologia, Vol. 28, pp. 181-186.
201. Venkat Reddy, P., Subramanyam, S. 1975b. Chromosome number of the paramphistome

© 2016 Global Journals Inc. (US)

Gigantocotyle explanatum Nasmark, 1937. Cur. Sci. (India). Vol. 44(11), pp. 400-401.
202. Von Kemnitz, G. A. 1913. Eibildung, Eireifung, Samenreifung und Befruchtung von *Brachyocoelium salamandrae* (Brachyocoelium crassicolle Rud). Arch. f. Zellf., Vol. 10, pp. 470-506.
203. Wassermann 1913. Die Oogenese des *Zoogonus minis* Lss. Arch. f. Mik. Anat., Vol. 83, pp. 1-140.
204. White, M. J. D. 1973. The chromosomes. 6th ed. Chapman and Hall, London.
205. Willey, C. H. and Godman, G. C. 1951. Gametogenesis, fertilization and cleavage in the trematoda *Zygocotyle lunata* (Paramphistomidae). J. Parasitol., Vol. 37(3), pp. 283-296.
206. Willmott, S. 1950. Gametogenesis and early development in *Gigantocotyle bathycotyle* (Fischoeder, 1901) Nasmark, 1937. J. Helminth., Vol. 24, pp. 1-14.
207. Woodhead, A. E. 1931. The germ cell cycle in the trematode family Bucephalidae. Trans. Amer. micr. Soc., Vol. 50, pp. 169-188.
208. Woodhead, A. E. 1957. Germ-cell development in the first and second generations of *Schistosomatium douthitti* (Cort, 1914) Price, 1931. Trans. Am. Micr. Soc., Vol. 76, pp. 173-176.
209. Yin, H. Z and Ye, B. Y. 1990. Studies on the karyotypes of *Fasciola* spp. J. Parasitol. Parasitic. Diseases (Shanghai), Vol. 8, pp. 124-126.
210. Zadesenets, K. S.; Katokhin, A. V.; Mordvinov, V. A. and Rubtsov, N. B. 2012. Comparative cytogenetics of Opisthorchid species (Trematoda, Opisthorchiidae). Parasitology International, Vol. 61(1), pp. 81-83.

© 2016 Global Journals Inc. (US)

This page is intentionally left blank

© 2016 Global Journals Inc. (US)

Global Journal of Medical Research: G
Veterinary Science and Veterinary Medicine
Volume 16 Issue 1 Version 1.0 Year 2016
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Survey on the Status of Abandoned Animals in Seoul City, 2013

By Bo Ram Hwang, Young Ah Lee, Hyung Joon Kim, Yoon Ju Cho, Hyunjhung Jhun, Sang Cheol Kim, Jin Seon Bae & Jin Soo Han

College of Veterinary Medicine, Konkuk University, Korea

Abstract- In Seoul City, the rate of households with a cat or dog was 17.9%, and the number of households with a companion animal was estimated to 3,590,000 in 2013. But, In Seoul City, more than 13,000 animals are abandoned every year.

In this study, the occurrence, monthly population change, breed, sex, age, and health condition of abandoned dogs and cats in each district of Seoul City in 2013 were surveyed based on data obtained from the website of the Animal Protection Management System, Animal and Plant Quarantine Agency (www.animal.go.kr).

In 2013, out of 11,320 abandoned animals in total, 7,772 (68.66%) were dogs and 3,548 (31.34%) were cats. Regarding dogs, 5,450 (70.12%) were purebred and 2,322 (29.88%) were crossbred; 5,279 (67.92%) were in normal health condition and 2,493 (32.08%) were in abnormal health condition; and 3,473 (44.69%) were 0-2 years old.

Keywords: abandoned animal population; animal protection law; animal registration system; APMS; TNR.

GJMR-G Classification : NLMC Code: QW 70

Strictly as per the compliance and regulations of:

© 2016. Bo Ram Hwang, Young Ah Lee, Hyung Joon Kim, Yoon Ju Cho, Hyunjhung Jhun, Sang Cheol Kim, Jin Seon Bae & Jin Soo Han. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/licenses/by-nc/3.0/), permitting all non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Survey on the Status of Abandoned Animals in Seoul City, 2013

Bo Ram Hwang [α], Young Ah Lee [σ], Hyung Joon Kim [ρ], Yoon Ju Cho [ω], Hyunjhung Jhun [¥], Sang Cheol Kim [§], Jin Seon Bae [χ] & Jin Soo Han [v]

Abstract- In Seoul City, the rate of households with a cat or dog was 17.9%, and the number of households with a companion animal was estimated to 3,590,000 in 2013. But, In Seoul City, more than 13,000 animals are abandoned every year.

In this study, the occurrence, monthly population change, breed, sex, age, and health condition of abandoned dogs and cats in each district of Seoul City in 2013 were surveyed based on data obtained from the website of the Animal Protection Management System, Animal and Plant Quarantine Agency (www.animal.go.kr).

In 2013, out of 11,320 abandoned animals in total, 7,772 (68.66%) were dogs and 3,548 (31.34%) were cats. Regarding dogs, 5,450 (70.12%) were purebred and 2,322 (29.88%) were crossbred; 5,279 (67.92%) were in normal health condition and 2,493 (32.08%) were in abnormal health condition; and 3,473 (44.69%) were 0-2 years old.

Regarding cats, 3,121 (87.97%) were Korean Shorthair, 308 (8.68%) were purebred, and 119 (3.35%) were crossbred; 3,325 (93.71%) were 0-2 years old, while 2,375 (71.43%) were 0-3 months old.

Results of this study will be used to effectively tackle the problem of abandoned animals in Seoul City and hence in Korea.

Keywords: abandoned animal population; animal protection law; animal registration system; APMS; TNR.

I. Introduction

In the past few year, the standard of living increased in Korea along with the number of one-person households, which led to an increase in the number of companion animals that attracted increasingly more public attention (Korean Statistical Information Service, http://kosis.kr/). In Seoul City, the rate of households with a cat or dog was 17.9%, and the number of households with a companion animal was estimated to 3,590,000 in 2013. In the same year, the number of companion dogs was 4,400,000 and of companion cats was 1,160,000 in Korea (Korea Social Economic Institute 2012, unpublished data). Based on a survey of 1000 households in Seoul City, it was estimated that approximately 640,000 households (1 to 6) had a cat or dog. When we included the households that were willing to raise a companion animal, this number climbed to 800,000 households (1 in 5) [1].

In 2013, the total number of abandoned animals was 97,197 in Seoul City, of which, 62,119 (63.9%) were dogs and 34,103 (35.1%) were cats, and the cost to manage abandoned animals climbed to 1 billion Korean Won (KW) per year. Lately, conflicts between residents, who have companion animals and those who do not, are very frequent. Additionally, intentional abandonment of animals is an increasing phenomenon in Korea and worldwide [2, 3, 4, 5, 6, 7].

Abandoned animals raise social issues, because of the frequent incidents of infectious diseases, such as dog ascaris (Toxacara canis) and rabies [8, 9, 10], which are transmissible to humans from bite injuries by abandoned animals, or contaminated drinking water from the dead bodies of abandoned animals. Moreover, necessary financial and human resources have been continuously increasing, since the implementation of the Animal Protection Law, which regulates the management of abandoned animals from capture to care.

According to the Animal Protection Law, the abandonment of an animal can be reported to a district office. District officers go to the place of abandonment, capture the animal, take it to a shelter within the district, and place an announcement for at least 7 days, in order the owner to get informed and be able to take the animal back (Animal Care Management System, http://www.animal.go.kr). If the owner does not appear within 10 days from the day of the announcement, the District Government takes ownership of the animal and a post-management plan is applied, which includes adoption, natural death, or euthanasia. By 2013, 19 out of 25 District Governments in Seoul City had made trust agreements with the Korean Animal Rescue and Management Association (KARMA) and 6 District Governments had entrusted local veterinary clinics and the Veterinary Association with the care of abandoned animals (Seoul City Metropolitan Animal Protection 2013, unpublished data).

