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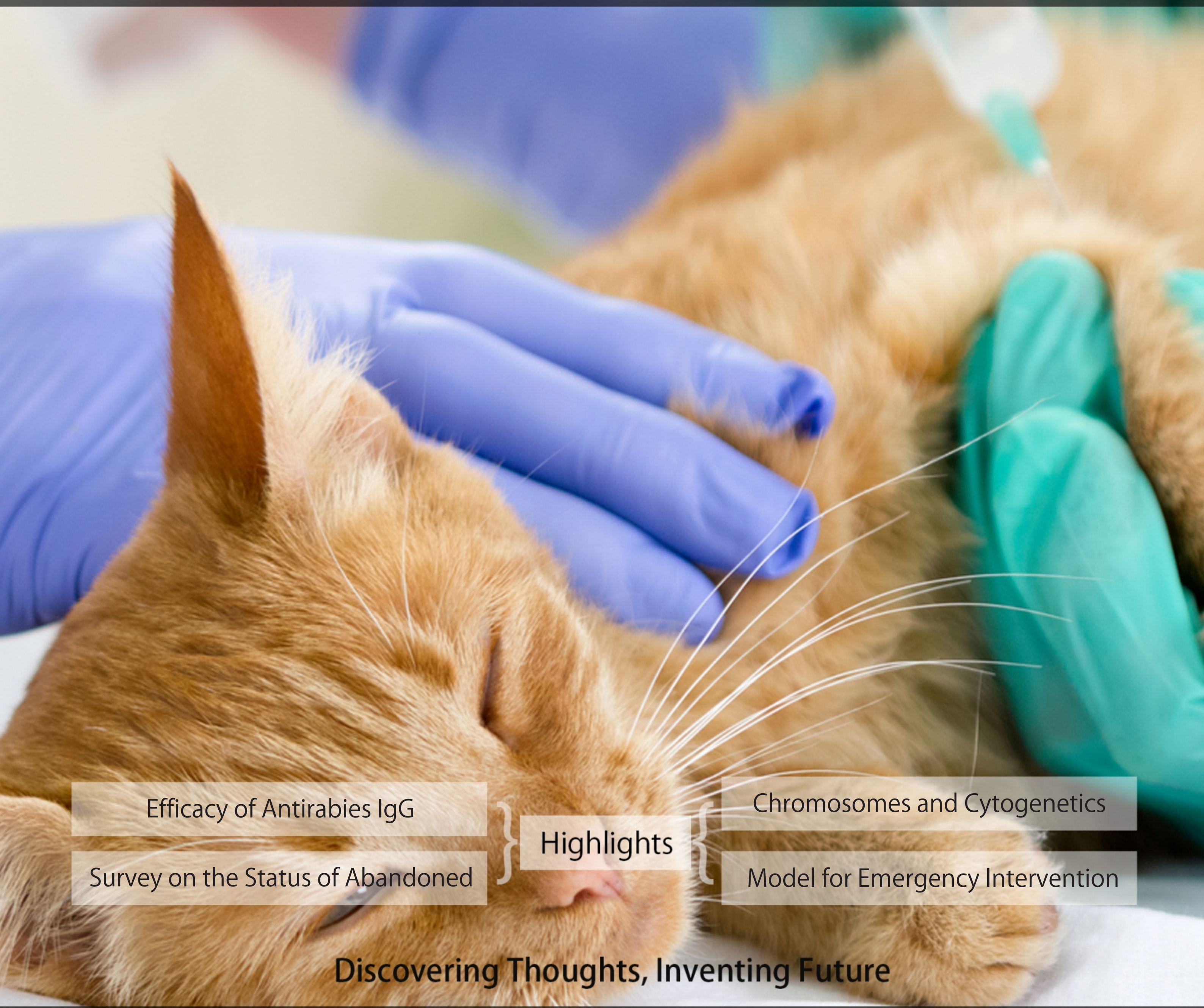

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Efficacy of Antirabies IgG and IgY on Protection of Mice Against Experimental Viral Infection as a Model for Emergency Intervention

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

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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.*

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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]

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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

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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

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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

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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 ¬ 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.

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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

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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 و128 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) بتجوبف 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 آمان تحضيره واستخدامه.

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

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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.*

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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 coryne-bacteria, 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 coryne-bacterial 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).

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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.

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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.

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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 coryne-bacteria 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.

