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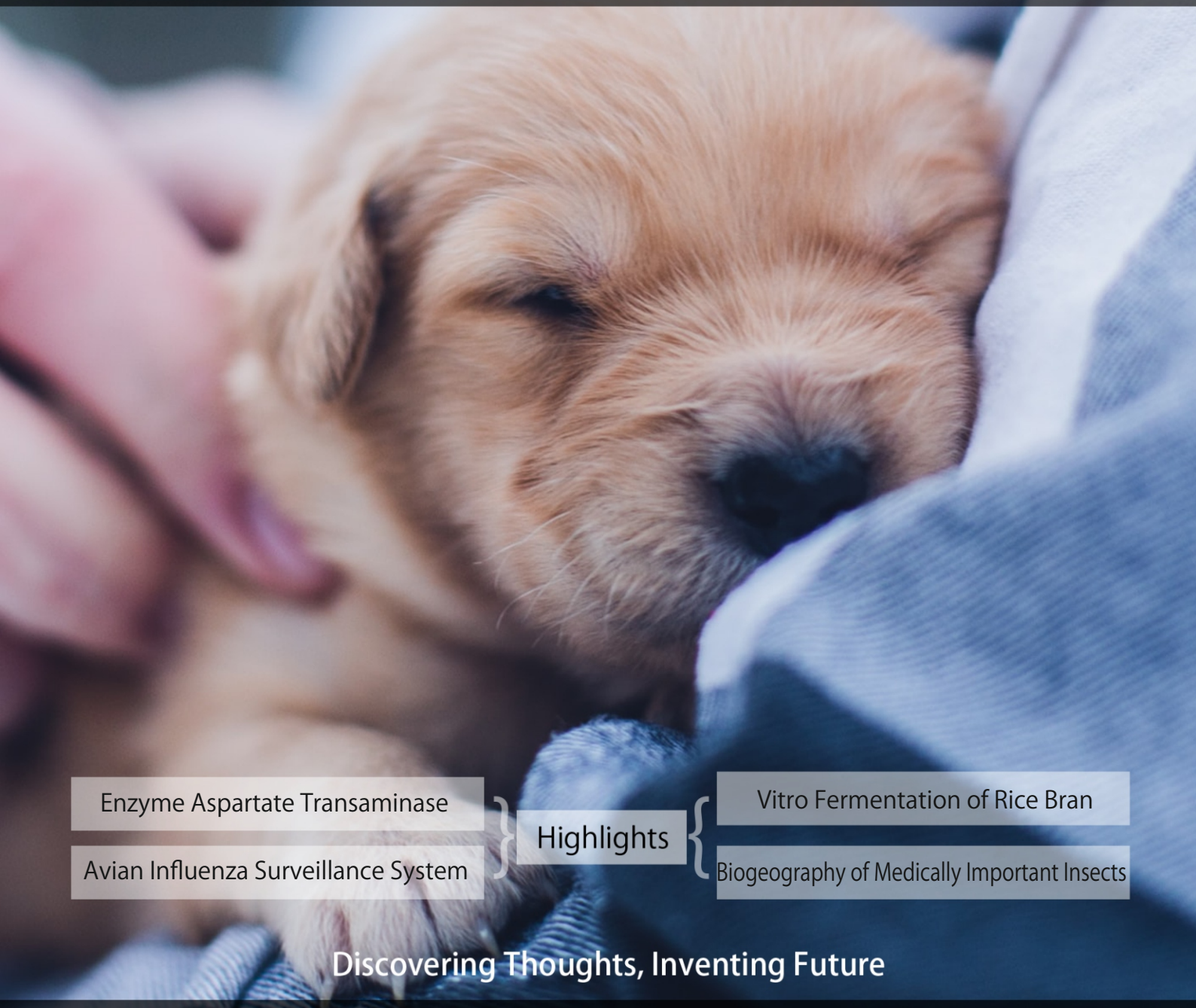

Enzyme Aspartate Transaminase

Avian Influenza Surveillance System

Highlights

Vitro Fermentation of Rice Bran

Biogeography of Medically Important Insects

Discovering Thoughts, Inventing Future

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Biogeography of Medically Important Insects using Quantitative Analysis

By Qi Shen, Zhixing You, Xiaojing Ma & Xiaocheng Shen
Henan University of Traditional Chinese Medicine

Abstract- We summarized distributional information of medically important insects from 76 families and 4531 genera occurring worldwide. The continents were divided into 67 basic geographical units. Using a new similarity formula and a new clustering method for quantitative analysis, 67 basic geographical units were clustered into 7 large unit groups and 20 small unit groups. The results were superior to the traditional single linkage method, average group linkage method, or sum of squares method. The cluster results were similar with the result of mainly phytophagous insects 104,344 genera in the world, but were different from the Wallace's mammal geographical division scheme. Based on these seemingly contradictory results, we infer that animals, insect and plants may have the same distribution pattern and that it is necessary to conduct precise quantitative analysis for animals and plants worldwide.

Keywords: biogeography; medical important insect; similarity general formula; multivariate similarity clustering analysis.

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Biogeography of Medically Important Insects using Quantitative Analysis

Qi Shen [α], Zhixing You [σ], Xiaojing Ma [ρ] & Xiaocheng Shen [ω]

Abstract- We summarized distributional information of medically important insects from 76 families and 4531 genera occurring worldwide. The continents were divided into 67 basic geographical units. Using a new similarity formula and a new clustering method for quantitative analysis, 67 basic geographical units were clustered into 7 large unit groups and 20 small unit groups. The results were superior to the traditional single linkage method, average group linkage method, or sum of squares method. The cluster results were similar with the result of mainly phytophagous insects 104,344 genera in the world, but were different from the Wallace's mammal geographical division scheme. Based on these seemingly contradictory results, we infer that animals, insect and plants may have the same distribution pattern and that it is necessary to conduct precise quantitative analysis for animals and plants worldwide.

Keywords: biogeography; medical important insect; similarity general formula; multivariate similarity clustering analysis.

I. Introduction

There are three categories of medically important insects: insects that feed on the blood of warm blooded animals (humans, mammals, and birds) and can transmit disease; insects that feed on the fur, feathers, and skin secretions of animals and birds, irritating the host; insects that live in the habitats of humans, mammals and birds, causing irritation and sometimes transmitting diseases. Since these insects have a close relationship with mammals and birds, they may have the same geographical distribution pattern with the mammals described by Wallace (1876). Computer and Internet technology has made it possible to collect and analyze large data sets and re-evaluate previous Wallace's scheme which are based on qualitative analysis (Olson et al., 2001; Procheş, 2005; Cox, 2010; Kreftet al., 2010; Procheşet al., 2012; Rueda et al., 2013; Whittaker et al., 2013; Holt et al., 2013; Peixotoet al., 2017). Kreftet al. (2010) and Holt et al. (2013) both used Simpson similarity formula and the UPGMA method for quantitative analysis of mammalian species distribution but they obtained different results. Different geographical division schemes for some insect orders and families have been proposed (Herman et al., 2001; Evans, 2007; Balianet al., 2008; Moor et al., 2008; Morse et al., 2011; Taegeret al., 2010). The results of most of these studies did not support the "Wallace line", that is Wallace's great contribution to the field. Among them, as medical important insects, Culinidae and Siphonaptera geographic division settings are also proposed the same questions (Siver, 2004; Vashchonovet al., 2013). The extensive attention and in-depth discussion in biogeography interpretation provides an exciting opportunity for evaluating insect distributions and geographical division plans.

We used the similarity general formula (SGF) proposed by Shenet al. (2008a) and multivariate similarity clustering analysis (MSCA) (Shenet al., 2008b) for quantitative analysis of the medically important insects in China (Shen, 2014). The results were unexpectedly similar to the results of all (93661) insect species in China (Shenet al., 2013a; 2013b; 2015), but different from the results of a Chinese mammalian species geographical division, made using qualitative analysis (Zhang, 2011). To study the relationship between the global distributions of medically important insects, phytophagous insects, and mammals, we used a variety of quantitative methods for this analysis.

II. Materials and Methods

a) Global medically important insect species

We used medically important insect distribution data from four resources: (1) World species and distribution data collected and summarized by entomologists, e.g. Knight et al., 1977, Durden et al., 1994, Currie et al., 2008, Adler et al., 2014; (2) Data from specific countries and regions, e.g. Seccombe et al., 1993, De Carvalho et al., 2005, Coscarón et al., 2008, Crespo et al., 2010, Chahari et al., 2015, Wolff et al., 2016, Takano et al., 2017; (3) Data from organizations studying biodiversity and academic websites, such as Beccdloni, 2014, Borkent, 2014, Pickering, 2014, Evenhuis, 2016, GBIF, 2017; (4) Recently published data for new species and new distribution records, such as Gustafsson et al.,2015, Najer et al., 2016, Szelag et al., 2016, Fuenzalidaet al., 2017, Huerta et al., 2017, Natarajanet al., 2017, Vidlčkaet al., 2017. The data cited above did not include marine species or fossil records. In total, 9 orders, 67 families, 4531 genera and 63470 species were included (Table 1). This was 4.3% of the

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total number of insect genera and 6.1% of the total insect species in the world. Because insects have small bodies, their species distribution is narrower compared with higher animals and plants (Shenet al., 2018). To improve the data utilization ratio and accuracy of the analysis, the genus was used as the basic biological unit (BBU).

Table 1: Global medically important insect species used for analysis

Orders	No. of families	No. of genera	No. of species	Main data sources
Blattodea	8	490	4428	Roth, 2003, Vidlička, 2013, 2017, Beccdloni, 2014, Vrsansky, 2010, 2012, 2013
Mallophaga	9	485	4565	Mey, 2004, Pickering, 2014, Gustafsson, *et al*., 2015
Anoplura	14	46	553	Durden *et al*., 1994, Sánchez-Montes *et al*., 2013
Hemiptera	1	22	74	Usinger, 1966,Iorio, 2012, GBIF, 2019c
Coleoptera	1	126	2480	GBIF, 2019a
Diptera	16	2337	36594	Knight *et al*., 1977,Seccombe *et al*., 1993,Currie, 2008, Adler *et al*., 2014,Borkent, 2014,Henriques, 2016, Evenhuis, 2016, Takaoka *et al*., 2017, GBIF, 2019b
Siphonaptera	20	241	2099	Acosta, 2003, Hastriter*et al*., 2006, Lewis *et al*., 2013,Vashchonok*et al*., 2013,Beaucournu*et al*., 2014
Lepidoptera	2	544	5969	GBIF, 2019e
Hymenoptera	5	440	6708	GBIF, 2019d
Total	76	4531	63470	

b) Division of basic geographical units (BGU) and building the databank

According to the terrain, climate, and other ecological conditions, we have divided the continents (except Antarctica) into 67 basic geographical units (BGU) (Fig. 1). Of these BGUs, 21 BGUs were mainly plain, 11 were mainly hills, 12 were mainly mountain, 11 were mainly plateau, five were mainly desert and seven were mainly islands. A total of 27 BGUs were in tropical zone, 34 were in temperate zones and six were extended to the frigid zone. The names and geographical ranges of the BGUs are listed in Table 2.

We used Microsoft Access as our database software. Each BGU was listed as the column and BBU was listed as the row. The distribution of different species belonging to the same genus was transferred to the BGU and summarized as the genus distribution. During the data entry, when there was a distribution, it was marked as 1; if there was no distribution, no record was entered. These basic distributional records (BDR) were the basis of quantitative analysis. Each BGU insect genus number is listed in Table 2.

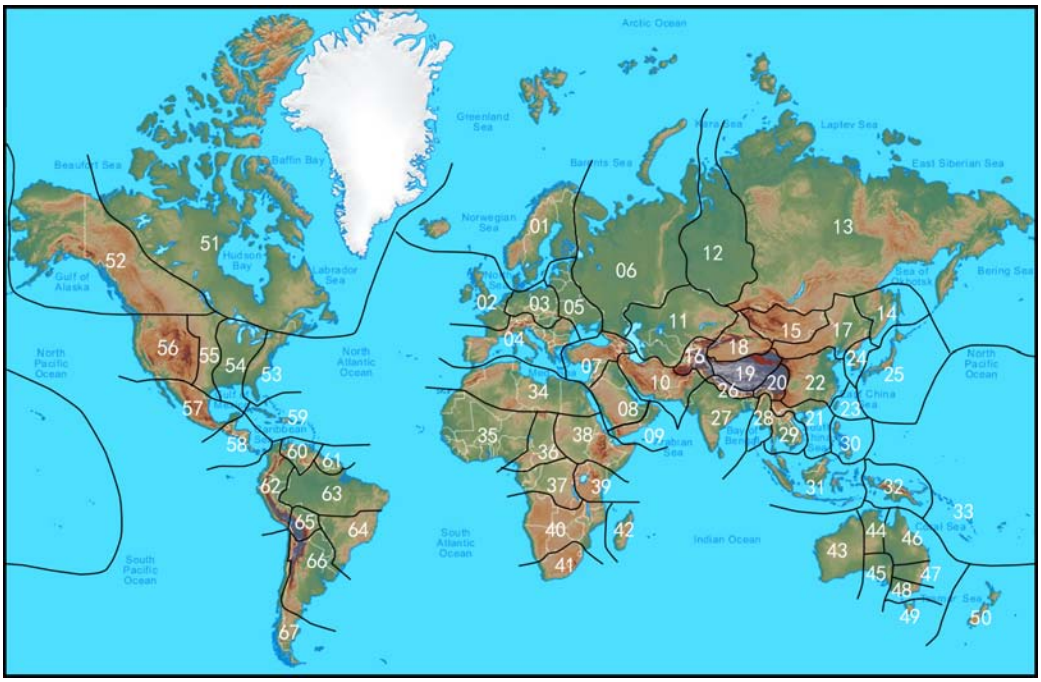


Fig. 1: BGUs of the World

01 Northern Europe, 02 Western Europe, 03 Central Europe, 04 Southern Europe, 05 Eastern Europe, 06 European Russia, 07 Middle East, 08 Saudi Arabia, 09 Yemen and Oman, 10 Plateau of Iran, 11 Central Asia, 12 Western Siberia, 13 Eastern Siberia, 14Ussuri region, 15 Mongolia, 16 Plateau of Pamir, 17Northeastern region of China, 18Northwestern region of China, 19 Qinghai-Xizang region of China, 20 Southwestern region of China, 21 Southern region of China, 22 Centre-eastern China, 23 Taiwan region of China, 24 Korea Peninsula, 25 Japan, 26 Himalayan region, 27 Indian and Sri Lanka, 28 Myanmar, 29 Indochina Peninsula, 30 Philippines, 31 Indonesia, 32 New Guinea, 33 Islands of Pacific Ocean, 34 Northern Africa, 35 Western Africa, 36 Central Africa, 37 Congo river basin, 38 Ethiopia region, 39 Tanzania region, 40 Angola region, 41 South Africa, 42 Madagascar, 43 Western Australia, 44 Northern Territory, 45 South Australia, 46 Queensland, 47 New South Wales, 48 Victoria, 49 Tasmania, 50 New Zealand, 51 Eastern Canada, 52 Western Canada,53 Mts. Eastern US, 54 Plain Central US, 55 Hills Central US, 56 Mts. Western US, 57 Mexico, 58 Central America region, 59 Caribbean Islands, 60 Venezuela, 61 Plateau Guyana, 62 Northern Mt. Andes, 63 Amazon Plain, 64 Plateau Brazil, 65 Bolivia, 66 Argentina, 67 Southern Mt. Andes