The rate of abandoned dogs and cats had been increasing in Korea, thus in 2004 a rescue program for

Author α ρ v: Department of Laboratory Animal Medicine & Institute for the 3Rs, College of Veterinary Medicine, Konkuk University, Seoul, 143-701, Korea. e-mail: labvet@konkuk.ac.kr
Author σ §: Department of Animal Sciences, Shingu College, Seongnam 462-743, Korea. e-mail: mingcabbage@hanmail.net
Author ω: 3Department of Pet Science, Seojeong College, Yangju 482-777, Korea. e-mail: vet-cho@hanmail.net
Author ¥: Department of Laboratory Animal Research Center, Konkuk University, Seoul, 143-701, Korea. e-mail: vetian@hanmail.net
Author χ: Department of Animal Protection, Seoul Metropolitan Government, Seoul, 100-744, Korea. e-mail: jinsbae1@seoul.go.kr

© 2016 Global Journals Inc. (US)

abandoned animals was implemented in 25 districts of Seoul City. The initial rate of abandoned animals was estimated at 13,000 per year, which increased significantly in 2010 at 18,624 and thereafter it decreased slowly at 15,229 in 2011 and at 13,556 in 2012 (Seoul City Metropolitan Animal Protection 2013, unpublished data).

Approximately 50% of the abandonments occur due to the problematic animal behavior, such as loud barking, improper urination, and excessive behaviors that offend their owners and destroy emotional bonds between humans and animals [11]. Nevertheless, the problem goes even deeper; many people buy companion animals imprudently just for their attractive look, do not train them and abandon them, when their behavior becomes problematic or their care is harder than expected, especially within a residential environment. Sympathy and responsibility for animals are two essential tools to tackle the problem of abandonment [12].

The objective of this study was to inform the public and to obtain necessary data for developing a policy to manage abandoned animals. We conducted a survey and data analysis on the occurrence, monthly population change, breed, sex, age, and health condition (normal or abnormal) of abandoned dogs and cats that were rescued and managed by KARMA, local veterinary clinics and the Veterinary Association in each district.

II. Methods

In this study, the occurrence, monthly population change, breed, sex, age, and health condition (normal or abnormal) of abandoned dogs and cats in each district of Seoul City in 2013 were surveyed based on data obtained from the website of the Animal Protection Management System, Animal and Plant Quarantine Agency (www.animal.go.kr). Health condition was divided into two categories, normal and abnormal. As abnormal was characterized any irreversible or critical condition, such as serious external injury (fracture or bleeding), virus infection, skin disease or when the animal was moribund.

III. Results

In 2013, out of 11,320 abandoned animals in total, 7,772 (68.66%) were dogs and 3,548 (31.34%) were cats. The occurrence of abandoned cats and dogs in each district was also analyzed and results are shown in Figure 1. The districts that had the highest occurrence of abandoned dogs were Gwanak-gu (528 dogs), Yangcheon-gu (460 dogs), and Eunpyeong-gu (448 dogs), while abandoned cats appeared more frequently in Yongsan-gu (606 cats), Mapo-gu (463 cats), and Gwanak-gu (412 cats). On the contrary, the districts that had the lowest occurrence of abandoned dogs were Jongno-gu (145 dogs), Jung-gu (148 dogs), and Seongdong-gu (192 dogs), while abandoned cats appeared less frequently in Gangdong-gu (17 cats), Dongjak-gu (19 cats), and Yangcheon-gu (28 cats).

The occurrence of abandoned cats and dogs per 1,000 households in each district was also analyzed and results are shown in Figure 2. The districts that had the highest occurrence of abandoned dogs per 1,000 households were Yongsan-gu (4.61 dogs), Gangbuk (3.43 dogs), and Jung-gu (3.24 dogs), while abandoned cats appeared more frequently per 1,000 households in Yongsan-gu (7.01 cats), Mapo-gu (3.27 cats), and Jung-gu (3.11 cats).). On the contrary, the districts that had the lowest occurrence of abandoned dogs per 1,000 households were Gangnam-gu (0.98 dogs), Songpa-gu (1.49 dogs), and Nowon-gu (1.53 dogs), while abandoned cats appeared less frequently per 1,000 households in Gangdong-gu (0.11 cats), Dongjak-gu (0.13 cats), and Yangcheon-gu (0.18 cats).

According to the Animal Protection Law and post-management plan of abandoned animals, out of 7,772 abandoned dogs 3,428 (44.11%) were euthanized, 2,044 (26.30%) were returned to their owners, 1679 (21.60%) were adopted, and 431 (5.80%) died naturally (Figure 3). The districts that had a higher rate of adoption compare to that of euthanasia were Gwanak-gu, Mapo-gu, and Yongsan-gu (Figure 4). Additionally, out of 3,351 abandoned cats, 837 (23.59%) were euthanized, 60 (1.69%) were returned to their owners, 897 (25.28%) were adopted, and 1,557 (43.88%) died naturally (Figure 3). The districts that had a higher rate of adoption compared to the rate of natural death were Gwanak-gu, Yongsan-gu, Dongjak-gu, Yangcheon-gu, and Gangnam-gu (Figure 5). It is noteworthy that the rate of dogs that returned to their owners was higher by 24.61% that that of cats (Figure 3).

The monthly occurrence of abandoned cats and dogs was analyzed and results are shown in Figure 6. The months that had the highest occurrence of abandoned dogs were August (11.34%), July (10.87%), and May (10.36%), abandoned cats appeared more frequently in May (16.91%), June (14.88%), and July (14.15%).

A summary of data on breed, sex and health condition of abandoned dogs are presented in Table 1. Out of 7,772 abandoned dogs, 5,450 (70.12%) were purebreds and 2,322 (29.88%) were crossbreds took up of 7,772 abandoned dogs, while 3,446 (44.34%) were female and 4,326 (55.66%) were male. The number of male dogs was higher than that of female dogs. Normal dogs were 5,279 (67.92%) and abnormal dogs were 2,493 (32.08%). The number of normal dogs was double than that of abnormal dogs. Regarding the age of abandoned dogs, 3,473 (44.69%) were 0-2 years old, 1,999 (25.72%) were 3-5 years old, 1,214 (15.62%) were 6-8 years old, and 1086 (13.97%) were older than 9

© 2016 Global Journals Inc. (US)

years. Results showed that younger dogs were more likely to be abandoned than older dogs.

Out of 5,450 purebred dogs, 4,252 (78.02%) were small-sized dogs were, 1021 (18.73%) were middle-sized dogs, and 177 (3.25%) were big-sized dog. Out of 4,252 small-sized dogs, 1,454 (26.68%) were Maltese, 888 (16.29%) were Shih Tzu, 724 (13.28%) were Poodle, and 598 (10.97%) were Yorkshire Terrier (Table 2).

A summary of data on breed, sex and health condition of abandoned cats are presented in Table 3. Out of 3,548 abandoned cats, 308 (8.68%) were purebreds, 119 (3.35%) were crossbreds, and 3,121 (87.97%) were Korean Shorthair, while 1,748 (49.27%) were female and 1800 (50.73%) were male. The number of female cats did not differ significantly from the number of male cats. Normal cats were 1,990 (56.09%) and abnormal were 1,558 (43.91%). The number of normal cats was higher than that of abnormal cats. Regarding the age of abandoned cats, 3,325 (93.71%) were 0-2 years old, 183 (5.16%) were 3-5 years old, 38 (1.07%) were 6-8 years old and 2 (0.06%) were older than 9 years. Results showed that the majority of abandoned cats were infant cats. Out of 308 purebred cats, 126 (40.91%) were Persian and 88 (28.57%) were Turkish Angora (Table 4).