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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

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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

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Chromosomes and Cytogenetics of Trematodes

Fayaz Ahmad [α], Tanveer A. Sofi [σ], Khalid M. Fazili [ρ], Bashir A. Sheikh [Ѡ], 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

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(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;*

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

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

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(*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

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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)

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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)

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	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 corvus, Lymnaea palustris)	2n=20; 4m + 6sm+10a	Britt (1947)

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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)

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	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)

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Pleumocoeces similiplexus	2n = 22	Pennypacker (1940)
Pleumocoeces similiplexus	2n = 22	Britt (1947)
Trematorchis ranarum	2n=18	Subramanyam & Venkat Reddy (1977)
Glypthelmins guieta	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, ПHP*)	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)

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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);

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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)

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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)

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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)

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

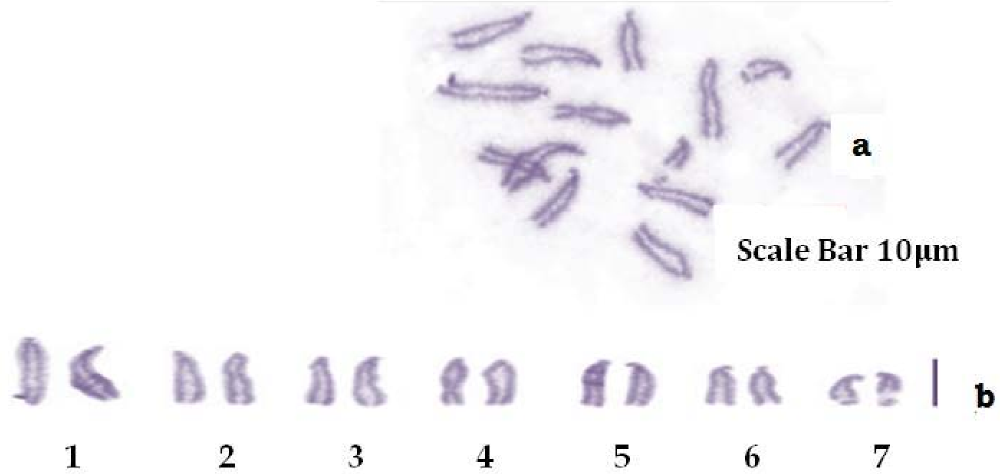

Figure 1 : (a)Chromosome preparation of *Diplozoon kashmirensis* (b) Ideogram constructed from mitotic cells of *Diplozoon kashmirensis* stained with Giemsa(a= acrocentric)