Table 2: The number of medical important insect genera of BGUs in the World

BGU	Number of genera	BGU	Number of genera	BGU	Number of genera	BGU	Number of genera
01	253	19	310	37	207	55	242
02	316	20	457	38	131	56	323
03	245	21	474	39	241	57	325
04	271	22	614	40	251	58	459
05	99	23	419	41	292	59	133
06	143	24	125	42	180	60	179
07	208	25	300	43	116	61	173
08	81	26	161	44	101	62	376
09	75	27	310	45	79	63	263
10	211	28	214	46	239	64	239
11	246	29	284	47	232	65	150
12	165	30	201	47	135	66	235
13	315	31	343	49	151	67	74
14	124	32	179	50	71	BBU	4531
15	103	33	168	51	205	BGU	67
16	127	34	209	52	256	BDR	15450
17	446	35	222	53	299	AR*	231
18	314	36	139	54	233	ADT**	3.41

**AR (average richness): BDR/BGU: **ADT (average distributional territory): BDR/BBU*

c) Clustering methods

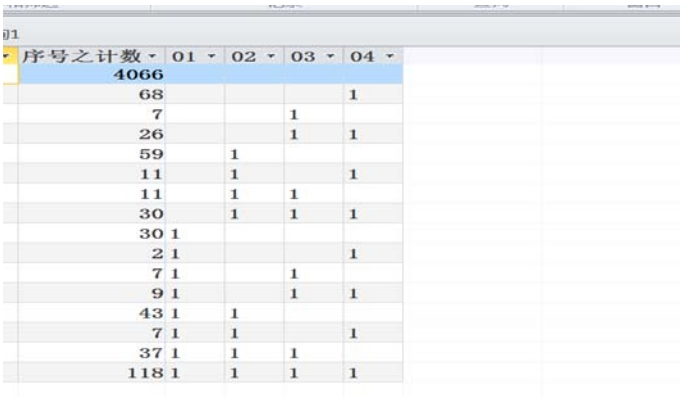
Shen's SGF is defined as follows: similarity coefficient among multiple regions is the ratio of the average species number shared by all regions to the total species number (Shenet al., 2008a):

$$SI_n = \sum H_i / nS_n = \sum (S_i - T_i)/nS_n$$

In this formula, $SI_n$is the similarity coefficient of n BGUs: S_i, H_i and T_i are i BGU species number, common species number and unique species number, respectively, and $H_i = S_i - T_i$: S_n is the total species number in n BGUs. For calculation, all values were obtained from the database search page. This was convenient for both manual and computer calculations.

The MSCA indicated that the similarity coefficient of any group could be calculated directly and not restrained by the clustering order. It even was possible the first to calculate the total similarity coefficient of the 67 BGUs.

For example, we calculated the similarity coefficient of four BGU from Europe (Fig. 2). The 4066 in the first column of the first row was the number of genera that have not distribution by all four BGUs. The first number in other each column was the unique species number of every BGU. The number 465, which was the total genus number 4531 minus 4066, was the total species number of all five BGUs. The genus numbers of four BGUs were 253, 316, 245, and 271 as shown in Table 2. Using a calculator for these steps 253+316+245+271−30−59−7−68=921, dividing by 4, then dividing by 465, produced a similarity coefficient 0.495. The process was simple compared to the processes of other clustering methods.



序号之计数	01	02	03	04
4066				
68				1
7			1	
26			1	1
59		1		
11		1		1
11		1	1	
30		1	1	1
30	1			
2	1			1
7	1		1	
9	1		1	1
43	1	1		
7	1	1		1
37	1	1	1	
118	1	1	1	1

Fig. 2: Screen cut for calculating No. 01–04 BGU similarity coefficient

To compare the analysis results, three of the most common hierarchical clustering methods were used (Kreft, 2010):

1) The single linkage method, also called nearest neighbor method, using the Jaccard (1901) similarity formula: SI=C/(A+B-C), which was the most basic clustering method;
2) Average group linkage method, which was also called unweighted pair group means algorithm (UPGMA) method, using the Szymkiewicz (1934) similarity formula, also called the Simpson (1947) formula: SI=C/min(A,B), which is the most popular clustering method.
3) Sum of squares method (Ward's method), using the Czekanowski (1913) similarity formula (also called Sørensen (1948) formula): SI=2C/(A+B). Using this method, better results can be obtained, but the calculation process is complicated.

The three similarity formulas were subjected to pairwise comparisons. A and B were species numbers in two regions and C was the species number shared by two regions.

III. Results

MSCA clustering results (Fig. 3) showed that the 67 BGUs total similarity coefficient was 0.089. At 0.370 similarity coefficient level, 67 BGUs were clustered as a–t 20 small unit groups, at 0.250 similarity coefficient level, 20 small unit groups were clustered as A–G 7 big unit groups. Unit of each group was neighbor and connected to each other, corresponding to the geographical principles. The similarity level was greater within the group than among different groups and the ecological condition of each group was independent, corresponding to the principles of statistics and ecology.

Clustering results showed high consistency with world insects clustering results (Shenet al., 2018). The numbers of big and small unit groups were the same, components of each big and small group were almost the same, and the structures between the groups were consistent. One difference was that the total similarity coefficient and similarity level of large and small unit groups of medically important insects were higher than all insects. This may be because medically important insects have generated more attention and research. The clustering location of individual units had moved: Unit 25# moved from the g small unit group to the f small unit group. Unit 37# moved from the h small unit group to the i small unit group. Unit 74# moved from the r small unit group to the s small unit group. These movements were between neighboring groups, consistent with geographical principles.

We compared mammalian division scheme (Wallace, 1876) and except for the D big unit group that was the same in the Ethiopian realm, all other groups were different. A and B big unit groups divided the Palearctic realm into east and west sections. C and E big groups incorporated New Guinea and Pacific islands from the Australian realm into the Oriental realm. F and G big groups incorporated Central America in the Neotropic realm into the Nearctic realm.

Compared with current world plant division proposed by Cox (Cox, 2001), the C, D, E big unit groups were the same as the India–Pacific kingdom, Afritropic kingdom, and Australian kingdom. The difference was that the A, B and F big unit groups divided the Holarctic realm into three parts. F and G big groups categorized Central America in the Neotropic kingdom into the Nearctic kingdom.

Compared with the current several insect groups division, our results support the following: the Palearctic realm is divided into two parts by Trichoptera and Aleyrodidae(Morse et al., 2011; Evans, 2007). The Siphonaptera and Trichopteracategorize New Guinea and the Pacific Islands into the Oriental realm (Vashchonoket al., 2013; Moor et al., 2008). The results also support separating Pacific Islands from the Australia realm by Staphylinidae, Aleyrodidaeand aquatic insects (Herman et al., 2001; Evans, 2007; Balianet al., 2008); incorporation of Yemen and Oman into the Palearctic realm by Symphyta and Culicidae (Taegeret al., 2010; Silver, 2004); And incorporation of Mexico into Nearctic realm by the Culicidae (Silver,

2004). However, the results did not support assigning New Zealand, Madagascar and Antarctic as separate realms.

The traditional methods did not produce accurate, sensitive, and precise results. The results of the single linkage method (Fig. 4) were chaotic with no distinct layers. Many geographical units could not be clustered, such as units 20, 31, 49, 58, 69, 78, that were called “noise”. The average group linkage method (Fig. 5) was better than the single linkage method and removed most of the “noise”. At the distant level of 0.63 the BGUs could be clustered into six unit groups, five of which had significant geographical meaning. The letters corresponding to the areas in Fig. 3, the largest group which was composed of 26 BGUs, were chaotic and lacked geographical values. More precise division did not improve this. The sum of squares method (Fig. 6) had better clustering results. At the distance of 1.2, the BGUs could be clustered into eight groups and the first seven had geographical meaning. The last group did not conform to the principle of geography and it was difficult to achieve precise clustering.