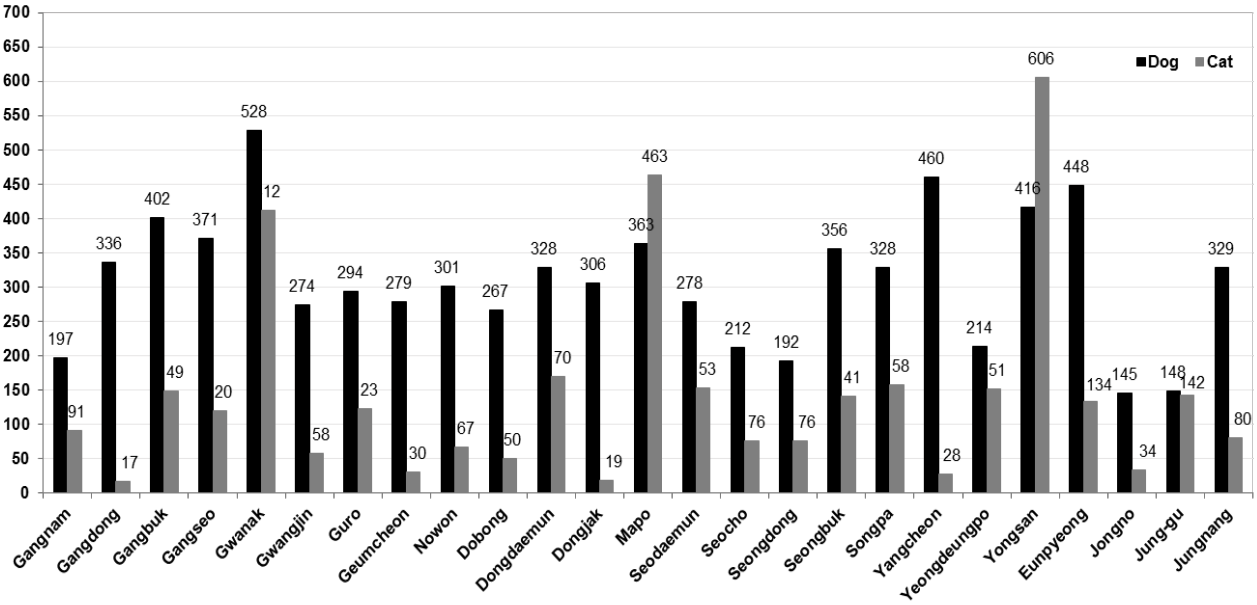


Figure 1 : Occurrence of abandoned dogs and cats in 25 districts of Seoul City in 2013

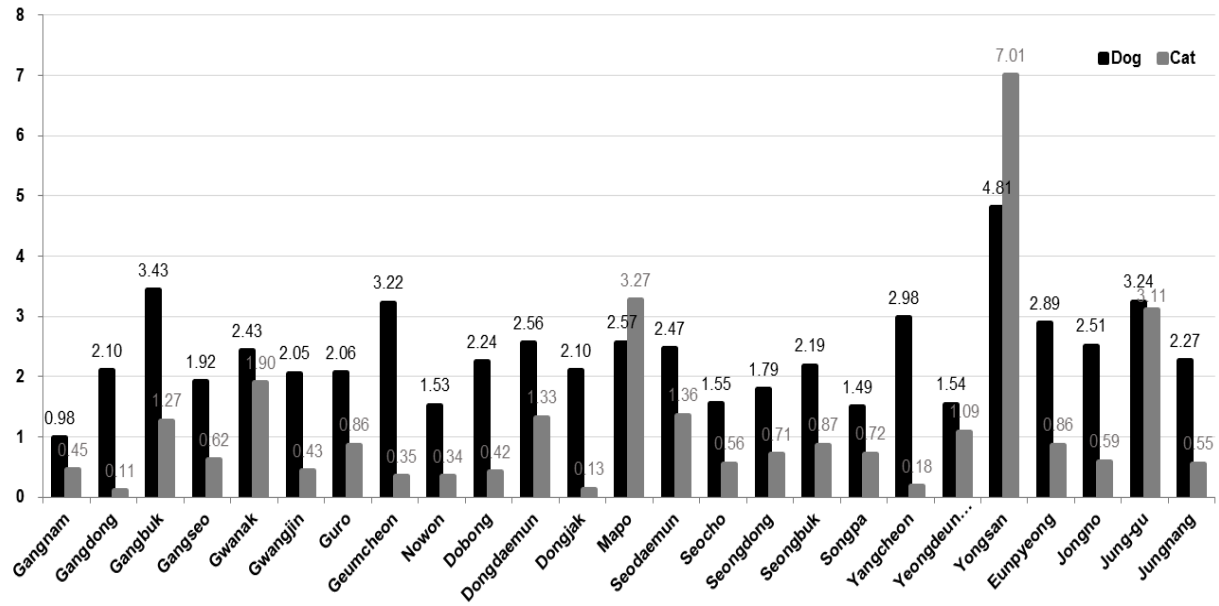


Figure 2 : Occurrence of abandoned animals dogs and cats per 1000 households in 25 districts of Seoul City in 2013

© 2016 Global Journals Inc. (US)

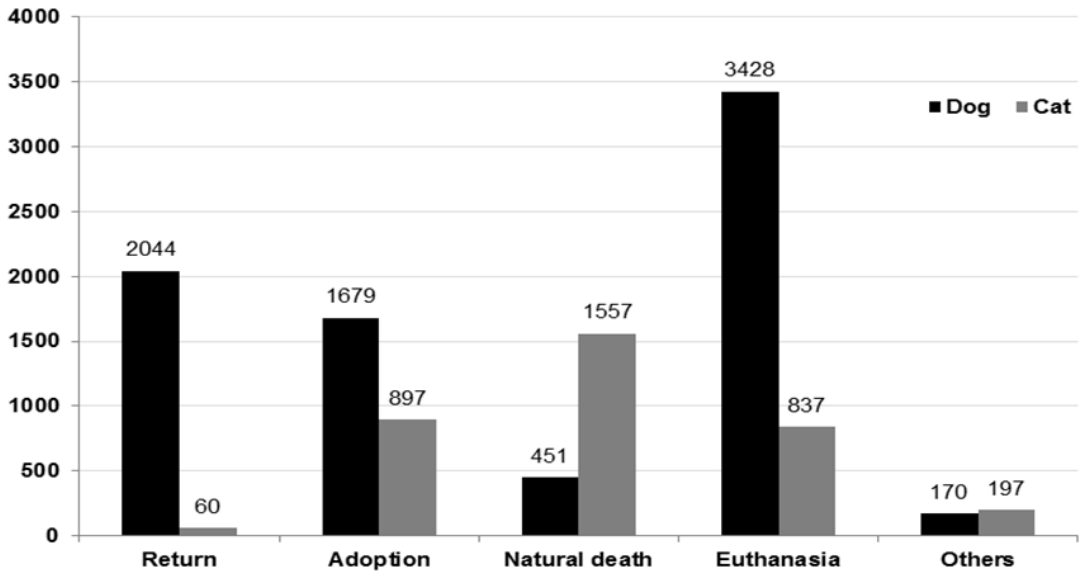


Figure 3 : Management of abandoned dogs and cats in 25 districts of Seoul City in 2013

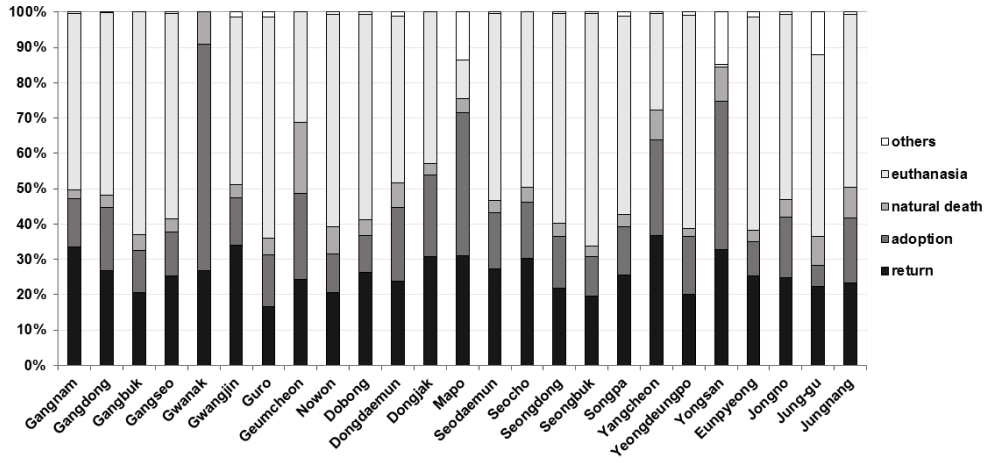


Figure 4 : Management of abandoned dogs in each of 25 districts of Seoul City in 2013