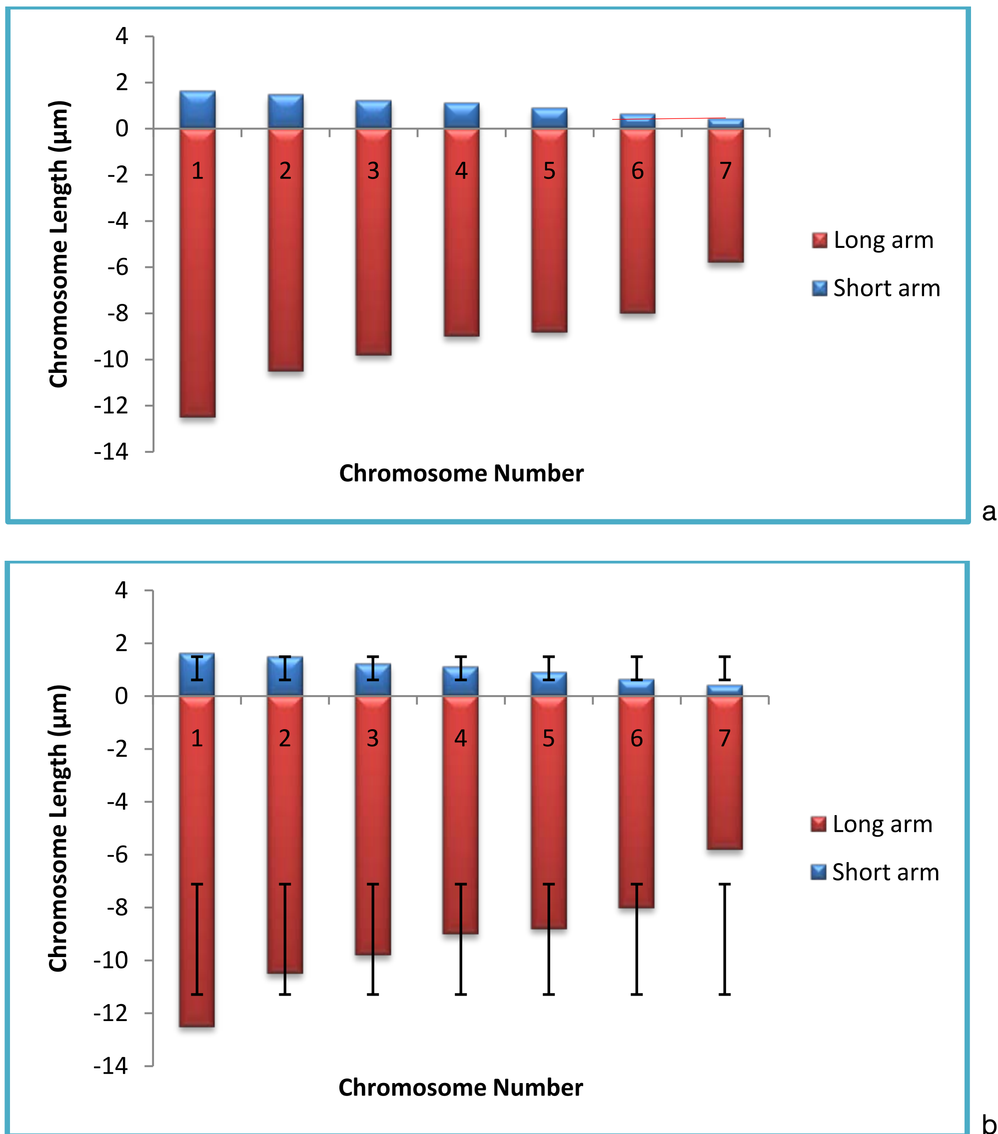

Figure 2 : (a) Idiogram of *Diplozoon kashmirensis* (b) Error Bars with Standard Deviation of *Diplozoon kashmirensis*

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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

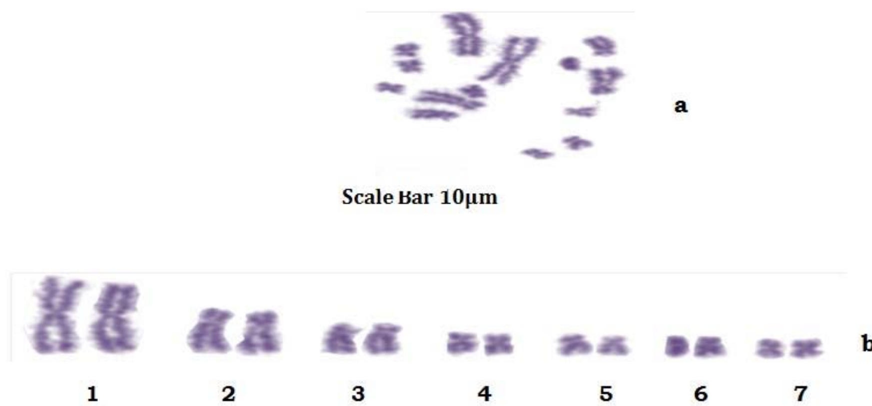

Figure 3 : (a) Chromosome preparation of *Diplozoon aegyptensis* (b) Karyotype constructed from mitotic cells of *Diplozoon aegyptensis* stained with Giemsa (m= metacentric; a= acrocentric;)

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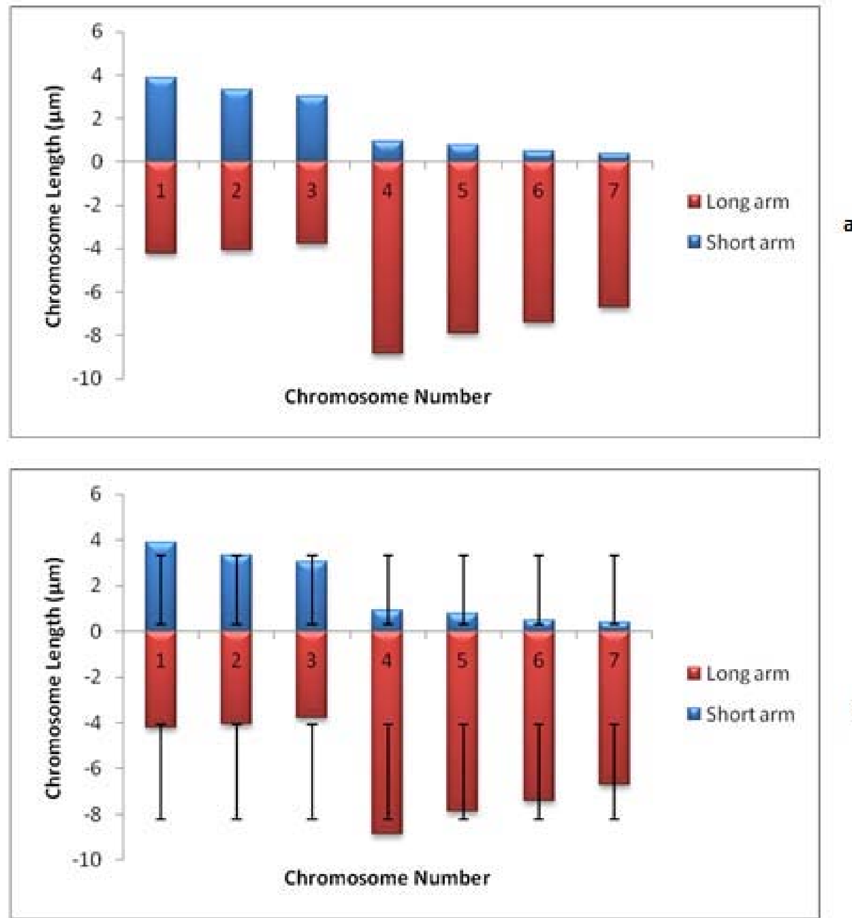