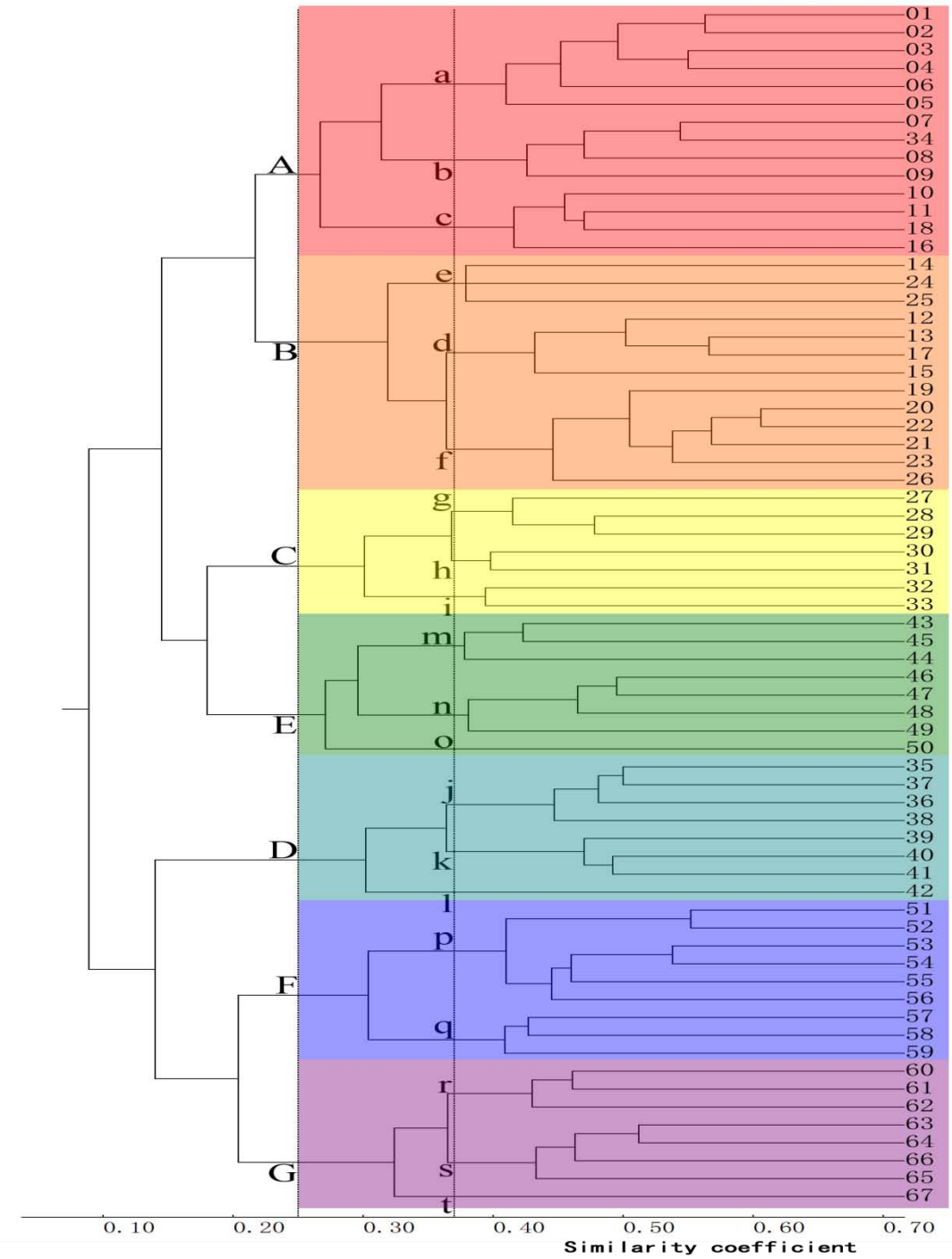


Fig. 3: Clustering tree of Medical insect of World by MSCA

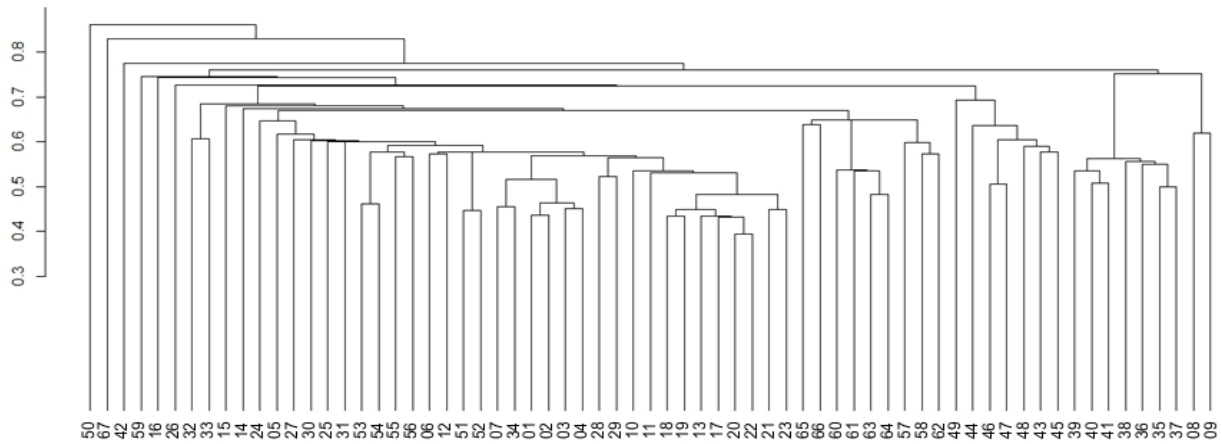


Fig. 4: Clustering tree by single linkage method

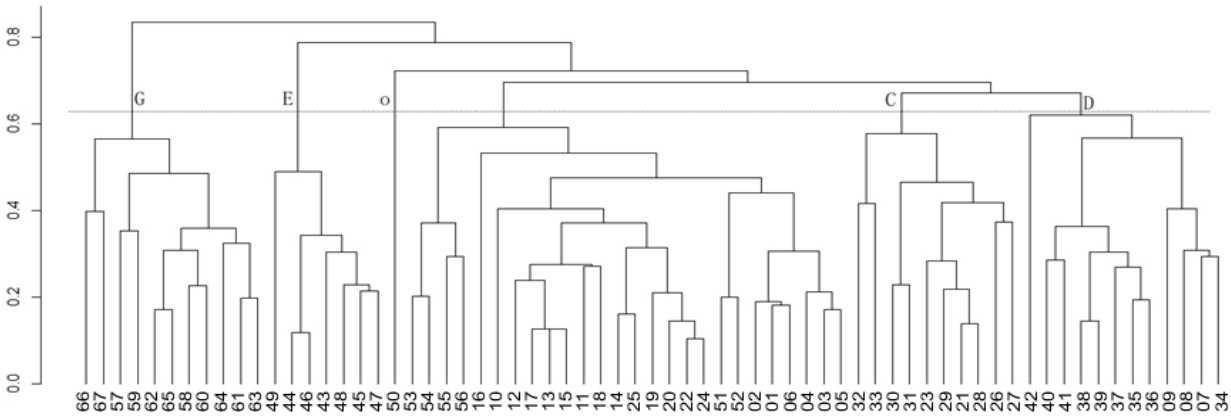


Fig. 5: Clustering tree by Average group linkage method

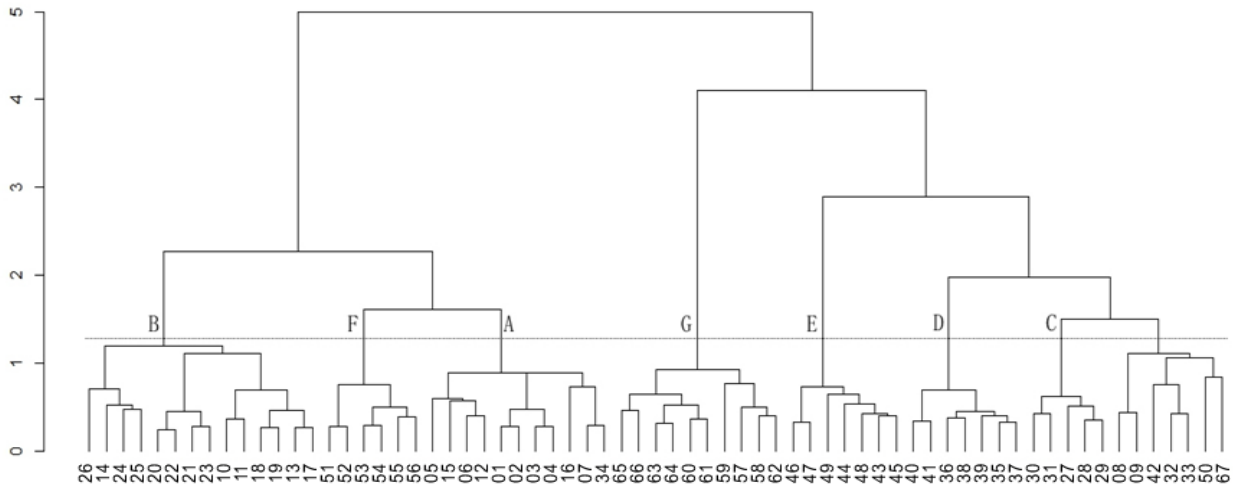


Fig. 6: Clustering tree by Sum of squares method

IV. Discussion

This study demonstrated that the distribution pattern of medically important insects is consistent with that of phytophagous insects. However it is indisputable fact that the medical insects have a close relationship of food chain with higher animals. Therefore, we can speculated that most insects are phytophagous and the distribution pattern is the same as for plants. Although the feeding habits of mammals are complex and some

of them are carnivorous, the final food sources are plants. Their distribution pattern should be the same as plants. Thus, medical important insects belong to the bi-direction food chain of animals → plants ← phytophagous insects and showed the same results as the total insect distribution. Without doubt this hypothesis requires confirmation from quantitative analysis of plants and mammals and the first step would be to select and standardize the different methods. The comparisons made in this study showed that MSCA method can be useful. We look forward to establishing a consistent model of the distribution patterns of plants, mammals, and insects across the world.

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Myiasis of Domestic Animals in Iraq

By M. O. Al-Ani, S. F. Abbas, T. H. Hamzza, A. Hatem, A. A. Shubar & M.S. Abdul-Rassoul

Abstract- This work carried out to detect dipterous fly agents of Myiasis of domestic animals in Iraq during the year 2017. Seventy cases of Myiasis were determined in the domestic animals distributed in five provinces: Baghdad, Diyala, Wasit, Diwaniya and Basrah. Fifty one cases were wound Myiasis, and 19 were various Myiasis cases for aural five rectal, four urogenita, three ophthalmic and one oral. Four species of dipterous flies larvae: *Chrysomya bezziana*, *Chrysomya megacephala*, *Lucilia sericata*, *Chrysomya albiceps* were identified as Myiasis agents. *Chrysomyabezziana* was most prevalent species has been recording 50 injured, 27 of them injured in sheep. The larvae were collected from six species of domestic animals; sheep were more susceptible to Myiasis followed by cattle, dog, goat, buffalo and cat.

GJMR-G Classification: *NLMC Code: QW 70*

Myiasis of Domestic Animals in Iraq

M. O. Al-Ani [α], S. F. Abbas [σ], T. H. Hamzza [ρ], A. Hatem [ω], A. A. Shubar [¥] & M.S. Abdul-Rassoul [§]

Abstract- This work carried out to detect dipterous fly agents of Myiasis of domestic animals in Iraq during the year 2017. Seventy cases of Myiasis were determined in the domestic animals distributed in five provinces: Baghdad, Diyala, Wasit, Diwaniya and Basrah. Fifty one cases were wound Myiasis, and 19 were various Myiasis cases for aural five rectal, four urogenita, three ophthalmic and one oral. Four species of dipterous flies larvae: *Chrysomya bezziana*, *Chrysomya megacephala*, *Lucilia sericata*, *Chrysomya albiceps* were identified as Myiasis agents. *Chrysomyabezziana* was most prevalent species has been recording 50 injured, 27 of them injured in sheep. The larvae were collected from six species of domestic animals; sheep were more susceptible to Myiasis followed by cattle, dog, goat, buffalo and cat.

I. Introduction

Myiasis is the infestation of live animals with dipterous larvae which at least for a certain period feed on the host dead or living tissues ;liquid body substance or ingested food (Zumpt;1956). No attention has been given to myiasis in domestic animal in Iraq until the firest recorded cases of chrysomya bezziana in 1996 in Baghdad (OIE1996, Abdul-Rassol 1996) (Al.ani 1997).Veterinary directorate informed the national organization FAO,OIE ,AOAD to control the outbreak with international effort. A result of the survillence program was held through a team in the veterinary directorate with representive veterinarian in each vet hospital in each province and the distribution of Traps at all vet. dispensaries through all provinces to detect the different type of fly under the leading of the first auther and the diagnosis of the adult fly and larvae by the staff of Entomology unit and the confirmation the last auther.this paper discuss the myiasis cases detected in domestic animals in Iraq during the year 2017 and to dedicated the last auther who died during preparation of the this paper at 2018.

II. Materials and Methods

Myiasis cases were obtained from the national team veterinarias in each province from veterinary hospitals extracting larvae from animals and send them to central veterinary Diagnostic Laboratory, Entomology unit for the identification with complete history of the cases. Larvae were collected from deep wound at least ten larvae from each case dippend in worm water and then in 70% alcohol and examined by stereomi-croscope, diagnosis of larvae will be according to Spradbery1991.

Table (1): Myiasis cases of domestic animals in Iraq

No.	Kind of Myiasis	Myiasis agent		Host		Date of collection	Locality
		Species name	Larval stage	Species name	Site of infestation		
1	Wound	*Ch. bezziana*	Third	Sheep	Fatty tale	O8.O1.2017	Basrah
2	Wound	*Ch. bezziana*	Third	Sheep	Fatty tale	17.01.2017	Rashdiya,Baghdad
3	Wound	*Ch. bezziana*	Third	Sheep	Fatty tale	24.01.2017	Al-Wehda, Baghdad
4	Wound	*L. sericata*	Third	Cattle	Fatty tale	24.01.2017	Al-Taji, Baghdad
5	Wound	*Ch. bezziana*	Third	Sheep	Fatty tale	06.02.2017	Basrah
6	Wound	*L. sericata*	Third	Sheep	Leg	28.03.2017	14July, Baghdad
7	Wound	*Ch. bezziana*	Third	Dog	Head	03,04.2027	Al-Jablia, Basrah
8	Wound	*Ch. bezziana*	Third	Sheep	Fatty tale	17.04.2017	Basrah
9	Wound	*Ch. bezziana*	Third	Sheep	Fatty tale	26.04.2017	Abu Al-KhasibBasrah
10	Ophthalmic	*Ch. bezziana*	Third	Cattle	Eye	26.04.2017	Shatt Al-Arab, Basrah
11	Wound	*Ch.megacephala*	Third	Sheep	Leg	02.05.2017	Rashdiya,Baghdad
12	Wound	*Ch. bezziana*	Third	Cattle	Thigh	09.05.2017	Shatt Al-Arab, Basrah
13	Wound	*Ch. megacephala*	Third	Sheep	Fatty tale	10.05.2017	Wasit
14	Aural	*Ch. bezziana*	Third	Dog	Ear	14.05.2017	Lilian, Basrah
15	Wound	*Ch. bezziana*	Third	Sheep	Fatty tale	21.05.2017	Al-Mohanawiya,Diwaniya

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16	Urogenital	*Ch. bezziana*	Third	Cattle	vagina	23.05.2017	Al-Wehda, Baghdad
17	Wound	*Ch. bezziana*	Third	Buffalo	Abdomen	23.05.2017	Al-Wehda, Baghdad
18	Oral	*Ch. bezziana*	Third	Dog	Mouth	25.05.2017	Abu-Alkhasib, Basrah
19	Rectal	*Ch. megacephala*	Third	Cattle	Anus	29.05.2017	Rashdiya,Baghdad
20	Wound	*Ch. megacephala*	Third	Goat	Leg	29.05.2017	Rashdiya,Baghdad
21	Aural	*Ch. bezziana*	Third	Sheep	Ear	30.05.2917	Al-Qurnah, Basrah
22	Wound	*Ch. bezziana*	Third	Cattle Calf	Eye	11.06.2017	Al-Mohanawiya,Diwaniya
23	Wound	*Ch. bezziana*	Third	Sheep	Fatty tale	13.06.2017	Ghamas, Diwaniya
24	Aural	*Ch. bezziana*	Third	Dog	Ear	15.06.2017	Al-Moqdadiya, Diyala
25	Wound	*Ch. bezziana*	Third	Cattle Calf	Umbilicus	04.07.2017	Al-Wehda, Baghdad
26	Urogenital	*Ch. bezziana*	Third	Cattle	Vagina	04.07.2017	Al-Wehda, Baghdad
27	Wound	*Ch. bezziana*	Third	Sheep	Fatty tale	04.07.2017	14July, Baghdad
28	Wound	*L. sericata*	Third	Sheep	Horn	04.07.2017	14July, Baghdad
29	Wound	*L. sericata*	Third	Sheep Ewe	Udder	04.07.2017	14July, Baghdad
30	Wound	*Ch. bezziana*	Third	Cattle	Flank	12.07.2017	Baqoba, Diyala
31	Wound	*Ch. bezziana*	Third	Sheep	Fatty tale	16.07.2017	Rashdiya,Baghdad
32	Wound	*Ch. bezziana*	Third	Sheep	Fatty tale	01.08.2017	Rashdiya,Baghdad
33	Wound	*Ch. bezziana*	Third	Sheep Lamb	Umbilics	01.08.2017	Rashdiya,Baghdad
34	Aural	*Ch. bezziana*	Third	Sheep	Ear	06.08.2017	Abu-SaidaDiyala
35	Aural	*Ch. bezziana*	Third	Sheep	Ear	15.08.2017	Rashdiya,Baghdad
36	Wound	*Ch. bezziana*	Third	Sheep	Femoral	15.08.2017	Rashdiya,Baghdad
37	Wound	*Ch. bezziana*	Third	Cattle Calf	genu	14.09.2017	Jorf Al-Nadaf, Baghdad
38	Aural	*Ch. bezziana*	Third	Sheep	Ear	18.09.2017	Rashdiya,Baghdad
39	Rectal	*Ch. bezziana*	Third	Cattle	Anus	18.09.2017	Rashdiya,Baghdad
40	Rectal	*Ch. bezziana*	Third	Cattle	Anus	19.09.2017	Al-Taji, Baghdad
41	Wound	*Ch. bezziana*	Third	Cattle	Genu	18.10.2017	Diyala bridge
42	Wound	*Ch. bezziana*	Third	Sheep Lamb	Fatty tale	18.10.2017	Diyala bridge
43	Rectal	*Ch. albiceps*	Second	Cat	Anus	19.10.2017	Yarmuk, Baghdad
44	Wound	*Ch. megacephala*	Third	Sheep Ewe	Udder	21.10.2017	14July, Baghdad
45	Wound	*Ch. megacephala + Ch. alpiceps*	Third	Sheep	Thigh	21.10.2017	14July, Baghdad
46	Wound	*Ch. Megacephala + Ch. alpiceps*	Third	Cattle Calf	Umbilics	22.10.2017	14July, Baghdad
47	Wound	*Ch. Megacephala*	Third	Sheep Ram	Thigh	22.10.2017	14July, Baghdad
48	Ophthalmic	*Ch. Megacephala + Ch. alpiceps*	Third	Cattle Calf	Eye	24.10.2017	14July, Baghdad
49	Wound	*Luciliasericata+Ch. albiceps*	Third	Sheep Ewe	Thigh	25.10.2017	14July, Baghdad
50	Urogenital	*Ch. Megacephala+Ch. albiceps*	Third	Cattle	Vagina	26.10.2017	14July, Baghdad
51	Wound	*Ch. Megacephala+Ch. albiceps*	Third	Sheep Ewe	Fatty tale	26.10.2017	14July, Baghdad
52	Urogenital	*Ch. bezziana*	Third	Goat	Vagina	31.10.2017	Baghdad
53	Wound	*Ch. bezziana*	Third	Goat	Udder	01.11..2017	14July, Baghdad
54	Wound	*Ch. bezziana*	Third	Sheep Ewe	Fatty tale	01.11.2017	14July, Baghdad
55	Wound	*Ch. bezziana*	Third	Sheep	Fatty tale	01.11.2017	14July, Baghdad
56	Ophthalmic	*Ch. bezziana*	Third	Cattle Calf	Eye	01.11.2017	14July, Baghdad
57	Wound	*Ch. bezziana*	Third	Sheep	Fatty tale	05.11.2017	14July, Baghdad

58	Wound	*Ch. bezziana*	Third	Cattle Calf	Umbilicus	05.11.2017	14July, Baghdad
59	Wound	*Ch. bezziana*	Third	Sheep Ewe	Thigh	12.11.2017	Abu-Ghraib, Baghdad
60	Wound	*Ch. bezziana*	Third	Cattle	Flank	12.11.2017	ShikhHamad, Baghdad
61	Wound	*Ch. bezziana*	Third	Dog	Fore leg	12.11.2017	Baghdad
62	Wound	*Ch. bezziana*	Third	Sheep	Thigh	13.11.2017	Rashdiya,Baghdad
63	Rectal	*Ch. bezziana*	Third	Goat	Anus	21.11.2017	Abu-SaidaDiyala
64	Wound	*Ch.megacephala* + *Ch. albiceps*	Third	Cattle	Neck	23.11.2017	ShikhHamad, Baghdad
65	Wound	*L. sericata*	Third	Sheep	Back	27.11.2017	Rashdiya, Baghdad
66	Wound	*Ch. bezziana*	Third	Sheep	Fatty tale	07.12.2017	Al-Mohanawiya, Diwaniya
67	Wound	*Ch. bezziana*	Third	Sheep	Fatty tale	11.12.2017	Al-Taji, Baghdad
68	Wound	*Ch. megacephala* *L. sericata*	Third	Sheep Ewe	Fatty tale	11.12.2017	Al-Taji, Baghdad
69	Wound	*Ch. bezziana*	Third	Sheep	Hind leg	11.12.2017	Al-Dora, Baghdad
70	Wound	*Ch. bezziana*	Third	Sheep	Fatty tale	12.12.2017	Rashdiya,Baghdad

III. Result and Discussions

A total of seventy cases of Myiasis have been collected in study area.