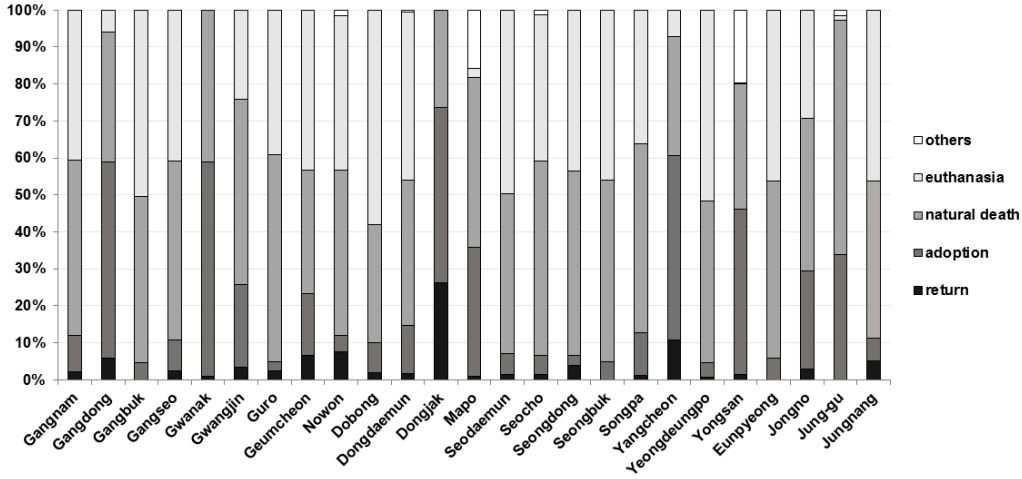


Figure 5 : Management of abandoned cats in each of 25 districts of Seoul City in 2013

© 2016 Global Journals Inc. (US)

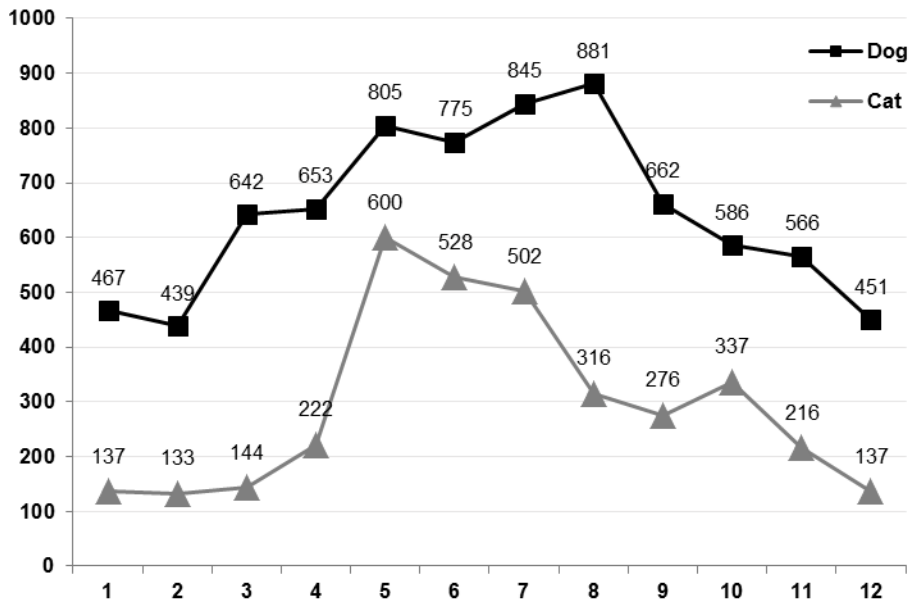


Figure 6 : Monthly occurrence of abandoned dogs and cats in 25 districts of Seoul City in 2013

Table 1 : Data summary (number and frequency) on breed, sex and health condition of abandoned dogs in 25 districts of Seoul City in 2013

	Breed		Sex		Physical Condition observed		Age(year)			
	Pure	Cross	Female	Male	normal	abnormal	0-2	3-5	6-8	over 9
(No.)	5,450	2,322	3,446	4,326	5,279	2,493	3,473	1,999	1,214	1,086
(%)	70.12	29.88	44.34	55.66	67.92	32.08	44.69	25.72	15.62	13.97

Table 2 : Data summary (number and frequency) on breeds and sizes of abandoned dogs in 25 districts of Seoul City in 2013

Small dog (< 10kg)			Middle dog (< 25kg)			Big dog (> 25kg)		
Breed	No. (%)		Breed	No. (%)		Breed	No. (%)	
Maltese	**1,454**	**(26.68)**	Jindo	264	(4.84)	Golden Retriever	46	(0.84)
Shih Tzu	**888**	**(16.29)**	Schnauzer	254	(4.66)	Siberian Husky	31	(0.57)
Poodle (Toy)	**724**	**(13.28)**	Coker Spaniel	220	(4.04)	Alaskan Malamute	28	(0.51)
Yorkshire Terrier	**598**	**(10.97)**	Spitz	125	(2.29)	Samoyed	18	(0.33)
Miniature Pinscher	156	(2.86)	Beagle	77	(1.41)	Labrador Retriever	10	(0.18)
Pomeranian	151	(2.77)	Welsh Corgi	19	(0.35)	Great Pyrenees	7	(0.13)
Pekingese	94	(1.72)	Bulldog	14	(0.26)	German Shepherd	6	(0.11)
Dachshund	78	(1.43)	Shar-pei	9	(0.17)	Collie	6	(0.11)
Chihuahua	68	(1.25)	Shetland Sheepdog	7	(0.24)	Pointer	5	(0.09)
Others	41	(0.75)	Others	32	(0.94)	Others	20	(0.17)
Subtotal	**4,252**	**(78.02)**	**Subtotal**	**1021**	**(18.73)**	**Subtotal**	**177**	**(3.25)**
						Total No. (%)	**5,450**	**(100)**

- **Standard for FCI (Federation Cynologique Internationale)*

© 2016 Global Journals Inc. (US)

Table 3 : Data summary (number and frequency) on breed, sex and health condition of abandoned cats in 25 districts of Seoul City in 2013

	Breed			Sex		Physical Condition observed		Age(year)			
	Pure	Cross	Korean short hair	Female	Male	normal	abnormal	0-2	3-5	6-8	over 9
(No.)	308	119	3,121	1,748	1,800	1,990	1,558	3,325	183	38	2
(%)	8.68	3.35	87.97	49.27	50.73	56.09	43.91	93.71	5.16	1.07	0.06

Table 4 : Data summary (number and frequency) on breeds and sizes of abandoned cats in 25 districts of Seoul City in 2013

Breed	No. (%)	
Persian	126	(40.91)
Turkish Angora	88	(28.57)
Russian Blue	39	(12.66)
Siamese	29	(9.42)
Scottish Fold	8	(2.60)
American Shorthair	7	(2.27)
Abyssinian	5	(1.62)
Norwegian Forest	2	(0.65)
Others	4	(1.30)
Total	308	(100)

Others; American Curl, Balinese, Bengal, Ragdoll

IV. Discussion

The objective of this study was to obtain necessary data for effectively tackling the problem of abandoned animals in Seoul City. The occurrence, monthly population change, breed, sex, age, and health condition (normal or abnormal) of abandoned dogs and cats in each district of Seoul City in 2013 were surveyed based on data obtained from the website of the Animal Protection Management System, Animal and Plant Quarantine Agency (www.animal.go.kr). Overall, out of 11,320 abandoned animals, 7,772 (68.66%) were dogs and 3,548 (31.34%) were cats.