Figure 4 : (a) Ideogram of *Diplozoon aegyptensis* (b) Error Bars with Standard Deviation of *Diplozoon aegyptensis*

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

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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

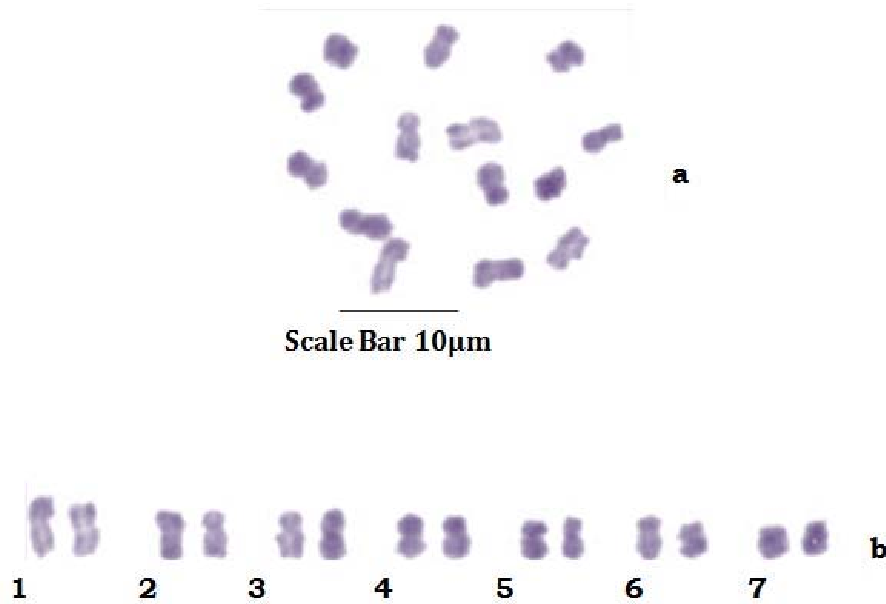

Figure 5 : (a) Chromosome preparation of *Diplozoon guptai* (b) Karyotype constructed from mitotic cells of *Diplozoon guptai* stained with Giemsa (m= metacentric; sm= Submetacentric; st= Subtelocentric; a= acrocentric;)

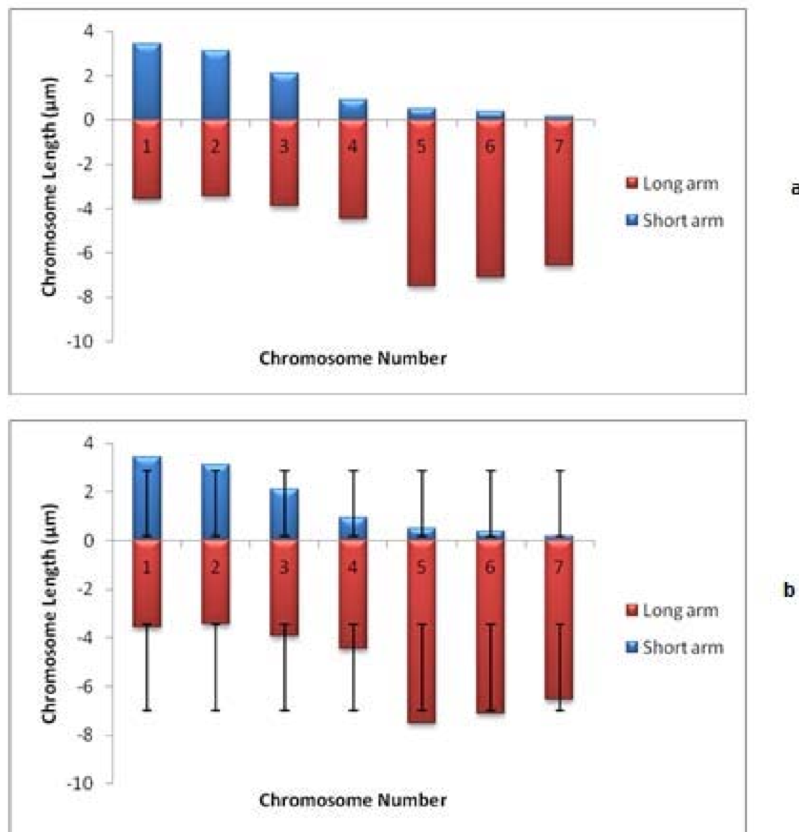

Figure 6 : (a) Ideogram of *Diplozoon guptai* (b) Error Bars with Standard Deviation *Diplozoon guptai*