Table (2): Number of animal Myiasis cases according to the causal agent and hosts detection, Iraq

Agent species	Host						Sum	Percent (%)
	Buffalo	Cat	Cattel	Dog	Goat	Sheep		
Ch. albiceps	00	01	02	00	00	02	05	6.67
Ch. bezziana	01	00	14	05	03	27	50	66.67
Ch. megacephala	00	00	05	00	01	07	13	17.34
L. sericata	00	00	01	00	00	06	07	9.34

As show in table no-1wound Myiasis 51 Cases, Aural 6 cases, Rectal 5 cases, Urogenital 4, cases, Ophthalmic 3 cases, Oral 1 cases.

In table no(.2) Four species chrysomybezziana, ch.megacephala, ch.albices and lucilia sericata were identified as etiological agent of the myiasis, Ch. Bezziana (50) cases represent 66.67%, ch.megacephala (13),17.34% L.sericata (7), 9.34%, ch.alpiceps (5), 6.67%.

Among animals most myiasis were determined in sheep (39), represent 5.57%, then catlle (20), 2.85%, dog (5),0.71% ,Goat (4),0.57%, buffalo (1),0.14% and cats1.70 .*Ch. bezziana* (50), *Ch. megacephala* (13), *L. sericata (*7), *Ch. albiceps* 5. 75 *Ch. bezziana* 66.67%, *Ch. megacephala* 17.34%, *L. sericata* 9.34%, *Ch. albiceps* 6.67%) 3. Sheep 39, Cattle 20, Dog 5, Goat 4, Buffalo 1, Cat 1.70

Among animal the part of animal body envolved as fallow Fatty tail 22 case, Thigh 6 case, Ear 6 case, Anus 5 case, Leg 5 case, Umbolicus 4 case, Vagina 4 case, Eye 4 case, Udder 3 case, Genu 2 case, Flank 2 case and one case for each of: Abdomen, Back, Femoral, Head, Horn, Mouth, Nek. Among months the number of regesterd cases are in November 13 case, October 12 case, May 11 case, July 7 case, August 5 case, December 5 case, September 4 case, Janeuary 4 cases, Apri 4 casel, June 3 case, February 1 case, March 1 case.

There are many literature about myiasis in animals or human worldwide (Zumpts, 1965 and spradbery, 1991)inIraq. Abul-hab, 1980, Al-Ani 2014, Abdul-rassoul etal 2018) In this studay chrysomya bezziana still the most important causes of myiasis and was the predominant species in Iraq. L.sericata was also detected as a myiasis causing agent and this in agreement with result of abdul_rassoul etal, 2018. It was observed during the diagnosis of larvae that the third stage larvae were found in the most of the cases, 1stage and second stage larvae were very less detected fallow up myiasis in Iraq still continuing through a strict programe during every year.

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In Vitro Fermentation of Rice Bran by *Ruminococcus* Sp. for Desirable Chemical Changes as Feed for Livestock

By M A Alam, M S R Khan, K M S Islam, U Dickhöfer & M A Grashorn

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Abstract- Using rice bran in broiler diets has limitation due higher content of fiber and lower availability of few micronutrients including phosphorus. So, they were fermented anaerobically using 10% *Ruminococcus albus* isolated from rumen of cattle to get fermented value-added feed ingredient. It was fermented for 48 hours at 39°C giving 60% moisture at different conditions like FRB (Rice Bran treated with *Ruminococcus* sp.), UFRB (Rice Bran treated with 2.0% urea using *Ruminococcus* sp.), MFRB (Rice Bran treated with 5.0% molasses using *Ruminococcus* sp.), UMFRB (Rice bran treated with 2% urea & 5% molasses using *Ruminococcus* sp.). The protein content was increased in UFRB (18.43%), UMFRB (17.19%) in comparison to RB group (14.42%) where UFRB showed highest crude protein ($p<0.05$). The crude fiber was decreased in FRB (11.64), UFRB (9.92), MFRB (11.67), and UMFRB (10.83) in comparison to RB (12.57%). Phytate-P was also decreasing in UFRB (1.00%), MFRB (1.00%), UMFRB (0.82%) then to RB (1.13%). So, in vitro fermentation using *Ruminococcus spp.* reduces phytate-P and fiber content (CF and ADF) and increase crude protein of RB and UFRB.

Keywords: in vitro, fermentation, ruminococcus spp. rice bran, feed.

GJMR-G Classification: NLMC Code: QW 4

Strictly as per the compliance and regulations of:

In Vitro Fermentation of Rice Bran by *Ruminococcus* Sp. for Desirable Chemical Changes as Feed for Livestock

M A Alam [α], M S R Khan [σ], K M S Islam [ρ], U Dickhöfer [ω] & M A Grashorn[¥]

Abstract- Using rice bran in broiler diets has limitation due higher content of fiber and lower availability of few micronutrients including phosphorus. So, they were fermented anaerobically using 10% *Ruminococcus albus* isolated from rumen of cattle to get fermented value-added feed ingredient. It was fermented for 48 hours at 39˚C giving 60% moisture at different conditions like FRB (Rice Bran treated with *Ruminococcus* sp.), UFRB (Rice Bran treated with 2.0% urea using *Ruminococcus* sp.), MFRB (Rice Bran treated with 5.0% molasses using *Ruminococcus* sp.), UMFRB (Rice bran treated with 2% urea & 5% molasses using *Ruminococcus* sp.). The protein content was increased in UFRB (18.43%), UMFRB (17.19%) in comparison to RB group (14.42%) where UFRB showed highest crude protein ($p<0.05$). The crude fiber was decreased in FRB (11.64), UFRB (9.92), MFRB (11.67), and UMFRB (10.83) in comparison to RB (12.57%). Phytate-P was also decreasing in UFRB (1.00%), MFRB (1.00%), UMFRB (0.82%) then to RB (1.13%). So, in vitro fermentation using *Ruminococcus spp.* reduces phytate-P and fiber content (CF and ADF) and increase crude protein of RB and UFRB.

Keywords: in vitro, fermentation, ruminococcus spp. rice bran, feed.

I. Introduction

Rice bran is a major cereal agricultural by-products in rice-based agricultural countries like Bangladesh and has the potential as a feed ingredient. However, its utilization, especially for poultry is limited. The limitation of its use was due to its high fiber content, low protein and antinutritional factors such as Phytic acid as phytate. These antinutritive factors have been reported by Khalique *et al*., (2003) cause reduction of feed intake and depressed performance of broiler.

Nutritionally, several factors limited its use in poultry, especially broiler chicken diet. Almost half of phosphorous are in phytates form. Hull adulteration is a factor reducing the quality of rice bran (Farrell, 1994). High level of ash content indicates high level of hull (Warren and Farrell, 1990). Previous researches had attempted to use different techniques like fermentation (wizna *et al*., 2012), enzyme supplementation (Tirajoh *et al*., 2010) and the inclusion of fermented product (Kompiang *et al*., 1995) in increasing rice bran utilization for poultry feed.

Fermentation is one of the most advantageous approaches to improve the nutritive value of rice bran (Hardini, 2010). Microorganisms induced fermentation processes transformations of their metabolic activity and also increase the availability of nutrients in raw materials (Pelizer, Pontieri, & Moraes, 2007) which has been widely adopted to develop novel functional ingredients because this process may promote their functional quality such as antioxidant (Lee *et al*., 2008; Hardini, 2010; Wang *et al*., 2011; Cao *et al*., 2012; Kim *et al*., 2012) and optimize the use of rice bran in poultry feeding. Bidura *et al*., (2012) found that inclusion of yeast (*Saccharomyces cerevisiae*) increases the bioavailability of minerals and nutrients of rice bran and increase growth performance of male bali duckling. Also, fermentation of rice bran with *Aspergillus niger* caused change of nutrient content as poultry feed (Hardini, 2010).

Rice bran consisting of cellulose as the major component composed of cellulose, hemicellulose and lignin are coarse fiber which has some the limitations of the use of rice bran as feed in the broiler due to lack of lignocellulosic enzymes producing by digest tract but enzymes can be aided to hydrolyze the cellulose. This is different to ruminants (cattle, sheep, goats), rumen microbes producing lignocellulosic enzymes help the degradation of cellulose and hemicellulose (Muthukrishnan, 2007) by the species of cellulolytic bacteria which are *Fibrobacter succinogenes*, *Ruminococcus flavefaciens* and *R. albus* (Julliand *et al*., 1999; Koike *et al*., 2000; Chen and Weimer, 2001; Koike and Kobayashi, 2001). Cellulolytic ruminococci play a major role in the breakdown of plant cell wall material in the rumen (Bryant *et al.,* 1958; Dehority *et al.,* 1967; Sijpesteijn *et al.,* 1951; Flint *et al.,* 2008) that effectively reduced fiber and increased crude protein from corn stacks with the supplementation of Urea (3% w/w) and Molasses (5% w/w) (Gado *et al*., 2007; Supyiyati., 2012) due to effect of the non-protein nitrogen contribution from urea (Fontenot *et al*., 1983) also serves an important role in the metabolism of nitrogen-containing

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compounds by animals (Wizna *et al*., 2012) that increases the crude protein content of feed materials including rice milling waste (Amaefule *et al*., (2003).

In this present study, a fermentation technique was used in an attempt to improve the quality of rice bran. *Ruminococcus* sp. was used as the inoculum since it had been reported to produce the various cellulosomal types of enzyme complex which possesses a potential to degrade fiber (Flint *et al.,* 1997) supplementation with urea (3% w/w) and molasses (5% w/w) which supports fermentation media and stimulate the growth of microorganisms to change the nutritional value of rice bran.

II. Materials and Methods

The present study was carried out at the Department of Microbiology and Hygiene, Faculty of Veterinary Science and Department of Animal Nutrition, Faculty of Animal Husbandry, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

a) Bacterial Culture

Rumen ingesta was obtained through a permanent rumen fistula from the Sahjalal Animal Nutrition Field Laboratory to the analytical laboratory of the Department of Animal Nutrition Bangladesh Agricultural University, Mymensingh-2202 in strictly anaerobic conditions within half an hour for further processing.

Rumen liquor was obtained approximately 8hr. after feeding, strained through two thicknesses of cheesecloth, and collected in a 500 ml. centrifuge bottle. Air was excluded by completely filling the bottle, and closing it with a rubber stopper. The bottle was then held overnight at 2°C and centrifuged at *1200 g* for 10 min. before use.

Samples of rumen contents were 10 fold serial diluted in pre-reduced anaerobic diluents solutions (ADS) in the serum bottle with rubber stopper by anaerobic techniques up to 10^{-8} dilution (Hungate, 1966) then samples were cultured into the pre-reduced specific media contained serum bottle for rumen bacteria using 1mL syringe; Rumen Fluid Glucose Cellobiose agar (RGCA) medium which was prepared under continuous CO_2 flow and incubate in Anaerobic Jar (OXOID, England) at 39°C for 48 hours. Commercial CO_2 was freed from 0_2, by passing it over heated reduced copper gauze. The RGCA growth media contained: 15 mL Mineral Solution I (KH_2PO_4 3.0g; $(NH_4)_2SO_4$ 6.0g; NaCl 6.0g; $MgSO_4$ 0.6g; $CaCl_2{\cdot}2H_2O$ 0.795g p/L), 15 mL Mineral Solution II (K_2HPO_4 0.3 g/L), 0.25g Yeast Extract, 1g Tryptone, 0.1mL Resazurine (0.1%), 0.2mL Hemin (0.05%), 0.5g Microcrystaline Cellulose, 0.1g Cellobiose, 0.4g Sodium Carbonate, 20 mL Clear Rumen Fluid, 50mL Distilled Water and 50mg Cysteine Hydrochloride. Adjust pH to 6.7 with NaOH.

Morphological characteristics of the bacteria was cocci, single or pair, (figure-2) always gram-positive, non-motile. Some were rod shape. Short chain also found. None produced catalase, indole. Those bacteria were fermented cellulose and cellobiose. The acid produced from glucose, d-xylose and cellobiose. Hydrolysis of starch and gelatin liquefaction occurred. Presence of zone of clears around the colony and produce yellow pigment. Positive biochemical test samples were kept for DNA preparation and PCR.

The ADS media contained; 350 mL distilled H_2O, 0.1349g K_2HPO_4, 0.1349g KH_2PO_4, 0.2697g NaCl, 0.02697g $MgSO_4$, 0.0357g $CaCl_2{\cdot}2H_2O$, 0.2697g $(NH_4)_2SO_4$, 3 drops of 0.1% resazurin. After boiling and cooling, slowly add 0.9g Na_2CO_3. Bubble overnight (until color turns pink). Then add 5 mL of 3% (w/v) L-cysteine hydrochloride. Continue bubbling until colorless (usually requires 1 to 4 h). Dispense to serum bottle and autoclave.