In 19 districts abandoned animals were rescued and managed by KARMA and in 6 districts (Gwanak-gu, Geumcheon-gu, Dongjak-gu, Mapo-gu, Yangcheon-gu, and Yongsan-gu) by local veterinary clinics and the local Veterinary Association. In these 6 districts, the occurrence of abandoned animals was higher, but at the same the rate of adoption was also higher, while the rates of euthanasia or of natural death were lower than in the other 19 districts, which shows that management of abandoned animals was more effective.

Out of 7,772 abandoned dogs, 4,252 (78.02%) were purebred, small-sized dogs, probably because they are more suitable for residential environment such as apartments or villas. Abandoned purebred dogs were Maltese, Shih Tzu, Poodle, Yorkshire Terrier, and Miniature Schnauzer, breeds that constitute 70% (210,000 dogs) of the total companion dog sales in Korea (Korea Industry and Economy Laboratory 2006).

Out of 7,772 abandoned dogs, 3,446 (44.34%) were female and 4,326 (55.66%) were male. The number of male dogs was higher than that of female dogs; because it is harder to toilet-train male dogs than female dogs, and also they are more loud and wander around in heat, characteristics that annoy both the owners and their neighbors [13].

Data showed that 5,279 (67.92%) dogs were normal, 2,385 (30.69%) dogs were under 1 year old, and most of them were abandoned in July and August. These results showed that dogs were abandoned during the summer vacation period, not because they were unhealthy, but possibly because their care was harder than expected.

Out of 3,548 abandoned cats, 308 (8.68%) were purebreds, 119 (3.35%) were crossbreds, and 3,121 (87.97%) were Korean Shorthair. In Korea, the rate of purebred cats has been increasing, but Korean Shorthair is still the most popular breed [14]. It is generally difficult to distinguish abandoned and feral Korean Shorthair, because the latter breed naturally and

© 2016 Global Journals Inc. (US)

live independently in Seoul City. It is noteworthy that the rate of dogs that returned to their owners was higher by 24.61% that that of cats, which may suggest that most of Korean Shorthair were feral and not abandoned cats. Abandoned cats under 3 months old were 2,375 (71.43%) and cats that died naturally were 1557 (43.88%). March, May and June are the major reproduction moths in cats [15], so this explains the reason that Korean Shorthair occurred most often in May (16.91%), June (14.88%), and July (14.15%), and also the high probability of feral infant cats (except of those subjected to TNR) to be classified and managed as abandoned cats.

According to the Animal Protection Law (Article 13), cats that breed naturally and live independently are not subjects of protection, but of TNR to control their population and are excluded from rescue and care. But as a result feral cats are still treated as abandoned animals.

Animal registration system has been implemented in 53 cities and counties since 2008 and 195,808 dogs were registered until 2011. As a result, the rate of abandoned animals that returned to their owners increased, return time shorted and the occurrence of abandoned dogs decreased. Animal registration system has been extended nationwide since January 1, 2013. Out of 1,273,563 dogs, 479,147 have been registered since then and the rate of registration has been continuously increasing.

In Taipei, Taiwan, the Bureau of Animal Protection tightened up animal care education and Federal Government supported neutralization of animals. As a result, 70% of companion animals were registered and the occurrence of abandoned animals decreased sharply (Taipei City Animal Protection Office, Ministry of Health and Welfare 2013, unpublished data).

In Tokyo, Japan, dog registration system has been implemented since 1985. As a result, more than 50% of dogs have been registered, and the occurrence of abandoned dogs decreased by 83% (Tokyo Animal Center 2012, unpublished data).

In Ottawa, Canada and New South Wales, Australia, animal registration system has been implemented both for dogs and cats (Seoul Metropolitan Government 2013, unpublished data). If animal registration system for cats is enforced, it will help to distinguish domestic cats from feral cats, manage them more effectively and decrease the occurrence of feral cats.

In Conclusion, to tackle the problem of abandoned animals, first, a new regulation of companion animal sellers and owners should be included in the Animal Protection Law to strengthen their responsibility; second, education programs on animal rights should be implemented; third, potential owners should be informed on the basic physiological and ethological characteristics of companion animals before hey adopt them. Additionally, an effective management system should be imposed on every phase of animal production, distribution, and sale.

Abandoned animal management can be improved, if new animal care centers are developed and managed directly by Seoul City Metropolitan Government as happens in Taipei and Tokyo, and also volunteer work is encouraged for a more transparent and effective management. We suggest that new convenient animal care centers should be constructed, a positive animal protection policy should be established and education on animal rights should be promoted.

This is the first report that surveyed the characteristics of abandoned animals that rescued and managed by Seoul City Metropolitan Government, and results can be used to effectively tackle the problem of abandoned animals in Seoul City and hence in Korea.

V. Acknowledgments

This research was supported by Seoul Metropolitan Government, Korea in 2013

References Références Referencias

1. Yoo, K. Y., Cho, S. H., Gin, Y., & Lee, Y. J. (2004). Strategic guidelines to protect and manage pet animals in Seoul. Seoul Development Institute.
2. Carter, C. N. (1990). Pet population control: Another decade without solutions?. Journal of the American Veterinary Medical Association (USA).
3. Gibson, K. L., Keizer, K., & Golding, C. (2002). A trap, neuter, and release program for feral cats on Prince Edward Island. The Canadian Veterinary Journal, 43(9), 695.
4. Levy, J. K., Gale, D. W., & Gale, L. A. (2003). Evaluation of the effect of a long-term trap-neuter-return and adoption program on a free-roaming cat population. Journal of the American Veterinary Medical Association, 222(1), 42-46.
5. Rowan, A. N. (1992). Shelters and pet overpopulation: A statistical black hole. Anthrozoös, 5(3), 140-143.
6. Wallace, J. L., & Levy, J. K. (2006). Population characteristics of feral cats admitted to seven trap-neuter-return programs in the United States. Journal of Feline Medicine and Surgery, 8(4), 279-284.
7. Zawistowski, S., Morris, J., Salman, M. D., & Ruch-Gallie, R. (1998). Population dynamics, overpopulation, and the welfare of companion animals: new insights on old and new data. Journal of Applied Animal Welfare Science, 1(3), 193-206.
8. Beran, G. W., & Frith, M. (1988). Domestic animal rabies control: an overview. Review of Infectious Diseases, 10(Supplement 4), S672-S677.
9. Butcher, R. (1999). Stray dogs-- a worldwide problem. The Journal of small animal practice, 40(9), 458.

© 2016 Global Journals Inc. (US)

10. Daryani, A., Sharif, M., Amouei, A., & Gholami, S. (2009). Prevalence of Toxocara canis in stray dogs, northern Iran. Pakistan Journal of Biological Sciences, 12(14), 1031.
11. Kim, Y. M., Abd El-Aty, A. M., Hwang, S. H., & Lee, J. H. (2009). Risk factors of relinquishment regarding canine behavior problems in South Korea. Weshalb geben private Hundehalter in Südkorea ihren Hund an ein Tierheim ab: Verhaltensprobleme und Risikofaktoren. Berl. Münch. Tierärztl. Wschr., 7(1-2), 1-7.
12. Voslářvá, E., & Passantino, A. (2012). Stray dog and cat laws and enforcement in Czech Republic and in Italy. Annali dell'Istituto superiore di sanitÃ, 48(1), 97-104.
13. Sung, G. C. (2010). Survey on the status of abandoned animals. Journal of Korean veterinary medical association, 42, 274-279.
14. Myung, B. Y., Gwangju Animal Shelter, G., Kim, S. E., Shim, K. M., Ryu, J. W., Kim, S. H., ... & Park, H. J. (2010). Clinical characteristics of oral diseases on stray cats in Gwangju. Korean Journal of Veterinary Research.
15. Nutter, F. B., Levine, J. F., & Stoskopf, M. K. (2004). Reproductive capacity of free-roaming domestic cats and kitten survival rate. Journal of the American Veterinary Medical Association, 225(9), 1399-1402.