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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

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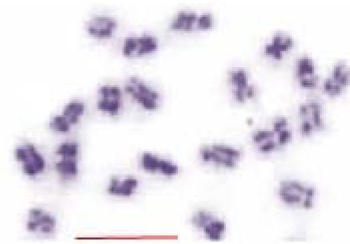
absolute length and centromeric position the chromosomes have been arranged in order of decreasing length in an ideogram and the karyotype formula is; **Karyotype Formula : (K) 2n=20=1m+ 2sm+3t+4a.**

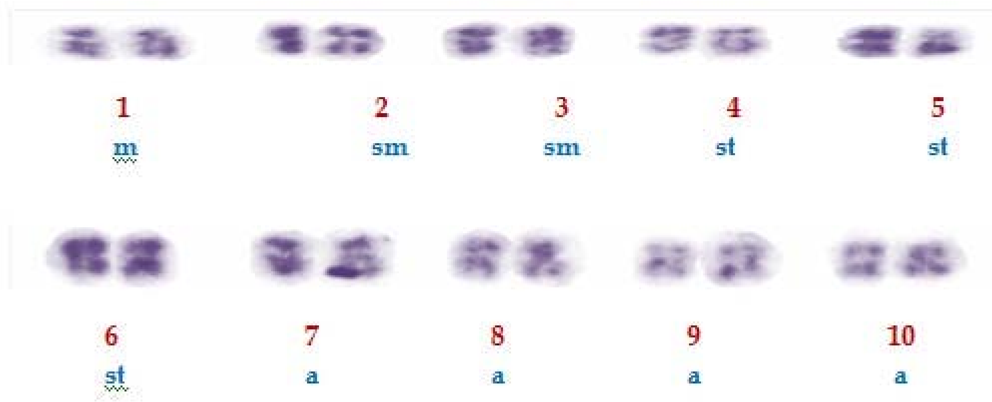
Table 5 : Measurements and classification of chromosomes of *Clinostomum schizothoraxi* Kaw, 1950

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	



Scale Bar 10µm

a



b

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)

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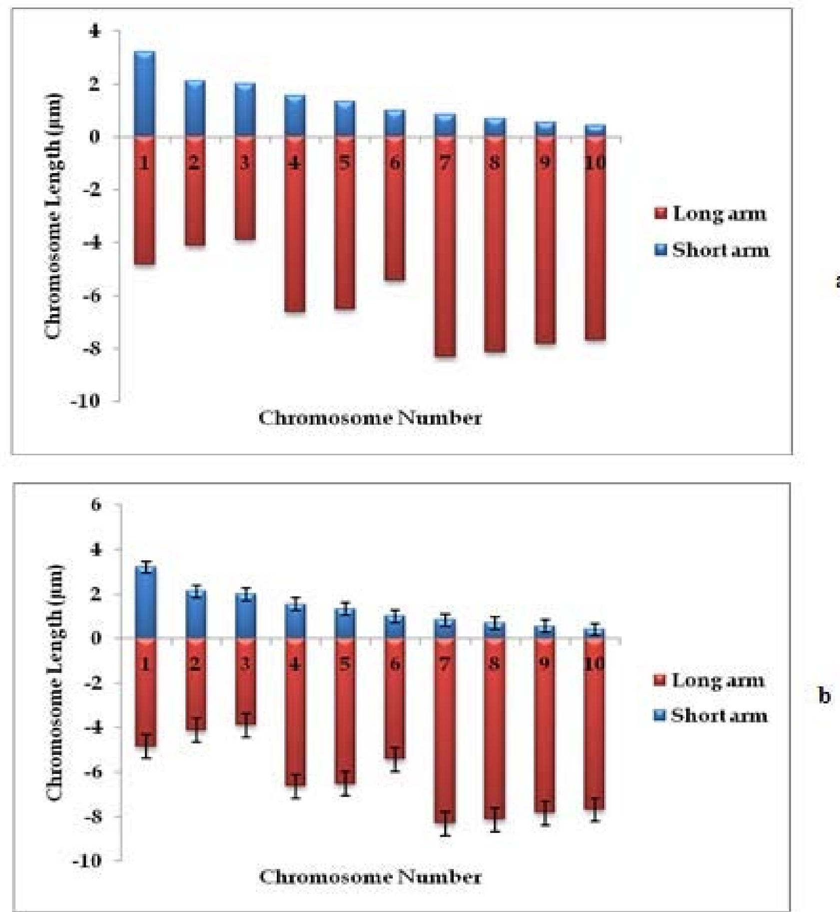

Figure 8 : (a) Haploid idiogram, (b) Error bars with standard error of *Clinostomum schizothoraxi* Kaw, 1950

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

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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

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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-

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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.

