SHORT COMMUNICATION

Culiseta annulata – just a biting nuisance or a deadly foe?

Martin Ward^a and Giovanni Benelli^{b,c}

^aThe Pain Clinic, Bolsover, UK; ^bDepartment of Agriculture, Food and Environment, University of Pisa, Pisa, Italy; ^cThe BioRobotics Institute, Scuola Superiore Sant'Anna, Pisa, Italy

KEYWORDS Cancer; Culicidae; Hepatitis C; Human Papilloma virus; *Clonorchis sinensi*

1. Introduction

Our basic knowledge on the possible relationship between biting activity of mosquito vectors and the spread of cancer is scarce and patchy [1–3]. Depinay et al. [4] and Schneider and Higgs [5] have shown that mosquito saliva has the capacity to suppress the human immune system, which is regarded as a pre-requisite to infectious agents causing cancers. Furthermore, it has been argued that anopheline mosquitoes could vector overlooked viruses, which can act as carcinogenic agents [6,7]. In addition, earlier it has been shown that *Aedes aegypti* (L.) is able to transfer tumour cells of hamster reticulum cell sarcoma through subsequent feeding events on vertebrate hosts [8,9].

But what do we really know about the ability of other – overlooked – mosquito species that can vector potentially carcinogenic agents? Recently, patients presenting to a small UK pain clinic who reported a history of cancer were asked if they were prone to mosquito bites, and many said they were “plagued” by them. One patient stated during the taking of a case history that he had three instances of cancer and that in his opinion mosquitoes were the cause. The patient based this claim on a set of symptoms arising within days of being bitten, which matched those of his experiences with cancer (M. Ward, pers. comm.). Therefore, we asked patients to search their gardens and houses for mosquitoes and a total of 12 mosquitoes (including some alive individuals) were brought to the clinic. The largest proportion of the insects were identified as *Culiseta annulata* (Schrank) (Diptera: Culicidae) [10].

There is an increasing body of research showing that EMF or similar devices using pulses (PEMFs) are now being used experimentally in university settings with

respect to cancer, both with and without chemotherapy, and particularly where standard treatments have failed or patients are unable to tolerate other treatments. In essence, EMF devices can be likened to a radio receiver, which is connected to a human transmitter. If the device is ‘tuned’ to 313,800 Hz and a signal is received from the patient then it is likely that the radio is tuned to the – for example – *S. mitis* transmission station. Heimes [11, p.22] provides the example of the skin that has an integrated regulatory system that starts the process of tanning at the exact frequency of sunlight. If the skin tanned because of simply warming then we would develop a tan in the sauna. A pulsed EMF device has been used in a collaborative project between the Universities of San Paulo, the University of Alabama at Birmingham and UAB Comprehensive Cancer Centre, as well as Swiss and French Institutes [12–14]. The study by Barbault et al. [12] for example, found a total of 1524 tumour frequencies ranging from 0.1 Hz to 114 Khz of which 77.6% were tumour specific. Blackman [15] commenting on their work, points out that clinical benefits were observed with a reduction in gene expression and increases in mitotic spindle dysfunction. Crocetti et al. [16] report PEMFs having a significant effect on breast cancer and Pasi et al. [17] have shown that PEMFs with temozolomide can elicit an epigenetic pro-apoptotic effect in the chemo- and radio-resistant T98G glioblastoma cell line. These papers conform with many basic scientific principles, in that they have formed hypotheses, conducted experiments, analysed the data, used specific principles of reasoning and drawn conclusions. Collectively they have further illustrated a cornerstone of science, namely a biological change and reproducibility. Besides, some general practitioners are utilising EMF devices [18] as are many complementary practitioners. Several German manufacturers of these devices have been in business for over thirty years and thus have a reliable track record.

In this research, an initial testing of both the mosquitoes and patients with a history of cancer was undertaken using an electromagnetic frequency device (EMF), which revealed a matching pattern of infectious agents in both patients and one particular mosquito species – *C. annulata*. We then considered numerous other, more orthodox, methodologies for testing this association. With respect to analysis of human blood, urine or saliva samples, ethical problems were raised and the exercise was projected to be extremely costly. A practical concern we had was that testing blood samples for the presence of *Plasmodium* may prove negative because some types of *Plasmodium*, for example *Plasmodium vivax*, are often found dormant in the liver rather than having a presence in blood, saliva or urine. Analysis of mosquito DNA, utilising PCR techniques, was also considered but the estimated funding cost of £70–100,000 for a full analysis has so far proved impossible to raise. Epidemiological studies would also be very expensive and considering the long lead time between mosquito bite and clinical symptoms of cancer there would be many confounding factors, for example environmental changes that could alter where mosquitoes were at the time of the bite and where they were at the onset of cancer. What we present here is, therefore, only the experiment undertaken with the EMF device and should be regarded as a pilot or preliminary study.