© 2016 Global Journals Inc. (US)

Global Journals Inc. (US) Guidelines Handbook 2016

www.GlobalJournals.org

FELLOWS

FELLOW OF ASSOCIATION OF RESEARCH SOCIETY IN MEDICAL (FARSM)

Global Journals Incorporate (USA) is accredited by Open Association of Research Society (OARS), U.S.A and in turn, awards "FARSM" title to individuals.The'FARSM' title is accorded to a selected professional after the approval of the Editor-in-Chief/Editorial Board Members/Dean.

- The "FARSM" is a dignified title which is accorded to a person's name viz. Dr. John E. Hall,Ph.D., FARSS or William Walldroff, M.S., FARSM.

FARSM accrediting is an honor. It authenticates your research activities. After recognition as FARSM, you can add 'FARSM' title with your name as you use this recognition as additional suffix to your status. This will definitely enhance and add more value and repute to your name. You may use it on your professional Counseling Materials such as CV, Resume, and Visiting Card etc.

The following benefits can be availed by you only for next three years from the date of certification:

FARSM designated members are entitled to avail a 40% discount while publishing their research papers (of a single author) with Global Journals Incorporation (USA), if the same is accepted by Editorial Board/Peer Reviewers. If you are a main author or co-author in case of multiple authors, you will be entitled to avail discount of 10%.

Once FARSM title is accorded, the Fellow is authorized to organize a symposium/seminar/conference on behalf of Global Journal Incorporation (USA). The Fellow can also participate in conference/seminar/symposium organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent.

You may join as member of the Editorial Board of Global Journals Incorporation (USA) after successful completion of three years as Fellow and as Peer Reviewer. In addition, it is also desirable that you should organize seminar/symposium/conference at least once.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time.This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

The FARSM can go through standards of OARS. You can also play vital role if you have any suggestions so that proper amendment can take place to improve the same for the benefit of entire research community.

As FARSM, you will be given a renowned, secure and free professional email addres with 100 GB of space e.g. johnhall@globaljournals.org. This will include Webmail, Spam Assassin, Email Forwarders,Auto-Responders, Email Delivery Route tracing, etc.

The FARSM will be eligible for a free application of standardization of their researches. Standardization of research will be subject to acceptability within stipulated norms as the next step after publishing in a journal. We shall depute a team of specialized research professionals who will render their services for elevating your researches to next higher level, which is worldwide open standardization.

The FARSM member can apply for grading and certification of standards of their educational and Institutional Degrees to Open Association of Research, Society U.S.A. Once you are designated as FARSM, you may send us a scanned copy of all of you credentials. OARS will verify, grade and certify them. This will be based on your academic records, quality of research papers published by you, and some more criteria. After certification of all your credentials by OARS, they will be published on your Fellow Profile link on website https://associationofresearch.org which will be helpful to upgrade the dignity.

The FARSM members can avail the benefits of free research podcasting in Global Research Radio with their research documents. After publishing the work, (including published elsewhere worldwide with proper authorization) you can upload your research paper with your recorded voice or you can utilize chargeable services of our professional RJs to record your paper in their voice on request.

The FARSM member also entitled to get the benefits of free research podcasting of their research documents through video clips. We can also streamline your conference videos and display your slides/ online slides and online research video clips at reasonable charges, on request.

© Copyright by Global Journals Inc.(US)| Guidelines Handbook

The FARSM is eligible to earn from sales proceeds of his/her researches/reference/review Books or literature, while publishing with Global Journals. The FARSS can decide whether he/she would like to publish his/her research in a closed manner. In this case, whenever readers purchase that individual research paper for reading, maximum 60% of its profit earned as royalty by Global Journals, will be credited to his/her bank account. The entire entitled amount will be credited to his/her bank account exceeding limit of minimum fixed balance. There is no minimum time limit for collection. The FARSM member can decide its price and we can help in making the right decision.

The FARSM member is eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get remuneration of 15% of author fees, taken from the author of a respective paper. After reviewing 5 or more papers you can request to transfer the amount to your bank account.

MEMBER OF ASSOCIATION OF RESEARCH SOCIETY IN MEDICAL (MARSM)

The ' MARSM ' title is accorded to a selected professional after the approval of the Editor-in-Chief / Editorial Board Members/Dean.

The “MARSM” is a dignified ornament which is accorded to a person’s name viz. Dr. John E. Hall, Ph.D., MARSM or William Walldroff, M.S., MARSM.

MARSM accrediting is an honor. It authenticates your research activities. Afterbecoming MARSM,you can add 'MARSM' title with your name as you use this recognition as additional suffix to your status. This will definitely enhance and add more value and repute to your name. You may use it on your professional Counseling Materials such as CV, Resume, Visiting Card and Name Plate etc.

The following benefitscan be availed by you only for next three years from the date of certification.

MARSM designated members are entitled to avail a 25% discount whilepublishing their research papers (of a single author) in Global Journals Inc., if the same is accepted by our Editorial Board and Peer Reviewers. If you are a main author or co-author of a group of authors, you will get discount of 10%.

As MARSM, you willbe given a renowned, secure and free professional email address with 30 GB of space e.g. johnhall@globaljournals.org. This will include Webmail, Spam Assassin, Email Forwarders,Auto-Responders, Email Delivery Route tracing, etc.

© Copyright by Global Journals Inc.(US) | Guidelines Handbook

We shall provide you intimation regarding launching of e-version of journal of your stream time to time.This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

The MARSM member can apply for approval, grading and certification of standards of their educational and Institutional Degrees to Open Association of Research, Society U.S.A.

Once you are designated as MARSM, you may send us a scanned copy of all of your credentials. OARS will verify, grade and certify them. This will be based on your academic records, quality of research papers published by you, and some more criteria.

It is mandatory to read all terms and conditions carefully.

Auxiliary Memberships

Institutional Fellow of Open Association of Research Society (USA) - OARS (USA)

Global Journals Incorporation (USA) is accredited by Open Association of Research Society, U.S.A (OARS) and in turn, affiliates research institutions as "Institutional Fellow of Open Association of Research Society" (IFOARS).

The "FARSC" is a dignified title which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., FARSC or William Walldroff, M.S., FARSC.

The IFOARS institution is entitled to form a Board comprised of one Chairperson and three to five board members preferably from different streams. The Board will be recognized as "Institutional Board of Open Association of Research Society"-(IBOARS).

The Institute will be entitled to following benefits:

The IBOARS can initially review research papers of their institute and recommend them to publish with respective journal of Global Journals. It can also review the papers of other institutions after obtaining our consent. The second review will be done by peer reviewer of Global Journals Incorporation (USA) The Board is at liberty to appoint a peer reviewer with the approval of chairperson after consulting us.

The author fees of such paper may be waived off up to 40%.

The Global Journals Incorporation (USA) at its discretion can also refer double blind peer reviewed paper at their end to the board for the verification and to get recommendation for final stage of acceptance of publication.

The IBOARS can organize symposium/seminar/conference in their country on behalf of Global Journals Incorporation (USA)-OARS (USA). The terms and conditions can be discussed separately.

The Board can also play vital role by exploring and giving valuable suggestions regarding the Standards of "Open Association of Research Society, U.S.A (OARS)" so that proper amendment can take place for the benefit of entire research community. We shall provide details of particular standard only on receipt of request from the Board.