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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.

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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

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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,

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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

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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*

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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 rnonsoni* 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

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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.

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

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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*

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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

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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

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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).

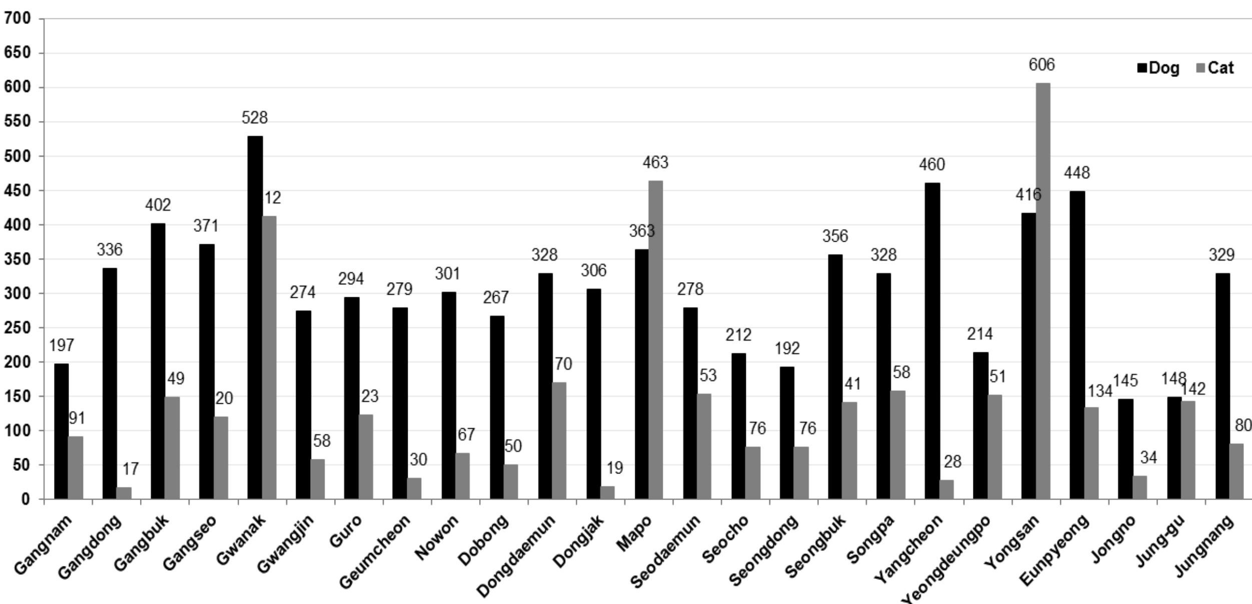

Figure 1 : Occurrence of abandoned dogs and cats in 25 districts of Seoul City in 2013