2. Experimental

We utilised a German EMF device (Rayonex Polar 1000 Evolution) together with frequencies derived from Heimes [11] and frequencies for Simian virus 40 (SV40) from Sylver [19], to summarise the findings from four case studies. Our primary focus was on infectious agents already linked to cancer, some of the other infectious agents we have found are listed solely for their possible involvement in cancers or to illustrate the mosquito connection. Each of the patients gave written authorisation for their data to be used (anonymously) for research purposes and none were treated for cancer per se, but sought overall health advice and assistance.

Case no. 1 is a male in his late sixties with a recent diagnosis of cancer of the prostate and of the 8th thoracic vertebra. Case no. 2 is a lady in her sixties with a recent diagnosis of bladder cancer. Case no. 3 is a male in his sixties with lung cancer that has suddenly become more active, and case no. 4 is a female in her late sixties with a past record of breast cancer. Whilst it cannot be ruled out that patient 4 was bitten after she developed cancer she is repeatedly bitten by mosquitoes and brought several *C. annulata* into the clinic. Two of the other three cases

had mosquito bites at the time of presentation to the clinic, which was within two weeks of cancer diagnosis.

The experimental work with the EMF device is both simple and straightforward. The Rayonex device contains a database of infectious agents. The IARC and other infectious agents associated with cancer were called up one by one from the database and the patient tested for its presence. By way of examples the frequency for *P. vivax* is 44.16 KHz. and for Human Papilloma virus is 40.43 KHz. The device provides a binary response, the patient showing a positive or negative answer to the presence of the frequency being tested. In the case of SV40, the device did not hold the relevant frequencies in its data-base so these were inserted manually in a programme designed for such contingencies. With respect to the testing of the mosquitoes, the device has a system for testing substances, called a relations test. This facility was used to test the mosquitoes for the presence of the same infectious agent frequencies found in the cancer patients.

3. Results and discussion

Table 1 reveals a consistent pattern between infectious agents found in four case studies of patients with different cancers and *C. annulata*. The first four infectious agents are part of the nine group 1 carcinogens identified by the IARC. Concerning the other infectious agents, six of them (i.e., *Balantidium coli*, *molluscum contagiosum*, *Streptococcus*, Simian virus 40, *Mycoplasma* and *Toxoplasma gondii*) have been identified in the literature as having a likely connection with cancer. Taken together ten of the thirty-three infectious agents linked with cancer (see Ward et al. [20]) appear to be present in just one species of mosquito. The prospect that SV40 is transmitted by mosquitoes, as suggested here for the first time, would, if confirmed by further research solve a long-standing enigma as to the origin of the virus's reservoir. *Toxoplasma gondii* and *Clonorchis sinensis* have not been linked with mosquitoes before, so this finding could be of additional importance.

HPV is associated with cervical, anogenital, head and neck cancers and has been found in mosquitoes [21]; hepatitis C is associated with non-Hodgkin lymphoma and cancers of the liver (found in mosquitoes by Chang et al. [22]); HTLV-1 is linked with adult T-cell leukaemia and lymphoma, and *Clonorchis sinensis* with cholangiocarcinoma and infections in the gallbladder and pancreas. Mycoplasmas have been associated with gastric carcinomas, oesophageal, lung, and breast cancers [23] and linked directly to mosquitoes [24]. *Molluscum contagiosum* has been linked with lung cancer, lymphoma, and leukemia [25] and with mosquitoes [21]. *Balantidium coli* has been linked in case studies with a leukemia, non-Hodgkins lymphoma and anal cancer [26–28]. Besides, *Streptococcus* has been found associated with gastric cancer cells [29]. This is an extensive list of cancers, covering numerous parts of the anatomy and all may be initiated by a bite from *C. annulata*.

Table 1. Infectious agents detected in four cancer cases and *Culiseta annulata* mosquitoes.

Infectious agent	<i>C. annulata</i>	Case 1	Case 2	Case 3	Case 4
Hepatitis C	yes	yes	yes	yes	yes
Human Papilloma virus	yes	yes	yes	yes	yes
HTLV type 1	yes	yes	yes	yes	yes
<i>Clonorchis sinensis</i>	yes	yes	yes	yes	yes
<i>Bacillus</i>	yes	yes	yes	yes	yes
<i>Balantidium coli</i>	yes	yes	yes	yes	yes
<i>Molluscum contagiosum</i>	yes	yes	yes	yes	yes
<i>Plasmodium vivax</i> *	yes	yes	yes	yes	yes
<i>Streptococcus mitis</i>	yes	yes	yes	yes	yes
Simian virus 40	yes	yes	yes	yes	yes
<i>Mycoplasma</i>	yes	yes	yes	yes	yes
<i>Salmonella enteritidis</i> *	yes	yes	yes	yes	yes
Herpes simplex*	yes*	yes	yes	yes	yes
<i>Schistosoma mansoni</i> *	yes	yes	yes	yes	yes
<i>Toxoplasma gondii</i>	yes	yes	yes	yes	yes
<i>Ancylostoma brasiliense</i>	yes	yes	yes	yes	yes

*alongside a given infectious agent indicates that the agent is of a family similar to a known cancerous agent.;

*alongside *C. annulata* indicates that the infectious agent was not found in all of the seven mosquitoes that were analysed.