The board members can also join us as Individual Fellow with 40% discount on total fees applicable to Individual Fellow. They will be entitled to avail all the benefits as declared. Please visit Individual Fellow-sub menu of GlobalJournals.org to have more relevant details.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time. This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

After nomination of your institution as "Institutional Fellow" and constantly functioning successfully for one year, we can consider giving recognition to your institute to function as Regional/Zonal office on our behalf.
The board can also take up the additional allied activities for betterment after our consultation.

The following entitlements are applicable to individual Fellows:

Open Association of Research Society, U.S.A (OARS) By-laws states that an individual Fellow may use the designations as applicable, or the corresponding initials. The Credentials of individual Fellow and Associate designations signify that the individual has gained knowledge of the fundamental concepts. One is magnanimous and proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice.

Open Association of Research Society (US)/ Global Journals Incorporation (USA), as described in Corporate Statements, are educational, research publishing and professional membership organizations. Achieving our individual Fellow or Associate status is based mainly on meeting stated educational research requirements.

Disbursement of 40% Royalty earned through Global Journals : Researcher = 50%, Peer Reviewer = 37.50%, Institution = 12.50% E.g. Out of 40%, the 20% benefit should be passed on to researcher, 15 % benefit towards remuneration should be given to a reviewer and remaining 5% is to be retained by the institution.

We shall provide print version of 12 issues of any three journals [as per your requirement] out of our 38 journals worth $ 2376 USD.

Other:

The individual Fellow and Associate designations accredited by Open Association of Research Society (US) credentials signify guarantees following achievements:

- The professional accredited with Fellow honor, is entitled to various benefits viz. name, fame, honor, regular flow of income, secured bright future, social status etc.

- In addition to above, if one is single author, then entitled to 40% discount on publishing research paper and can get 10%discount if one is co-author or main author among group of authors.
- The Fellow can organize symposium/seminar/conference on behalf of Global Journals Incorporation (USA) and he/she can also attend the same organized by other institutes on behalf of Global Journals.
- The Fellow can become member of Editorial Board Member after completing 3yrs.
- The Fellow can earn 60% of sales proceeds from the sale of reference/review books/literature/publishing of research paper.
- Fellow can also join as paid peer reviewer and earn 15% remuneration of author charges and can also get an opportunity to join as member of the Editorial Board of Global Journals Incorporation (USA)
- • This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in–depth understanding of the application of suitable techniques to a particular area of research practice.

Note :

"

- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone. "

Process of submission of Research Paper

The Area or field of specialization may or may not be of any category as mentioned in 'Scope of Journal' menu of the GlobalJournals.org website. There are 37 Research Journal categorized with Six parental Journals GJCST, GJMR, GJRE, GJMBR, GJSFR, GJHSS. For Authors should prefer the mentioned categories. There are three widely used systems UDC, DDC and LCC. The details are available as 'Knowledge Abstract' at Home page. The major advantage of this coding is that, the research work will be exposed to and shared with all over the world as we are being abstracted and indexed worldwide.

The paper should be in proper format. The format can be downloaded from first page of 'Author Guideline' Menu. The Author is expected to follow the general rules as mentioned in this menu. The paper should be written in MS-Word Format (*.DOC,*.DOCX).

The Author can submit the paper either online or offline. The authors should prefer online submission.Online Submission: There are three ways to submit your paper:

(A) (I) First, register yourself using top right corner of Home page then Login. If you are already registered, then login using your username and password.

(II) Choose corresponding Journal.

(III) Click 'Submit Manuscript'. Fill required information and Upload the paper.

(B) If you are using Internet Explorer, then Direct Submission through Homepage is also available.

(C) If these two are not conveninet , and then email the paper directly to dean@globaljournals.org.

Offline Submission: Author can send the typed form of paper by Post. However, online submission should be preferred.

Preferred Author Guidelines

MANUSCRIPT STYLE INSTRUCTION (Must be strictly followed)

Page Size: 8.27" X 11'"

- Left Margin: 0.65
- Right Margin: 0.65
- Top Margin: 0.75
- Bottom Margin: 0.75
- Font type of all text should be Swis 721 Lt BT.
- Paper Title should be of Font Size 24 with one Column section.
- Author Name in Font Size of 11 with one column as of Title.
- Abstract Font size of 9 Bold, "Abstract" word in Italic Bold.
- Main Text: Font size 10 with justified two columns section
- Two Column with Equal Column with of 3.38 and Gaping of .2
- First Character must be three lines Drop capped.
- Paragraph before Spacing of 1 pt and After of 0 pt.
- Line Spacing of 1 pt
- Large Images must be in One Column
- Numbering of First Main Headings (Heading 1) must be in Roman Letters, Capital Letter, and Font Size of 10.
- Numbering of Second Main Headings (Heading 2) must be in Alphabets, Italic, and Font Size of 10.

You can use your own standard format also.
Author Guidelines:

1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

1. GENERAL

Before submitting your research paper, one is advised to go through the details as mentioned in following heads. It will be beneficial, while peer reviewer justify your paper for publication.

Scope

The Global Journals Inc. (US) welcome the submission of original paper, review paper, survey article relevant to the all the streams of Philosophy and knowledge. The Global Journals Inc. (US) is parental platform for Global Journal of Computer Science and Technology, Researches in Engineering, Medical Research, Science Frontier Research, Human Social Science, Management, and Business organization. The choice of specific field can be done otherwise as following in Abstracting and Indexing Page on this Website. As the all Global

Journals Inc. (US) are being abstracted and indexed (in process) by most of the reputed organizations. Topics of only narrow interest will not be accepted unless they have wider potential or consequences.

2. ETHICAL GUIDELINES

Authors should follow the ethical guidelines as mentioned below for publication of research paper and research activities.

Papers are accepted on strict understanding that the material in whole or in part has not been, nor is being, considered for publication elsewhere. If the paper once accepted by Global Journals Inc. (US) and Editorial Board, will become the copyright of the Global Journals Inc. (US).

Authorship: The authors and coauthors should have active contribution to conception design, analysis and interpretation of findings. They should critically review the contents and drafting of the paper. All should approve the final version of the paper before submission

The Global Journals Inc. (US) follows the definition of authorship set up by the Global Academy of Research and Development. According to the Global Academy of R&D authorship, criteria must be based on:

1) Substantial contributions to conception and acquisition of data, analysis and interpretation of the findings.

2) Drafting the paper and revising it critically regarding important academic content.

3) Final approval of the version of the paper to be published.

All authors should have been credited according to their appropriate contribution in research activity and preparing paper. Contributors who do not match the criteria as authors may be mentioned under Acknowledgement.

Acknowledgements: Contributors to the research other than authors credited should be mentioned under acknowledgement. The specifications of the source of funding for the research if appropriate can be included. Suppliers of resources may be mentioned along with address.

Appeal of Decision: The Editorial Board's decision on publication of the paper is final and cannot be appealed elsewhere.

Permissions: It is the author's responsibility to have prior permission if all or parts of earlier published illustrations are used in this paper.

Please mention proper reference and appropriate acknowledgements wherever expected.

If all or parts of previously published illustrations are used, permission must be taken from the copyright holder concerned. It is the author's responsibility to take these in writing.

Approval for reproduction/modification of any information (including figures and tables) published elsewhere must be obtained by the authors/copyright holders before submission of the manuscript. Contributors (Authors) are responsible for any copyright fee involved.

3. SUBMISSION OF MANUSCRIPTS

Manuscripts should be uploaded via this online submission page. The online submission is most efficient method for submission of papers, as it enables rapid distribution of manuscripts and consequently speeds up the review procedure. It also enables authors to know the status of their own manuscripts by emailing us. Complete instructions for submitting a paper is available below.

Manuscript submission is a systematic procedure and little preparation is required beyond having all parts of your manuscript in a given format and a computer with an Internet connection and a Web browser. Full help and instructions are provided on-screen. As an author, you will be prompted for login and manuscript details as Field of Paper and then to upload your manuscript file(s) according to the instructions.