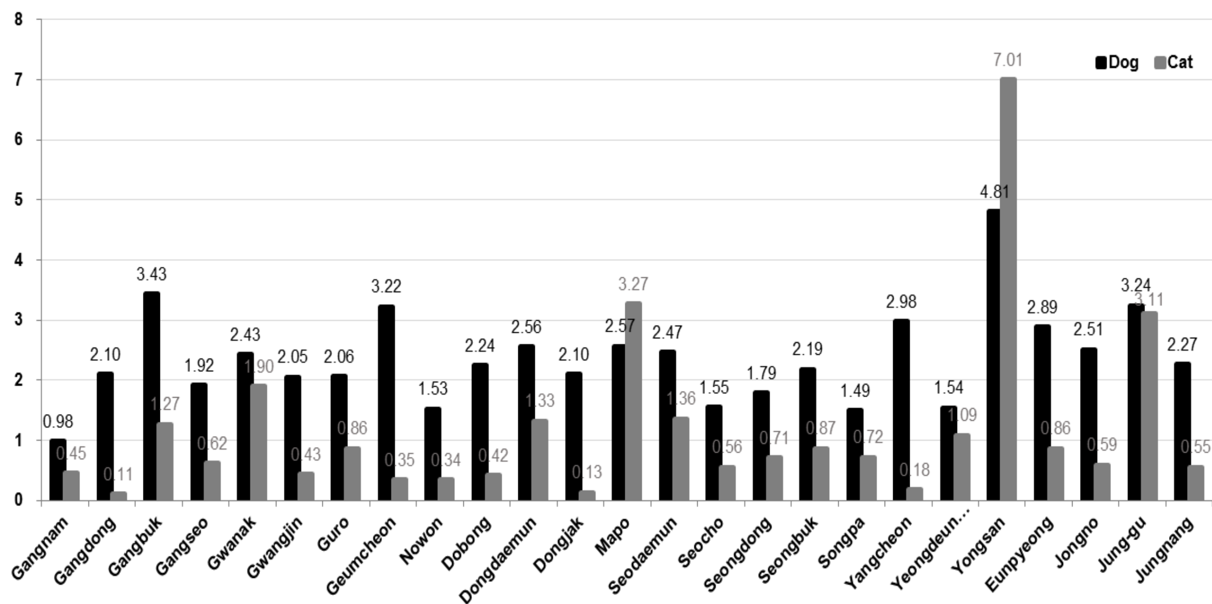

Figure 2 : Occurrence of abandoned animals dogs and cats per 1000 households in 25 districts of Seoul City in 2013

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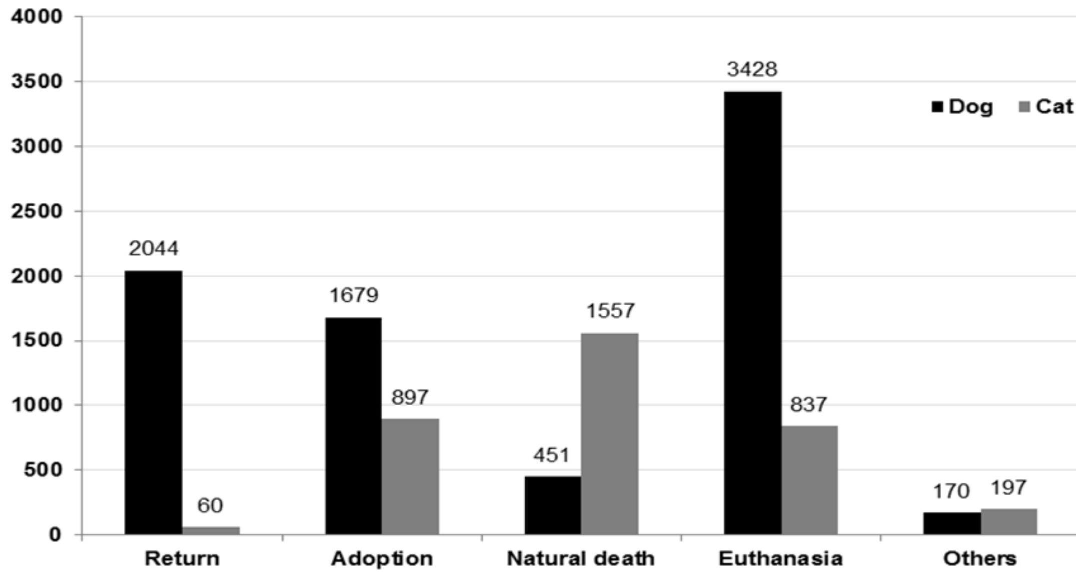

Figure 3 : Management of abandoned dogs and cats in 25 districts of Seoul City in 2013

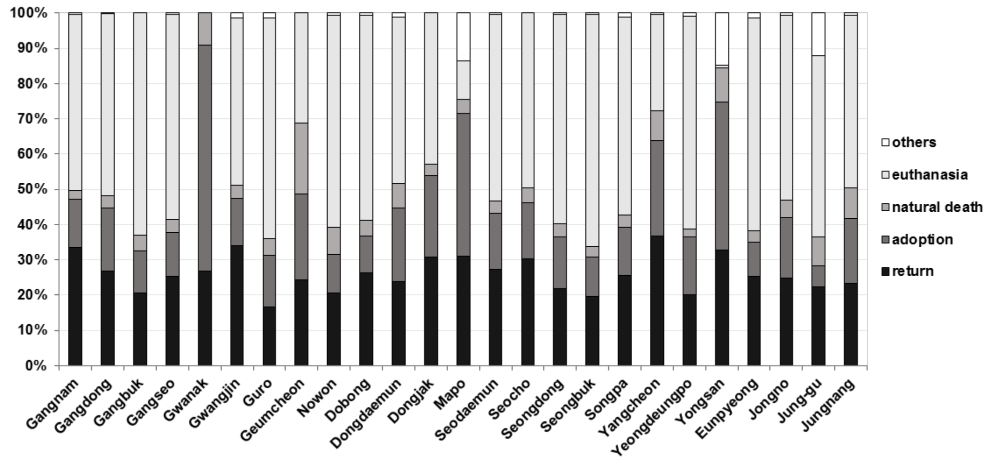

Figure 4 : Management of abandoned dogs in each of 25 districts of Seoul City in 2013