DNA Isolation and PCR Amplification: Total DNA extraction was performed with the QIAamp DNA Stool Kit (QIAGEN, Germany). Species-specific primer sets that amplify 16S rRNA of *Ruminococcus albus,* available to detect these species in rumen microbial ecosystems (Tajima *et al*., 2001; Koike and Kobayashi, 2001). The PCR mixture was performed using 1X PCR buffer (60 mM Tris-SO_4 pH 8.9, 18mM ammonium sulphate), 0.25mM dNTPs, 2mM $MgSO_4$, 0.2 mM primer, 1U of Platinum Taq High Fidelity (Invitrogen, USA), 20ng of genomic DNA and DNA/RNA free water adjusted to a total volume of 50µL. The PCR condition was 95°C for 5 min followed by 30 cycles of 94°C for 30 sec/cycle for denaturing, annealing at 60°C(Table 1) for 30 sec and finally 68°C 45sec for elongation, using a PxE 0.2 thermal cycler (Thermo electron corporation, USA). The PCR products were separated by 2% agarose gel electrophoresis using the molecular weight marker 100bp Ladder (Promega, USA) and the image was captured with a gel image analyzer. The purified PCR product was stored and will be sent for sequencing. The isolates were again confirmed by using the specific primer of bacteria.

The DNA fragments of the expected size (Table 1) were amplified from all the samples tested a representative image of the amplification after gel electrophoresis is shown in Figure 1.

Table-01: Species-Specific primers sequences for 16S RNA genes used in this study

Bacterium	Primer name	Sequence (5´-3´)	Annealing temp. (°C)	Product size (bp)	Ref.
Ruminococcus albus	Ra1281 f	CCCTAAAAGCAGTCTTAGTTCG	60	175	Koike and Kobayashi, 2001
	Ra1439 r	CCTCCTTGCGGTTAGAACA			

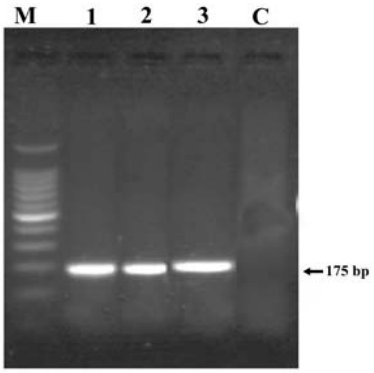

Figure 1: PCR detection of cellulytic bacteria from the rumen contents using species-specific primers (175bp) for *Ruminococcus albus* (1 & 2) MWM: 100bp ladder molecular weight marker.

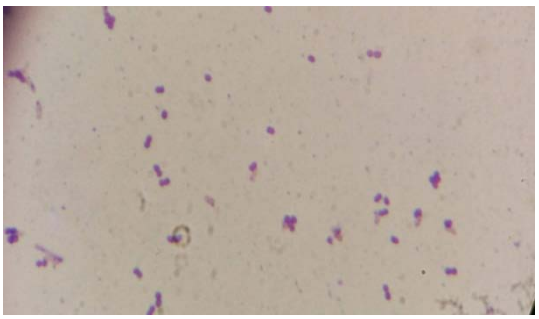

Figure 2: *Ruminococcus spp x 100*

b) Fermentation of Rice Bran

Rice bran was used throughout the study and was gathered from a local market and screened to remove any impurities and dirt through a sieve. It was kept in a clean polythene bag in the laboratory until used. Rice bran was diluted using carbonated water to get different moisture content at 60% level. 10% bacterial inoculum (on DM basis) were added in the diluted rice bran mixed with 2% urea (UFRB), 5% molasses (MFRB) and 2% urea plus 5% molasses (UMFRB) separately or combinedly. Anaerobic fermentation continued for a period of 48 hours at 39°C in sealed serum bottle. After fermentation of fermented rice bran was immediately transferred to the refrigerator to stop further fermentation. pH, Proximate components (CP, CF, ADF, NDF and Ash), Total-P and Phytate-P were determined before and after fermentation of rice bran in accordance with AOAC (2005). These are the fermentated groups; *RB*: Rice Bran (control), *RBB*: Rice Bran treated with *Ruminococcus* sp. *UFRB*: Rice Bran treated with 2% urea using *Ruminococcus* sp. *MFRB*: Rice Bran treated with 5% molasses using *Ruminococcus* sp. *UMFRB*: treated with 2% urea & 5% molasses using *Ruminococcus* sp.

c) Chemical analysis

The proximate analysis of ingredients was measured by AOAC (2005). The crude protein content was measured by macro Kjehdahl digestion unit using Kjeltec 1030 and Auto analyzer procedure using autoanalyzer. Total phosphorus was measured according to AOAC (1980) and Phytate-phosphorus was determined according to Latta and Eskin, (1980).

d) Statistical analysis

All variables were subjected to analysis of variance (ANOVA) (Duncan, 1955) in a completely randomized design (CRD) by the statistical package using statistical computer package program (SPSS). Tukey pairwise comparisons were used to compare treatment means (Steel and Torrie, 1980).

III. Results and Discussion

According to Morphological characteristics they were all gram positive coccoid and showed catalase & indole negative, cell arrangement were single or diplococci belong to the genus *Ruminococcus* sp. (Bryant *et al*, 1959) (figure-2). This bacterium including species *(R. albus*) was confirmed identified by molecular techniques (Koike and Kobayashi, 2001) and used for the fermentation of rice bran.

Table-02: The composition of different Fermented Rice Bran

Parameters	Fermented groups				
	RB	RBB	UFRB	MFRB	UMFRB
pH	*6.62^{a}±0.03	5.44^{cd}±0.01	6.16^{b}±0.04	5.35^{d}±0.01	5.62^{c}±0.18
Crude Protein (CP)	14.42^{bc}±0.21	13.99^{c}±0.50	18.43^{a}±3.30	13.20^{c}±0.29	17.19^{ab}±0.44
Crude Fiber (CF)	12.57^{a}±0.22	11.64^{ab}±0.41	9.92^{b}±1.38	11.67^{ab}±0.79	10.83^{b}±0.09
Total Phosphorus	3.29±08.	2.99±0.30	3.36±0.34	3.28±0.55	2.95±0.29
Phytate-P	1.13^{a}±0.03	1.21^{a}±0.20	1.00^{ab}±0.07	1.00^{ab}±0.05	0.82^{b}±0.05
Ash	12.08±0.80	11.96±0.30	11.01±2.11	11.43±0.28	10.58±0.62
ADF	24.07±4.75	20.60±2.37	18.52±0.48	17.75±1.37	20.23±2.17

RB: Rice bran (control), RBB: Rice bran treated with *Ruminococcus* sp, UFRB: Rice bran treated with 2% urea using *Ruminococcus* sp. MFRB: Rice bran treated with 5% molasses using *Ruminococcus* sp.UMFRB: Rice bran treated with 2% urea & 5% molasses using *Ruminococcus* sp.

*Mean±SD; ^{abc}Means with dissimilar superscripts are significantly different ($p<0.05$)

Our Study observed that pH changes from 6.62 to 5.35 which were decreased. Results indicate that the phytate degrading enzymes from rice bran were active in the first six hours of the process. The pH changes during production of phytase in the rice bran media over 10 weeks were observed. Initial 3 weeks, a reduction in pH from pH 6 to pH 4.2 (Abd-ElAziem Farouk, 2017). The optimum initial pH for phytase production of *B. cereus* was pH 7.2 (Vohra and Satyanarayana, 2003). pH changes are considered to be due to the production of sugar molecule to an equimolar mixture of organic acids, ethanol and carbon dioxide by fermentation and the period of microbial growth during fermentation (Mackenzie, *et al*., 1965; Prabhu, *et al*., 2014).

In this study after 48 hours anaerobic fermentation of rice bran with *Ruminococcus albus*, the data of Table-2 showed that the crude protein was significantly increased in UFRB (18.43%), UMFRB (17.19%) than control RB (14.42%) but decreased in RBB (13.99), MFRB (13.20%). The highest crude protein was found in UFRB (17.19%) ($p<0.05$). On another hand, the data of table-02 clearly showed that crude fiber and phytate-P content was significantly decreased in all the treated groups RBB, UFRB, MFRB and UMFRB than RB control. The lowest crude fiber was found in UFRB (9.92%) ($p<0.05$).These results indicate that the cellulytic bacteria of rumen can improve the quality of rice bran that increased the CP with the addition of urea and molasses. The result also supported that rice bran contain cellulose as the major component, which is best for the growth of microorganisms and the production of single cell protein biomass (Yunus *et al*., 2015; Khin *et al*., 2011) which increase the crude protein content of rice bran (Sukaryana, 2001) with the addition of urea in the UFRB using cellulolytic bacteria *B. amyloliquefaciens* as an inoculum improved fermentation and its microbial population (Wizna *et al*. 2012). Protein content was also increased after fermentation of cassava waste Supriyati (2002) that agree with the result of the present experiment as protein content was increase when urea and molasses were added during fermentation (Supriyati and Kompiang, 2002). In this study, UFRB showed highest CP (18.435). *Ruminococcus* sp. produces the various cellulosomal type of enzyme complex which possesses a potential to degrade fiber (Flint, 2008). In this study, crude fiber was decreased in rice bran using *R. albus* which supports the results of Galil (2008), using bacterial treatments (*Ruminococcus albus and Cl. cellulovorans*) caused increases crude protein (from 1.45 to 15.16) and decreases in crude fiber (from 44.08 to 28.44%) of rice straw. Wizna *et al.,* (2009) also found that is inoculation of *B. amyloliqfacience* was increased enzymes activities during fermentation of cassava waste that produces many kinds of enzymes to decrease crude fiber.

On the other hand, MFRB and RBB could not increase crude protein due to lack of additional nitrogen source to grow microbes that nitrogen was a crucial component needed by ruminal microbes after carbon and oxygen (Griffin, 1991) which need a much amino acid higher.

There was a decrease in phytate-P in a definite order in UFRB (1%), MFRB (1%), UMFRB (0.82%) than RB (1.12%) control but increase in RBB (1.21%) ($p<0.05$). Ravindran (1995) reported that among the common feedstuff sesame meal and rice bran have the highest level of phytate but after fermentation by *Ruminococcus albus*, phytate-P was decreased. Yanke *et al.,* (1998) reported that the presence of phytase activity was investigated in 334 strains of 22 species of obligatory anaerobic bacteria that decrease phytate phosphorus in fermentation of rice bran by using rumen liquor. this results also agreed with hungate (1966) that Phytate phosphorus degrades by rumen microbes.

After fermentation of rice bran, there was no significant difference in total-P, ADF and ash content ($p<0.05$) but the difference in numerically. However, Total phosphorus content were within the range of 1.26-1.79% reported by Ukil (1999) and 1.62-1.81% reported by Warren and Farrel (1990c). The variations in nutrient composition might be due to the sources from which the bran was obtained. The chemical composition of rice bran varies due to the variation in the milling process and adulteration with hull (Warren and Farrel, 1990a). In this study, Total phosphorus was higher than that report.

IV. Conclusions

It can be concluded that under this study fermentation of rice bran using *Ruminococcus albus* isolate from rumen liquid from cattle might improve nutritional value i.e. increase crude protein and decrease crude fiber, Phytate-phosphorus. However, animal experiments are required to confirm the effectiveness of fermented rice bran using *Ruminococcus albus*.

Acknowledgments

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Performance of Avian Influenza Surveillance System, Ogun State Nigeria, 2015-2019

By Folajimi. O. Shorunke, Aisha Usman, Tade Adeniyi Olanrewaju, Ndadilnasiya Endie Waziri & Kia Grace S.N

Abstract- *Background:* In 2019, two Highly pathogenic avian influenza (HPAI) A(H5N8) outbreaks in poultry establishments in Bulgaria, two of wild birds in Denmark and one low pathogenic avian influenza (LPAI) A(H5N3) in captive birds in the Netherlands were reported. Nigeria recorded the first outbreak of Highly Pathogenic Avian Influenza (HPAI) in February 2006 in a commercial poultry farm. Nigerian Pandemic Preparedness and Action Plan for Avian Influenza were then used to respond.Although influenza sentinel surveillance has been established in several African countries including Nigeria, data about the performance of established surveillance systems are limited on the continent.We described the avian influenza (AI) surveillance system in Ogun State, accessed veterinary health workers and farmers knowledge, evaluated all its attributes and made recommendations to improve the AI surveillance system.

Methods: We adopted 2001 CDC Updated Guidelines for Evaluating Public Health Surveillance Systems. We reviewed and analyzed passive surveillance data from Ogun State Ministry of Agric, key informant interviews were conducted for relevant stakeholders at the state level and Local Government divisional veterinary clinics and farms to obtain additional information on the operations of the system.

Keywords: *avian influenza, surveillance, evaluation, ogun-state.*

GJMR-G Classification: *NLMC Code: WA 105*

Strictly as per the compliance and regulations of:

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Results: A total of 99,923 birds were affected during the period under review. The knowledge of AI and the six attributes of the Ogun State AI surveillance system evaluated include knowledge (2.4), simplicity (2.5), flexibility (2.3), acceptability (2.2) which were (moderate to good), sensitivity (1.7), stability (1.2) were (weak to moderate) and timeliness (1.0) was (weak). The overall score of the surveillance system was averaged at (1.9) indicating (weak to moderate).

Conclusion: AI surveillance system in Ogun State is simple, flexible and acceptable with good knowledge by officers, but requires improvement in timeliness of data, sensitivity of system, and stability. More training should be conducted quarterly, for all surveillance officers and system's ability to detect cases of AI should be improved by involving more poultry farm workers. More funds and stipends for surveillance officers to improve the stability of the system are desirable.

Keywords: avian influenza, surveillance, evaluation, ogun-state.

I. Introduction

Highly pathogenic avian influenza (HPAI) is a global threat to human and animal health, having high impacts on poor livestock keepers; it has the capacity to cripple the production line of even the most industrious poultry farmer(1). While billions have been spent on the disease by the WHO, FAO and other health partners, response to the epidemic remains fragmented and information channels slow(1), thereby leading to an increase in zoonotic emerging diseases. With the increasing human population over the years, encroachment into the normal habitats of animals keeps occurring and hence increased contacts between humans and their animal domestic and wild neighbors. As these interfaces between wildlife, domestic animals and humans increase an increase in wildlife involvement in emerging diseases can be envisaged(1), Expansion of livestock production, as a result of increase human agricultural needs, especially when the expansion is in proximity to wildlife habitats, has been responsible for disease transmission from wildlife to livestock and vice versa(2)this has increased the likelihood of livestock being reservoir for the evolution and transmission of infections normally restricted to wild life in the sylvatic cycle to human(2). Some wildlife species have adapted to and thrived in the ecological landscape created by human settlement and agriculture and has become reservoirs for disease in livestock and humans. These and other factors are responsible for the occurrence of HPAI and other emerging diseases.