To avoid postal delays, all transaction is preferred by e-mail. A finished manuscript submission is confirmed by e-mail immediately and your paper enters the editorial process with no postal delays. When a conclusion is made about the publication of your paper by our Editorial Board, revisions can be submitted online with the same procedure, with an occasion to view and respond to all comments.

Complete support for both authors and co-author is provided.

4. MANUSCRIPT'S CATEGORY

Based on potential and nature, the manuscript can be categorized under the following heads:

Original research paper: Such papers are reports of high-level significant original research work.

Review papers: These are concise, significant but helpful and decisive topics for young researchers.

Research articles: These are handled with small investigation and applications

Research letters: The letters are small and concise comments on previously published matters.

5.STRUCTURE AND FORMAT OF MANUSCRIPT

The recommended size of original research paper is less than seven thousand words, review papers fewer than seven thousands words also.Preparation of research paper or how to write research paper, are major hurdle, while writing manuscript. The research articles and research letters should be fewer than three thousand words, the structure original research paper; sometime review paper should be as follows:

Papers: These are reports of significant research (typically less than 7000 words equivalent, including tables, figures, references), and comprise:

(a)Title should be relevant and commensurate with the theme of the paper.

(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.

Structure

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

Title: The title page must carry an instructive title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) wherever the work was carried out. The full postal address in addition with the e-mail address of related author must be given. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining and indexing.

Abstract, used in Original Papers and Reviews:

Optimizing Abstract for Search Engines

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.

The Editorial Board and Global Journals Inc. (US) recommend that, citation of online-published papers and other material should be done via a DOI (digital object identifier). If an author cites anything, which does not have a DOI, they run the risk of the cited material not being noticeable.

The Editorial Board and Global Journals Inc. (US) recommend the use of a tool such as Reference Manager for reference management and formatting.

Tables, Figures and Figure Legends

Tables: Tables should be few in number, cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g. Table 4, a self-explanatory caption and be on a separate sheet. Vertical lines should not be used.

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.

Preparation of Electronic Figures for Publication

Even though low quality images are sufficient for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit (or e-mail) EPS (line art) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings) in relation to the imitation size. Please give the data for figures in black and white or submit a Color Work Agreement Form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution (at final image size) ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs) : >350 dpi; figures containing both halftone and line images: >650 dpi.

Color Charges: It is the rule of the Global Journals Inc. (US) for authors to pay the full cost for the reproduction of their color artwork. Hence, please note that, if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a color work agreement form before your paper can be published.

Figure Legends: Self-explanatory legends of all figures should be incorporated separately under the heading 'Legends to Figures'. In the full-text online edition of the journal, figure legends may possibly be truncated in abbreviated links to the full screen version. Therefore, the first 100 characters of any legend should notify the reader, about the key aspects of the figure.

6. AFTER ACCEPTANCE

Upon approval of a paper for publication, the manuscript will be forwarded to the dean, who is responsible for the publication of the Global Journals Inc. (US).

6.1 Proof Corrections

The corresponding author will receive an e-mail alert containing a link to a website or will be attached. A working e-mail address must therefore be provided for the related author.

Acrobat Reader will be required in order to read this file. This software can be downloaded

(Free of charge) from the following website:

www.adobe.com/products/acrobat/readstep2.html. This will facilitate the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof.

Proofs must be returned to the dean at dean@globaljournals.org within three days of receipt.

As changes to proofs are costly, we inquire that you only correct typesetting errors. All illustrations are retained by the publisher. Please note that the authors are responsible for all statements made in their work, including changes made by the copy editor.

6.2 Early View of Global Journals Inc. (US) (Publication Prior to Print)

The Global Journals Inc. (US) are enclosed by our publishing's Early View service. Early View articles are complete full-text articles sent in advance of their publication. Early View articles are absolute and final. They have been completely reviewed, revised and edited for publication, and the authors' final corrections have been incorporated. Because they are in final form, no changes can be made after sending them. The nature of Early View articles means that they do not yet have volume, issue or page numbers, so Early View articles cannot be cited in the conventional way.

6.3 Author Services

Online production tracking is available for your article through Author Services. Author Services enables authors to track their article - once it has been accepted - through the production process to publication online and in print. Authors can check the status of their articles online and choose to receive automated e-mails at key stages of production. The authors will receive an e-mail with a unique link that enables them to register and have their article automatically added to the system. Please ensure that a complete e-mail address is provided when submitting the manuscript.

6.4 Author Material Archive Policy

Please note that if not specifically requested, publisher will dispose off hardcopy & electronic information submitted, after the two months of publication. If you require the return of any information submitted, please inform the Editorial Board or dean as soon as possible.

6.5 Offprint and Extra Copies

A PDF offprint of the online-published article will be provided free of charge to the related author, and may be distributed according to the Publisher's terms and conditions. Additional paper offprint may be ordered by emailing us at: editor@globaljournals.org .

Before start writing a good quality Computer Science Research Paper, let us first understand what is Computer Science Research Paper? So, Computer Science Research Paper is the paper which is written by professionals or scientists who are associated to Computer Science and Information Technology, or doing research study in these areas. If you are novel to this field then you can consult about this field from your supervisor or guide.

TECHNIQUES FOR WRITING A GOOD QUALITY RESEARCH PAPER:

1. Choosing the topic: In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

2. Evaluators are human: First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

3. Think Like Evaluators: If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

4. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

5. Ask your Guides: If you are having any difficulty in your research, then do not hesitate to share your difficulty to your guide (if you have any). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work then ask the supervisor to help you with the alternative. He might also provide you the list of essential readings.

6. Use of computer is recommended: As you are doing research in the field of Computer Science, then this point is quite obvious.

7. Use right software: Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

8. Use the Internet for help: An excellent start for your paper can be by using the Google. It is an excellent search engine, where you can have your doubts resolved. You may also read some answers for the frequent question how to write my research paper or find model research paper. From the internet library you can download books. If you have all required books make important reading selecting and analyzing the specified information. Then put together research paper sketch out.

9. Use and get big pictures: Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

10. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right! It is a good habit, which helps to not to lose your continuity. You should always use bookmarks while searching on Internet also, which will make your search easier.

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.

14. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating "hotchpotch." So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

15. Use of direct quotes: When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

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.

19. Know what you know: Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

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.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

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.

Informal Guidelines of Research Paper Writing

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.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- 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

· Use standard writing style including articles ("a", "the," etc.)

· 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

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

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:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- 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.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

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.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- 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?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals Inc. (US):

Segment Draft and Final Research Paper: You have to strictly follow the template of research paper. If it is not done your paper may get rejected.

- The **major constraint** is that you must independently make all content, tables, graphs, and facts that are offered in the paper. You must write each part of the paper wholly on your own. The Peer-reviewers need to identify your own perceptive of the concepts in your own terms. NEVER extract straight from any foundation, and never rephrase someone else's analysis.

- Do not give permission to anyone else to "PROOFREAD" your manuscript.

- Methods to avoid Plagiarism is applied by us on every paper, if found guilty, you will be blacklisted by all of our collaborated research groups, your institution will be informed for this and strict legal actions will be taken immediately.)
- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

Criterion for Grading a Research Paper (Compilation) by Global Journals Inc. (US)

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals Inc. (US).

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
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
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

Index

A

Aegyptensis · 10, 30, 34, 35, 38, 40

C

Colchicine · 38
Corynebacterium · 7, 8, 9
Cytogenetics · 10, 45

D

Diphtheriae · 7

K

Karyological · 10, 37, 42, 43, 45, 46, 47
Kashmirensis · 10, 30, 31, 32, 33, 38, 40
Khotenovsky · 10, 45

L

Lymphadenitis · 7

P

Paramphistomatids · 11, 12
Pleomorphic · 8
Pneumonia · 8

R

Rhabdocoel · 12

S

Schizothorax · 10, 11, 38

save our planet

Global Journal of Medical Research

Visit us on the Web at www.GlobalJournals.org | www.MedicalResearchJournal.org
or email us at helpdesk@globaljournals.org

ISSN 9755896

© Global Journals