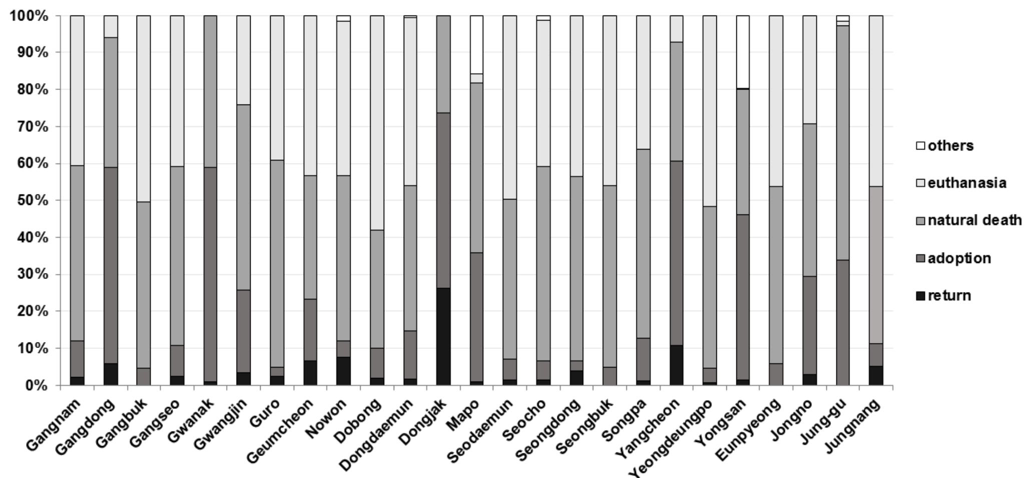

Figure 5 : Management of abandoned cats in each of 25 districts of Seoul City in 2013

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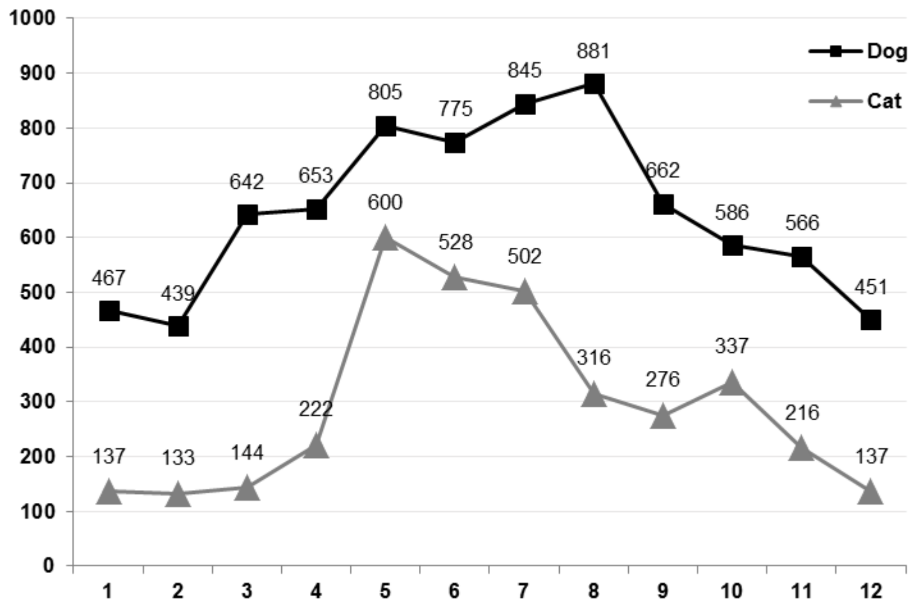

Figure 6 : Monthly occurrence of abandoned dogs and cats in 25 districts of Seoul City in 2013

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)*

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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

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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

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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.

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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.

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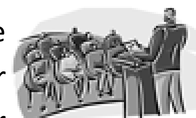

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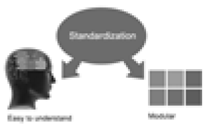

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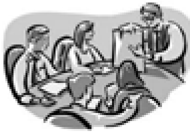

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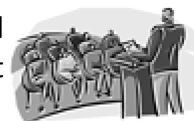

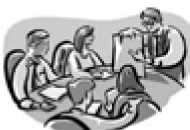

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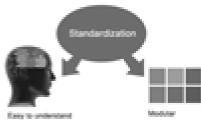

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4. Manuscript’s Category,

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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.

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Approach:

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Approach:

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Approach:

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Approach:

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References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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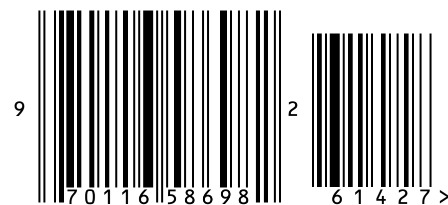

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