Influenza A viruses is one of the five genera in the Orthomyxoviridae family. They possess an eight-segment, negative- sense, ssRNA genome which is approximately 13 kb in size(3)(4). There are two main groups of influenza A viruses that are responsible for infecting poultry, subtypes H5 and H7, but not all of this two subtypes cause HPAI(5). Other viruses have been known to cause LPAI unless exacerbated by other factors like low immunity, in years past, HPAI viruses were rarely isolated from wild birds, but for LPAI viruses, extremely great isolation rates have been recorded in surveillance studies(6). Humans are solemnly

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responsible for the secondary spread of the disease, usually through movement of infected bird products from one farm to another or by facilitating transfer of infected bird feaces to susceptible birds, but sometimes wild birds could be involved. Different case definitions for AI were proposed by European Union and WHO stating that "For the purposes of this Terrestrial Code, avian influenza in its notifiable form (NAI) is defined as an infection of poultry caused by any influenza A virus of the H5 or H7 subtypes or by any AI virus with an intravenous pathogenicity index (IVPI) greater than 1.2 (or as an alternative at least 75% mortality)"(7)(6)(8).

In Europe, no HPAI infection have been detected in human due to wild birds and poultry outbreaks going on and the risk of zoonotic transmission to the European population is considered to be low(9).The initial incidence of the disease in Hong Kong, 1997 was prelude to the 2003 sporadic outbreaks in Asia. This was the precursor of the virus that was detected in Nigeria which also spread to other African countries like Egypt, Togo and Ivory- coast(10).

Nigeria recorded the first outbreak of HPAI in February 2006 in a commercial poultry farm in Northern Nigeria. The outbreak was not unexpected in the country, because many countries in the world has already started experiencing outbreaks of HPAI and already responded, hence providing Nigeria several templates on emergency preparedness(11). The preparedness plan were intended to be both flexible and dynamic, and includes preparedness and response components that are consistent with the general principles of disaster response and surveillance(8).

Surveillance of animal populations is critical to public health. Since any human pandemic virus is expected to first develop within an animal population and then cross the human-animal interface, the best hope of preventing an influenza outbreak is the early detection of such a virus within the affected animal population. Once identified, operations can be conducted to cull or vaccinate the animal population in which the disease is present and thus inhibit its ability to cross the human- animal interface and develop into a human influenza pandemic. Such surveillance system must be developed with attributes like; usefulness, sensitivity, data quality and the rest(12). Although influenza sentinel surveillance has been established in several African countries, data about the performance of established surveillance systems are limited on the continent(13)(14)(15). Such evaluations would enable countries to assess the performance of their surveillance systems, identify areas for improvement and provide evidence of data reliability for policymaking and public health interventions as well as compliance with international surveillance standards. The objective of Ogun state AI surveillance system includes; enabling quick response to outbreak; detecting trends of disease spread and containment of possible AI spread. We conducted Ogun state AI surveillance system evaluation from January 2015 to December 2019 to describe surveillance system, assess veterinary health workers and farmers knowledge of AI surveillance, assess key systems attributes and make appropriate recommendations on how to improve the surveillance system.

II. Methods

This surveillance system evaluation was conducted with guidance from2001 CDC Updated Guidelines for Evaluating Public Health Surveillance Systems(16).

a) *Study Area*

Ogun State is one of the 36 states in Nigeria, located in the southwestern geopolitical zones. It was created in 1976; it borders Lagos State to the south, Oyo and Osun states to the north, Ondo to the east and the Republic of Benin to the west. Abeokuta is the capital and largest city in the state. It has a total estimated population of 5,685,799 as at December 2019. The major occupation of the indigenes is farming and many are also civil servants. The state is divided into 3 senatorial districts with only 20 local government areas (LGA). There are 8 functional government veterinary clinics in the state which also function as reporting site, although each LGA had a reporting site in the past but all have collapsed due to lack of funds.

b) *Study Population*

We interviewed the Director of veterinary services and their assistants, veterinary officers at the state and zonal veterinary clinics, the state veterinary epidemiologist and poultry farmers with previous outbreak of AI in selected LGAs.

c) *Sampling Technique*

For this study, we divided Ogun state into five zones based on the availability of a government owned veterinary clinic and AI surveillance centre, these zones includes Remo, Ijebu, Yewa, Ota and Egba. Two LGA per zone was randomly selected with one farm and one veterinary health facility per LGA sampled. Farms selected include those with previous history of AI outbreak or those that regularly report related disease to the local veterinary authority (Figure 1).

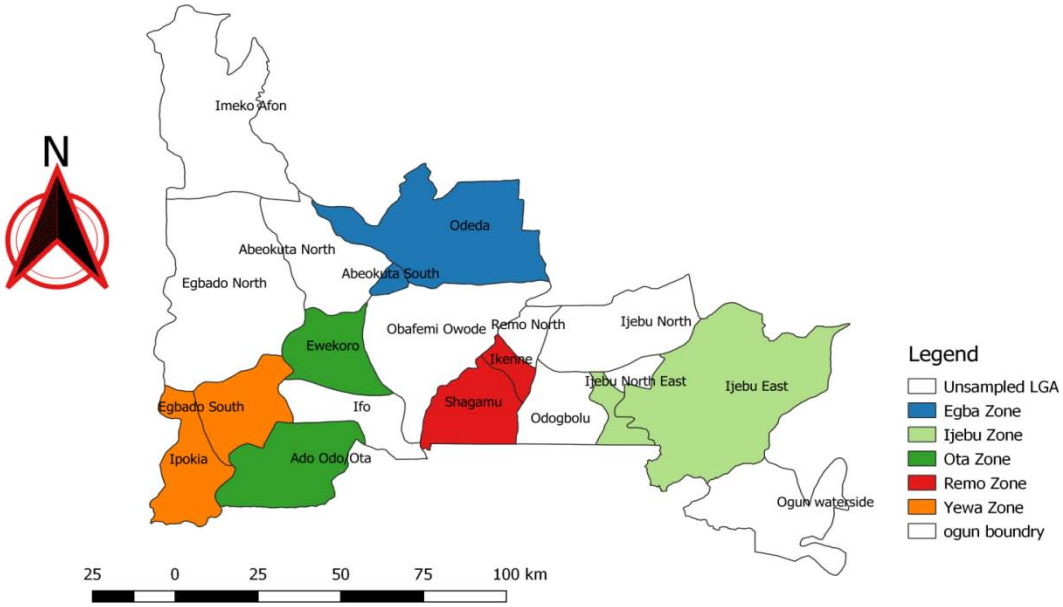

Figure 1: Showing different colors representing selected local government areas sampled per zone

III. Data Collection and Management

We reviewed available records on AI between 2015 and 2019. We extracted data from NADIS disease outbreak reporting forms and data collected from electronic reporting with ODK from the state, LGA, veterinary health facilities and local farms.

We conducted analysis using Microsoft Excel 2007 and Epi-Inf 7.0. Data output was summarized into descriptive forms using charts and tables.

We analyzed the questionnaires and scored the responses for various system attributes; Knowledge, Usefulness, Simplicity, Acceptability and Stability. For consistency and comparability of findings, we used the evaluation method and scoring system utilized for influenza surveillance evaluations conducted in other African countries(17). A scale from 1 to 3 was used to provide a score for each quantitative indicator as follows: < 60% scored 1 (poor performance); 60–79% scored 2 (moderate performance); ≥80% scored 3 (good performance)(17). Thereafter the scores assigned to each indicator were averaged for all indicators evaluated within each attribute to provide an overall score. The 7 evaluated attributes were then average to get an overall score for the surveillance system.

IV. Result

a) Operation of the AI surveillance system

In Ogun State, the AI surveillance system makes use of both active and passive surveillance methods to operate a multilevel and multi directional system.

The passive surveillance makes use of pre-visitation to farms by surveillance officers and state veterinary officers to check on their bio-security and also administer questionnaires to examine their knowledge, attitude and practice about AI. Workshops are conducted with farmers and other stake holders where new information about AI is disseminated and discussion on source of possible outbreaks are made. They are also regularly introduced to their surveillance agents (2 per LGA) for each zonal levels, who they will contact on the eventuality of an outbreak.

The Active surveillance however makes use of all veterinary officers in the 9 health facilities across the zonal levels in Ogun State. Informants (2per LGA) will first inform the closest veterinary health facility within their jurisdiction of the outbreak and veterinary doctors will then be deployed to collect samples from affected farm. This will then be forwarded to the veterinary research institute VOM by the AI desk officer and the state director of veterinary services (DVS), also a notification is forwarded to the National Avian influenza desk officer and the CVO. Results and feedbacks are sent by the reference lab VOM to the national AI desk officer, the affected state DVS and the national CVO. Feedback is also generated downwards towards the farmer (Figure 4).

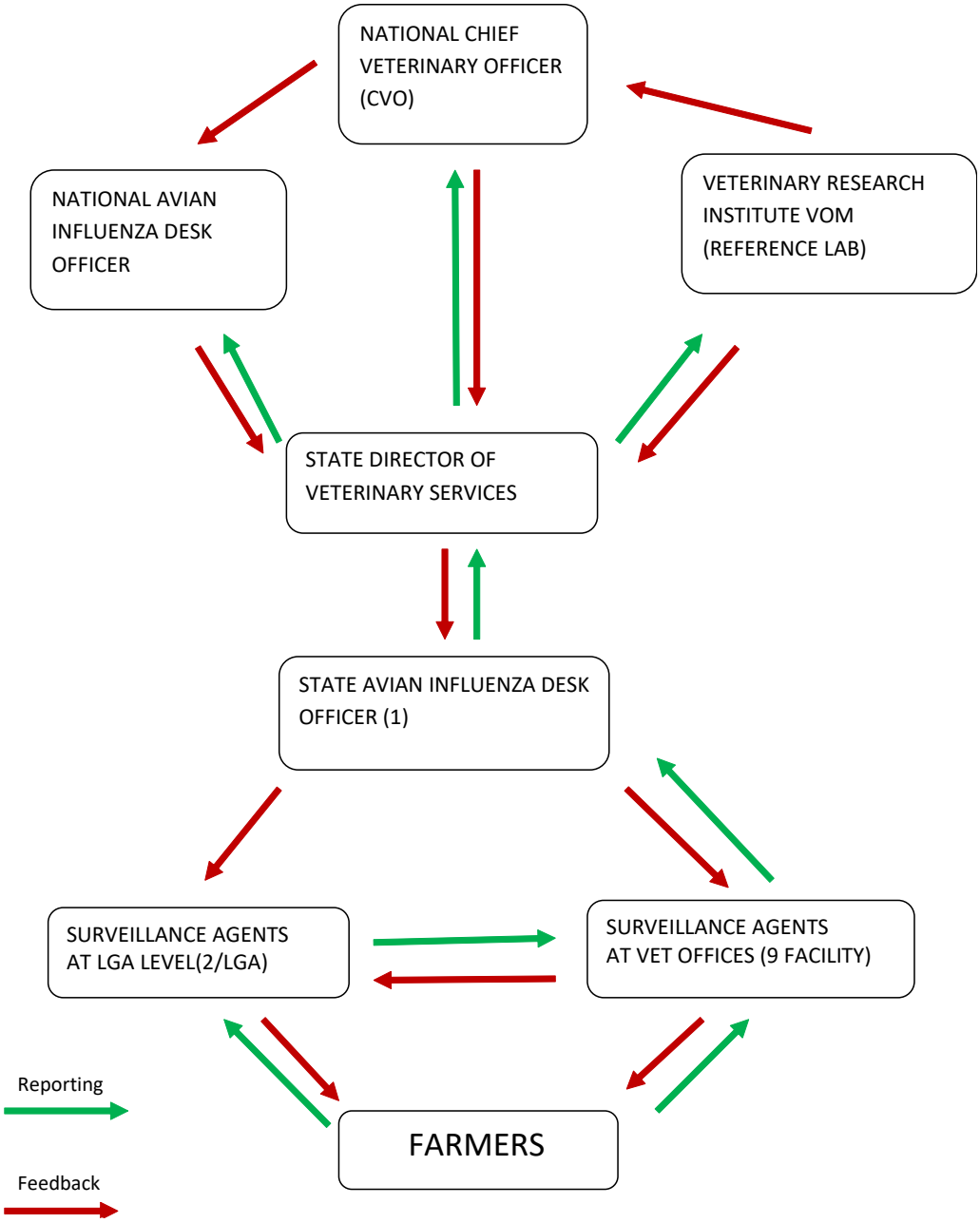

Figure 2: Showing Avian influenza surveillance system flow chart in Ogun State

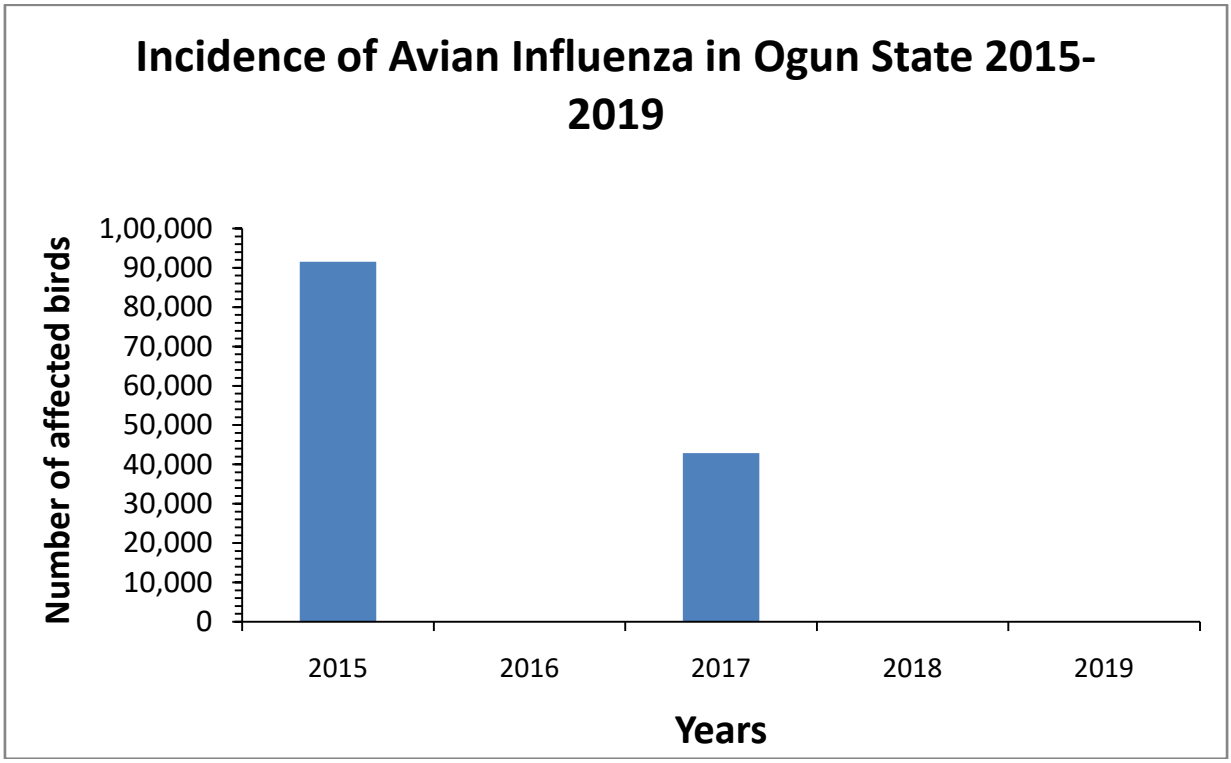

Figure 3: Showing number of affected birds with AI in Ogun State, 2015-2019.

Socio-demographic characteristics

Table 1: Socio-demographic characteristics of respondents

Characteristics		Frequency (n=36)	Proportion (%)
Sex	Male	23	63.9
	Female	13	36.1
Organization	Government hospital	29	80.6
	Poultry farm	7	19.4
Cadre of staff	Vet. doctor	18	50.0
	Vet. technician	8	22.2
	Vet. nurse	7	19.5
	Administrative staff	3	8.3
Years in service	<10 years	24	66.7
	≥10 years	10	27.8
	Unknown	2	5.5

b) System Attributes

Knowledge of AI and system attribute; Simplicity, Flexibility, Acceptability, Timeliness, Sensitivity and Stability were evaluated using extracted data and questionnaires.

Knowledge

Table 2: List of indicators and scores for knowledge, AI surveillance system evaluation Ogun State 2015-2019

Indicator	Calculation/data input	Indicator value	Score
Training			
Perception of surveillance staff on whether training is compulsory with policy.	Number of surveillance staff within each reported category/number interviewed	Yes: 52.9% No : 41.7% Unknown :5.5%	1
Perception of surveillance staff on whether they have been trained on AI surveillance.	Number of surveillance staff within each reported category/number interviewed	Yes: 80.6% No : 19.4%	3
Perception of surveillance staff on the type of training received.	Number of surveillance staff within each reported category/number with training	Formal : 75.9% Informal : 24.1%	2
Perception of surveillance staff on whether training improved performance	Number of surveillance staff within each reported category/number with training	Yes: 90.3% No : 9.7%	3
Perception of surveillance staff on needs for more training.	Number of surveillance staff within each reported category/number interviewed	Yes: 97.2 No : 2.8	3

An estimate of 80.6% of the respondents has received some form of training on AI surveillance, however only 61.1% were formally trained. A total of 35(97.2%) of the respondent affirmed there is need for more training; and (38.9%) agreed that the training should be Quarterly.

Simplicity

Table 3: List of indicators and scores for simplicity, AI surveillance system evaluation Ogun State 2016-2019

Indicator	Calculation/data input	Indicator value	Score
Simplicity			
Perception of surveillance staff on whether surveillance forms are easy to fill.	Number of surveillance staff within each reported category/number interviewed	Yes: 100.0% No : 0.0%	3
Perception of surveillance staff on time used in data collection.	Number of surveillance staff within each reported category (< 2 hours, 2- 8 hours, > 8 hours) / Number of surveillance staff interviewed	≤2 hours : 24.2% 3 - 8 hours : 66.8 >8 hours : 9.0%	2
Perception of surveillance staff on staff strength.	Number of surveillance staff within each reported category (≤ 2 staff, ≥3 staff) / Number of surveillance staff interviewed	≤ 2 staff:30% ≥ 3 staff:70%	2
Perception of surveillance staff on whether staffs are optimal.	Number of surveillance staff within each reported category/number interviewed	Yes: 83.3% No : 13.9% Unknown: 2.8	3

All 36 respondent to the questionnaire agreed that the forms used for AI surveillance were easy to fill. The median estimated time for collection, entering, editing, storing and analysis of surveillance data is 4(1-24) hours.

Flexibility

Table 4: List of indicators and scores for flexibility, AI surveillance system evaluation Ogun State 2016-2019

Indicator	Calculation/data input	Indicator value	Score
Flexibility			
Perception of surveillance staff on whether forms can accommodate change in surveillance system.	Number of surveillance staff within each reported category/ number interviewed	Yes: 94.4% No : 2.8% Unknown: 2.8%	3
Perception of surveillance staff on availability of staff for validating and completeness of data.	Number of surveillance staff within each reported category/ number interviewed	Yes: 91.7% No : 2.8% Unknown: 5.5%	3
Perception of surveillance staff on whether they have been supervised before.	Number of surveillance staff within each reported category/ number interviewed	Yes: 33.3% No : 63.9% Unknown: 2.8%	1

Acceptability

Table 5: List of indicators and scores for acceptability, AI surveillance system evaluation Ogun State 2016-2019

Indicator	Calculation/data input	Indicator value	Score
Acceptability			
Perception of surveillance staff on willingness to continue participation in surveillance.	Number of surveillance staff within each reported category/number interviewed	Yes: 94.4% No : 5.6%	3
Perception of surveillance staff on presence of challenges.	Number of surveillance staff within each reported category /number interviewed.	Yes: 55.6% No : 44.4%	1
Perception of surveillance staff on whether they are appreciated by system.	Number of surveillance staff within each reported category /number interviewed.	Yes: 72.2% No : 27.8%	2
Perception of surveillance staff on whether they have contributed to system.	Number of surveillance staff within each reported category /number interviewed.	Yes: 61.1% No : 33.3% Unknown: 5.6%	2
Perception of surveillance staff on whether suggestion was taken.	Number of surveillance staff within each reported category/ number that had made contribution	Yes: 81.3% No : 18.7%	3

20 (55.6%) agreed there were challenges in carrying out the job which was majorly financial (88.8%).

Timeliness

Table 6: List of indicators and scores for timeliness, AI surveillance system evaluation Ogun State 2016-2019

Indicator	Calculation/data input	Indicator value	Score
Timeliness			
Perception of surveillance staff on availability of policy on timeliness.	Number of surveillance staff within each reported category/number interviewed.	Yes: 47.2% No : 47.2% Unknown: 5.6%	1
Perception of surveillance staff on time it takes for data collation.	Number of surveillance staff within each reported category (≤30min, >30min)/ number interviewed.	≤30min: 36% >30min: 30% Unknown: 34%	1
Perception of surveillance staff on how soon monthly report completed in new month.	Number of surveillance staff within each reported category/ number interviewed.	1st 5 days: 47.2% end of 1st week:27.8% 2nd week:13.9% 3rd week:11.1%	3

The median time in minuets for collation of data is 30(10-300) minutes and (88.9%) of the respondent say they complete their report within the first 2 week'starget of a new month.

Sensitivity

Table 7: List of indicators and scores for sensitivity, AI surveillance system evaluation Ogun State 2016-2019

Indicator	Calculation/data input	Indicator value	Score
Sensitivity			
Perception of surveillance staff on whether they have submitted AI sample before.	Number of surveillance staff within each reported category/number interviewed.	Yes: 61.1% No : 36.1% Unknown: 2.8%	2
Perception of surveillance staff on laboratory diagnosis.	Number of surveillance staff within each reported category/number that have submitted sample.	Good: 63.6% Average : 36.4%	2
Perception of surveillance staff on whether system was able to detect all cases.	Number of surveillance staff within each reported category/number interviewed.	Yes: 36.1% No : 55.6% Unknown: 8.3%	1

Stability

Table 8: List of indicators and scores for stability, AI surveillance system evaluation Ogun State 2016-2019

Indicator	Calculation/data input	Indicator value	Score
Stability			
Perception of surveillance staff on duties with dedicated staff.	Number of surveillance staff within each reported category/number interviewed.	Data recording:66.7% Data storage:47.2% Data analysis:25.0% Data transfer:25.0%	1
Perception of surveillance staff on feedbacks from next level.	Number of surveillance staff within each reported category/number interviewed.	Yes: 55.6% No: 38.9% Unknown: 5.5%	1
Perception of surveillance staff on Interruption of system by inadequate staff.	Number of surveillance staff within each reported category/number interviewed.	Yes: 22.2% No: 75.0% Unknown: 2.8%	2
Perception of surveillance staff on Interruption of system by inadequate funds.	Number of surveillance staff within each reported category/number interviewed.	Yes: 69.4% No: 27.8% Unknown: 2.8%	1
Perception of surveillance staff on availability of stipends for surveillance duty.	Number of surveillance staff within each reported category/number interviewed.	Yes: 8.3% No: 88.9% Unknown: 2.8%	1

Only 3(8.3%) of the respondent said they store their data electronically in computer system 22(61.1%) use files and paper.

Knowledge, Simplicity, Flexibility, Acceptability, Timeliness, Sensitivity and Stability

Table 9: Mean indicators' scores (range 1–3) for each attribute, AI surveillance system evaluation Ogun State 2016-2019.

Attributes	Number of evaluated indicators	Mean score	Performance
Knowledge	5	2.4	Moderate to good
Simplicity	4	2.5	Moderate to good
Flexibility	3	2.3	Moderate to good
Acceptability	5	2.2	Moderate to good
Timeliness	3	1.7	Poor to Moderate
Sensitivity	3	1.7	Poor to Moderate
Stability	5	1.2	Poor to Moderate
Overall	23	1.9	Poor to Moderate

V. Discussion

The AI surveillance system in Ogun State between 2015 to 2019 can be said to have an average level of performance with an overall surveillance system evaluation score of 1.9 (poor to moderate) out of a scale of 3.0 (Table 9), and total number of affected birds of 99,923 within 5 years of surveillance (Figure 2), the system is presently not performing at its optimum to meet up with the objective of its establishment, which may be evident in the absence of reported cases and outbreaks in the year 2016, 2018 and 2019 which will hinder its contribution to the regional and global understanding of influenza epidemiology, including sharing of clinical samples with WHO collaborating center for annual selection of vaccine strains(18)(19)(20). This finding is different from that found in the national avian influenza surveillance evaluation published in 2014 (21) which suggested that AI surveillance systems across Nigeria were meeting the objectives of their establishment, a contrary result for Ogun state presently, however suggestive that the system is not receiving as much attention as it use to.

A key component of any surveillance system is the competency of surveillance officers in terms of knowledge of the basic objectives of the surveillance system and how the system should be operated(22). This is best learned through training of the surveillance officers, the Ogun State AI surveillance system has well trained staffs comprising 50% veterinary doctors (Table1) and other highly skilled professionals, with 61.1% of them having received formal training on AI surveillance (Table 2).However, there is still a large portion 38.9% that have not received formal training, this will definitely affect their eventual performance in the system.

The simplicity of this system and its processes engenders compliance which can facilitate the delivery of effective public health responses and ensure diseases are controlled in time to prevent further spread(23). All four indicators used to access the simplicity of Ogun State AI surveillance system showed between moderate to good score (Table 3) and all respondent to the questionnaire agreed that the form used for reporting are very easy to fill. This is also contrary to earlier studies (21)which was suggestive of a complex AI surveillance system. It may however be because this earlier studies were national based studies and the complexities were introduced at the federal level of the surveillance system.With an optimal staff strength and very simple reporting process other attributes of the surveillance system like acceptability and validity will be positively influenced(22)(24).

Similar to previous studies, the Ogun State AI surveillance system is flexible, having a second highest score of 2.3 among all the attributes (Table 4). The existence of optimal staff strengthis an advantage for the system, majority of whom agreed that forms used in collecting data can accommodate any change in the surveillance system. The lack of supervision noticed in the system is a set-back as staffs need to be supervised regularly to optimize the AI surveillance system in the state.

Majority of the respondents said they will continue with the surveillance system, giving a good score for the indicator (Table 5) and acceptability of the system. However, quite a substantial number said there were challenges in the AI surveillance system and finance was the most common challenge 88.8%. Compensation to farmers that have outbreaks of AI were insufficient considering the economic loss from depopulation of the poultry farm and the 3 months fallowing period before restocking(25)(26)(27). This will have adverse effect on willingness of farmers to report any outbreaks of AI in their farms; hence many are

resorting to vaccinating their birds(28)(29) which will be inimical to effort to eradicate the disease.

Timeliness of surveillance data was one of the weakest attribute with a score of 1.7. All the 3 indicator used to measure this attribute except timeliness of monthly reporting scores 1 (Table 6) this finding is similar to that published in 2014 where timeliness of AI surveillance data was also poor (21). With only 47% of the respondent having knowledge of the existence of a written policy on timeliness of data, meeting the first 2 week's monthly set target now becomes more un attainable.

Two out of the three indicators used to measure sensitivity had moderate to good score except for the indicator measuring the ability of the surveillance system to detect all cases which had a weak score (Table 7). With a weak to moderate sensitivity, more AI outbreaks go unnoticed, and this is capable of increasing the risk of occurrence of zoonotic human influenza which may progress to a global pandemic like the ongoing novel corona virus infection in china.

Of the five indicators used to evaluate for stability, four had weak score (Table 8), this is particularly due to the poor funding and lack of financial encouragement generally accorded surveillance officers in the veterinary services and animal disease surveillance systems(30)(31), which needs to change if the morale of surveillance officers towards AI surveillance in Ogun State is to be improved.

VI. Conclusions and Recommendation

This study showed that the existing AI surveillance system in Ogun State Nigeria is simple, flexible and acceptable with good knowledge by surveillance officers, but requires improvement in area of timeliness of data, sensitivity of the system, and stability through substantial funding to make it efficient for prevention and control of AI in Ogun state and avoid potential zoonotic transmission to man.

The study suggest that more training should be conducted, at least quarterly, for all surveillance officers, this will keep them abreast with present competencies in AI surveillance, including existing policies on timeliness. The system's ability to detect cases of AI should be improved by involving more farm workers in the AI surveillance system. More funding is also advocated for and collaboration of international partners such as FAO in terms of provision of stipends for surveillance officers to improve the stability of the system.

Data Dissemination

All findings of this surveillance system evaluation was shared with the Ogun State ministry of Agric through Director of Veterinary services, federal Veterinary Epidemiologist and other stakeholders, and training conducted for all AI surveillance officers in Ogun State by FELTP resident on surveillance processes and timeliness.

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Changes in the Activity of the Enzyme Aspartate Transaminase in the Blood of Goats Infected by Parasites

By Sh. A. Topchiyeva

Abstract- Experimental studies of the detection of aspartate transaminase activity in the blood of goats of Khizi-Khachmaz zone of Azerbaijan in different seasons of the year for the period from December 2017 to January 2018, depending on the degree of invasion by parasites of animals have been presented in the article. Determination of the enzymatic activity was carried out spectrophotometrically using a Folin reagent on a Specol 1500 spectrophotometer (Analitik Jena).

The maximum peak of intensity of aspartate transaminase enzymatic activity in blood of goats was revealed. The maximum value of the enzyme activity was reached in March equal to 94.5±3.5, and the minimum in November reaching 42.1±1.1 U/ L blood. It should be noted that their difference is significant (P>0.96).

Decrease in total protein in the blood of goats, depending on the degree of invasion by parasites was revealed. In the control groups of goats, the total protein corresponded to 80.6 ± 1.3 - 92.3 ± 1.8 g / l., While in the experimental groups of goats it was within 60.5 ± 0.9 -76.2 ± 2.0.

Keywords: *aspartate transaminase, blood, goats, parasites.*

GJMR-G Classification: *NLMC Code: QZ 85*

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Thus, experimental studies were conducted to identify aspartate transaminase activity in the blood and hepatic tissues of goats infested by parasites in different seasons of the year. Proceeding from the obtained data, it can be stated that the season of the year and the climatic conditions of their maintenance on farms significantly influences the aspartate transaminase enzymatic activity of goat homogenates.

Keywords: aspartate transaminase, blood, goats, parasites.

I. Introduction

One of the important factors determining the degree of spread and intensity of invasions is the time of year and the climatic conditions of farms. Parasite numbers rise with time when conditions are suitable and internal parasite burdens impact on the health and well-being of the animal when their numbers grow beyond what the animal can tolerate. Nematodes are pathogenic parasites, causing disease in the host. Usually they live in the digestive system of the host. Haemonchus contortus attaches to the wall of the abomasums in sheep and goats, feeding on the host's blood, causing aneamia. Other nematodes usurp the nutrients eaten by the host, causing weight loss (Hale, 2006) [1.2]

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In the literature, data has been given on the extent of the invasion, depending on climatic conditions. The difference in invasiveness is explained by unequal conditions of keeping, the degree of contamination of keeping and feeding areas of animals. The isolation of invasive elements in their opinion depends on the condition of the host organism, feeding, habitat conditions and abiotic factors. All these factors affect the viability of helminths in the external environment and the host organism [3, 4].

Serum enzyme activities of sorbitol dehydrogenase, glutamate dehydrogenase, gamma glutamyltransferase, alkaline phosphatase, aspartic aminotransferase, and creatine kinase, were measured in five clinically normal mixed-breed goats. Tissue activities of these enzymes were also measured in two goats. These basal serum values were then used to determine the response to treatment with carbon tetrachloride (CCl4). The basal value for serum and hepatic tissue sorbitol dehydrogenase were appreciably greater for goats than previously reported for sheep and cattle. The change in the above serum enzymes after CCl4 treatment resembled change in sheep, but the amount of sorbitol dehydrogenase increase was less than that in sheep.

This study established basal tissue and serum enzyme activity values and demonstrated the efficacy of the use of changes in serum S.D.H. and G.D.H. activity as indicators of acute hepatopathy in goats [5].

The blood, being the internal environment of the organism, has a relative constancy of its composition; nevertheless, it is a system that reflects in a varying degree all the changes that occur in the body. At the same time, its morphofunctional indicators are individual values (A.N. Kvochko, 2002). Blood content of red blood cells, hemoglobin and other hematological parameters, according to A.N. Kvochko (2001) varies with age, sex, level of feeding, content, productivity, and season of the year. Hematological parameters are interrelated with animal productivity. In this regard, for the early evaluation of the economically useful traits of animals, biochemical blood parameters are increasingly used.

Transamination processes are important in the developmental period of an organism, when it builds proteins of its tissues and organs from protein digestion products in the digestive tract, in which the full set of

amino acids necessary for synthesis is not always present (SE Vasilyeva, 1987). In this regard, the activity of serum transamination enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), whose concentration reflects the level of protein metabolism in the body, was studied.

The highest enzymatic activity of transaminases ALT (alanine aminotransferase) and AST (aspartate aminotransferase) was observed in animals obtained in the first two decades of kazleniya (group I and II), which was higher compared with group III animals, respectively, by 30.0.22.5% and 11.7% [6 , 7].

Alanine aminotransferase is an enzyme that catalyzes transamination processes. Indicators of activity of alanine transferase have differences in blood by sex, and also differ in the period of physiological development in young [8]

The synthetic function of the liver can be assessed by studying the activity of the transaminating enzymes AlAT and AsAT, the main function of which is the synthesis and breakdown of certain amino acids in the body. A significant increase in these indicators in the blood of animals of the experimental group is consistent with the data on the content of total protein, serum urea, and indicates a violation of liver function and intensity of protein metabolism.

The content of total protein in serum is an important indicator characterizing the level of metabolism in the body of an animal. Proteins are a building material for the cells of body tissues, they are actively involved in the formation of various types of products. A significant decrease in the total protein content in the blood of the experimental group when exposed to a negative temperature of -20 ° C below the physiological norm (70-80 g / l) can be associated with stress adaptation processes, in particular, with a decrease in the level of protein metabolism. Causes of hypoproteinonemia can be protein starvation or poor absorption of proteins from the feed due to disorders of the gastrointestinal tract, as well as mobilization of proteins as energy sources [9, 10].

Protein metabolism is a complex of transformations of proteins and amino acids in the body. It is known that serum proteins play a leading role in metabolic processes in animals and are functionally related to the development of their main economically valuable traits. These heterogeneous complexes contribute to the preservation of homeostasis, the transmission of hereditary information, provide the natural resistance of the organism [11-13].

Based on the above, the purpose of the research was to study the dynamics of total protein and enzymatic activity in healthy and parasite-infected goats.

II. Material and Research Methods

Experimental studies of the detection of aspartate transaminase activity in the blood of goats of Khizi-Khachmaz zone of Azerbaijan in different seasons of the year for the period from December 2017 to January 2018, depending on the degree of invasion by parasites of animals were held. Determination of the enzymatic activity and the dynamics of total protein in the blood was carried out spectrophotometrically using a Folin reagent on a Specol 1500 spectrophotometer (Analitik Jena). The assessment of the reliability of differences of compared samples was performed by Student's criterion.

III. Research Results and Discussion

We determined the dynamics of the activity of the enzyme aspartate aminotransferase (AcAT) in samples of serum of goats invaded by parasites, depending on the degree of parasite invasion at different times of the year.

It was found that in goats infested by parasites, the activity of AsAT in the blood in the winter in February is 2.14 times lower ($p < 0.05$) than in the first half of spring. The activity of AlAT in the serum of goats in September was 1.04 times ($p < 0.01$) higher than in February. In addition, in the blood of goats in the summer in June, there was a decrease in the activity of ALT by 1.38 times ($p < 0.05$), compared with the activity of the enzyme in the spring period (in March).

Analysis of the data obtained shows that the activity of enzymes that regulate the conjugation of protein and carbohydrate metabolism in the blood of goats during the period of invasion by parasites may be associated with their subsequent ability to survive of animals and their reproductive performance.

The maximum peak of intensity of aspartate transaminase enzymatic activity of blood of goats was revealed. The maximum value of the enzyme activity was reached in March equal to 94.5±3.5 U/ L of the blood, and the minimum in June reaching 72 U/ L of the blood (tabl.1, fig.1).

Thus, experimental studies were conducted to identify aspartate transaminase activity of blood in goats infested by parasites in different seasons of the year. Proceeding from the obtained data, it can be stated that the season of the year and the climatic conditions of their maintenance on farms significantly influences the aspartate transaminase enzymatic activity of goat homogenates. (tabl.1, fig.1)

Thus, experimental studies were conducted to identify aspartate transaminase activity of blood in goats infested by parasites in different seasons of the year. Proceeding from the obtained data, it can be stated that the season of the year and the climatic conditions of their maintenance on farms significantly influences the

aspartate transaminase enzymatic activity of goat homogenates.

The amount of activity of the enzyme aspartate aminotransferase (AST) in the blood of healthy goats was determined, which ranged from 20.8 ± 1.2 to 31.8 ± 1.3U / L.

It was experimentally revealed that in goats an increase in the enzymatic activity of AsAT in the blood during parasite invasion is detected. At the same time, the activity of AsAT in the blood at all seasons is significantly lower in healthy goats than in infected ones. The activity of AST in the blood of healthy goats is 1.39 times lower ($p < 0.05$) than in the winter period. The activity of AlAT in the serum of animals increased 2.97 times ($p < 0.02$) compared with the control group of animals.

Table 1: Data on aspartate-transaminase activity in the blood of goats, in different seasons of the year

Aspartate-transaminase activity of goat's blood enzymes, U/L							
Months							
Winter season		Autumn season		Spring season		Summer season	
December	42.8±2.3	September	46.2±2.1	March	94.5±3.5	June	68.3±3.4
January	43.4±1.6	October	44.2±1.3	April	72.4±2.1	July	59.5±2.2
February	44.1±2.4	November	42.1±1.1	May	70.6±1.8	August	48.5±2.5

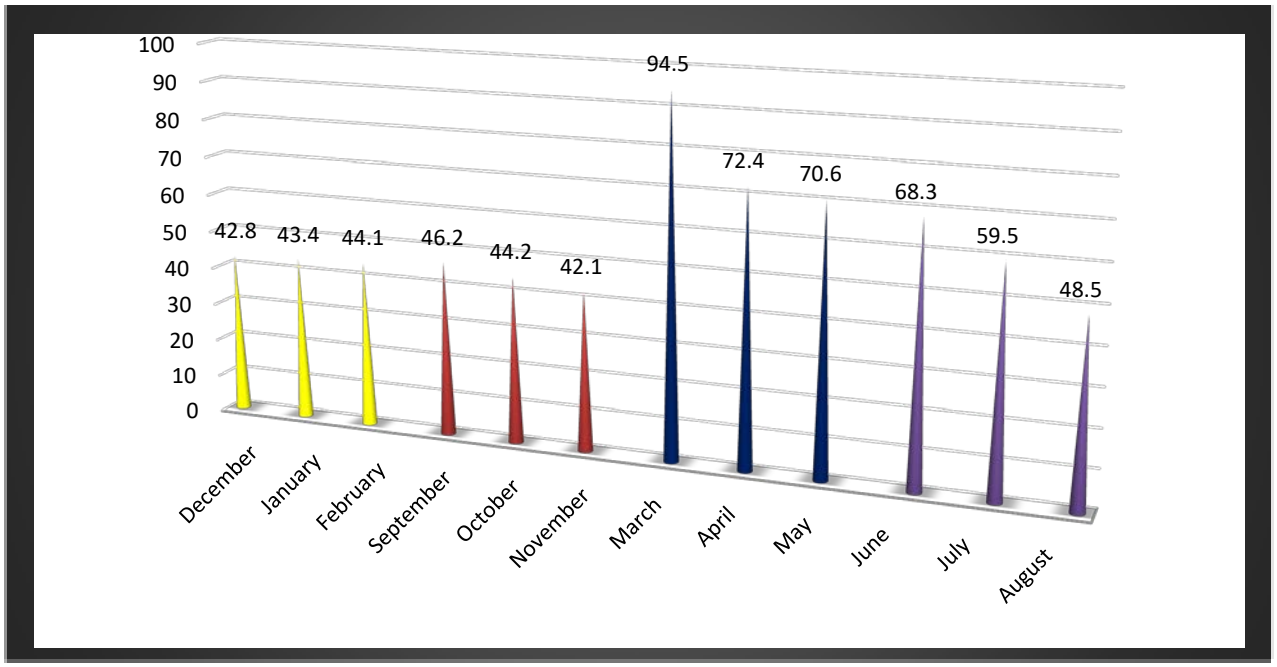

Fig.1: Data on aspartate-transaminase activity in the blood of goats, in different seasons of the year, related to the state of animal health

The dynamics of changes in the content of total protein in serum is an important indicator characterizing the level of metabolism in the animal. With the invasion of animals by parasites, there is a change in the total blood protein of the experimental animals. A significant decrease in the total protein content in the blood of the experimental group was noted, depending on the degree of infection of goats. При исследовании общего белка методом Фолина у контрольных групп коз общий белок колебался в пределах 80.6±1.3-92.3±1.8 г/л соответственно. In the study of total protein by the folin method in the control groups of goats, the total protein ranged from 80.6 ± 1.3-92.3 ± 1.8 g / l, respectively However, depending on the degree of parasite invasion, which affects the activity of the enzyme AsAT, a decrease in the total protein content was observed at the same time (Table 2), which is most likely due to the stress of adaptation processes, in particular, to a decrease in the level of protein metabolism during parasite invasion.

So, with an increase in enzymatic activity, there is a decrease in total protein in the blood of infested goats, which is associated with the degree of infection of experimental animals.

In conclusion, the change in the activity of the enzyme AsAT in the blood, depending on the degree of invasion and the season should be noted.

Analysis of the data also shows that the activity of enzymes that regulate the conjugation of protein and carbohydrate metabolism in the blood of goats during invasion by parasites may be associated with their subsequent ability to survive and reproduce. The indicators of total blood protein allow us to assess the physiological state of the body of goats, the functions of its organs and systems in the work of maintaining protein metabolism and are one of the main indicators for diagnosing various pathologies of animals.

Table 2: Data on the dynamics of total protein in the blood of infested goats

Total protein, g/l							
Months							
Winter season		Autum season		Spring season		Summer season	
December	70.8±1.3	September	76.2±2.0	March	64.2±1.5	June	68.3±1.4
January	71.4±1.9	October	74.2±1.2	April	62.1±1.3	July	69.5±1.1
February	71.1±1.5	November	72.1±1.5	May	60.5±0.9	August	68.5±0.9

Monitoring on the biochemical parameters of the blood of goats will allow for effective breeding activities to form the gene pool of an animal population with a high metabolic rate in order to improve the high productivity of small ruminants.

IV. Findings

1. The activity of the enzyme aspartate aminotransferase (AST) in the blood of healthy goats was detected, which ranged from 20.8 ± 1.2 to 31.8 ± 1.3U/L.
2. The activity of AsAT in the blood at all times of the year is significantly lower in healthy goats than in infected ones.
3. The maximum peak of intensity of aspartate transaminase enzymatic activity in blood of goats was revealed. The maximum value of the enzyme activity was reached in March equal to 94.5±3.5, and the minimum in November reaching 42.1±1.1 U/L blood.
4. Decrease in total protein in the blood of goats, depending on the degree of invasion by parasites was revealed. In the control groups of goats, the total protein corresponded to 80.6 ± 1.3 - 92.3 ± 1.8 g/l., While in the experimental groups of goats it was within 60.5 ± 0.9 -76.2 ± 2.0 g/l.
5. Research results confirm the importance of the role of protein metabolism, which is the basis of all vital processes in the body, with its transformation in the body associated with the processes of growth, development and productivity.

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1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

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

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

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

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

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

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

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

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

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

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

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

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

Informal Guidelines of Research Paper Writing

Key points to remember:

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

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

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

General style:

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

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

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

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

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

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

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

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

Results:

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

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

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

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

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

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

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

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