Notably, SV40 has been investigated for a possible connection with cancer for many years [30–32,33]. Now, it has been confirmed as a human virus and secondly associated with human osteosarcoma and non-Hodgkin lymphoma [34, 35,36].

Besides, the finding of *P. vivax* is interesting as this parasite is usually found in *Anopheles* mosquitoes. *P. vivax* is not found in some parts of the world so it is most likely that *C. annulata* carries not *P. vivax* but a homologous form of *Plasmodium* that is similar to *P. vivax* in its frequency bandwidth, and which may possibly be a mutation of *P. vivax*. *Plasmodium vivax* (or a homologous form) has some characteristics that would fit well with the possible mosquito-cancer connection. It can remain dormant in the host, has relapses, and may escape detection by not having a presence in blood [37–39,40].

Lastly, *T. gondii* is suspected of having a connection with cancer [41], and has also been related to depression [42–44,45], which in turn has related to cancer [46–48,49].

4. Conclusions

Culiseta annulata is the largest UK mosquito, known to bite humans and with a presence in the Palearctic region. Whilst known to infect humans with cellulitis, little else appears to be known about it and it has simply been regarded as a biting nuisance, rather than a deadly foe. Our findings, utilising a relatively novel detection technique suggest that it can carry a cocktail of infectious agents including four of the nine recognised group 1 carcinogens, as well as eight other infectious agents that the literature has linked with cancer. This mosquito may carry a greater number of potentially cancerous infectious agents as we lacked frequencies for some of the infectious agents linked with cancer and found other infectious agents present which may have cancerous potential, although not currently linked with cancer. Our findings suggest that SV40 is present in *C. annulata*, answering a long-standing question about its source and surprisingly that *C. sinensis* and *T. gondii* are also carried by this mosquito.

More research is urgently required, preferably, if funding can be found, by more orthodox means to either confirm or disprove our preliminary findings. If they are confirmed then a substantial amount of additional research needs to be undertaken to study the feeding, breeding and life cycle of this mosquito species, to maximise the potential for its control [50, 51].

Informed consent

Informed consent was obtained from all individual participants included in the study.

Geolocation

The work was done in the United Kingdom.

Acknowledgements

Andrea Crisanti and two anonymous reviewers improved an earlier version of our work. We would like to thank Alan Ward for his practical assistance with the analysis of both mosquitoes and patients.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

- [1] Benelli G, Lo Iacono A, Canale A, et al. Mosquito vectors and the spread of cancer: an overlooked connection? *Parasitol Res.* 2016;115:2131–2137.
- [2] Johansson O, Ward M. The human immune system's response to carcinogenic and other infectious agents transmitted by mosquito vectors. *Parasitol Res.* 2017;116:1–9. DOI:10.1007/s00436-016-5272-2
- [3] Ward M, Benelli G. Avian and simian malaria: do they have a cancer connection? *Parasitol Res.* 2017;116(3):839–845.
- [4] Depinay N, Hancini F, Begdadi W, et al. Mast cell dependent down regulation of antigen-specific immune responses by mosquito bites. *J Immunol.* 2006;176:4141–4146.

- [5] Schneider BS, Higgs S. The enhancement of arbovirus transmission and disease by mosquito saliva is associated with modulation of the host immune response. *Trans R Soc Trop Med Hyg.* **2008**;102(5):600–608.
- [6] Lehrer S. *Anopheles* mosquito transmission of brain tumor. *Med Hypotheses.* **2010**;74:167–168.
- [7] Benelli G. Green synthesized nanoparticles in the fight against mosquito-borne diseases and cancer – a brief review. *Enzyme Microbial Technol.* **2016**;95:58–68.
- [8] Banfield WG, Woke PA, MacKay CM, et al. Mosquito transmission of a reticulum cell sarcoma of hamsters. *Science.* **1965**;148:1239–1240.
- [9] Banfield WG, Woke PA, MacKay CM. Mosquito transmission of lymphomas. *Cancer.* **1966**;19:1333–1336.
- [10] Gaffigan TV, Wilkerson RC, Pecor JE, et al. Systematic catalog of Culicidae; **2015**. Available from: <http://www.mosquitocatalog.org/>
- [11] Heimes D. Bioresonance according to Paul Schmidt. Baunach: Spurbuchverlag; **2014**.
- [12] Barbault A, Costa FP, Bottger B, et al. Amplitude-modulated electromagnetic fields for the treatment of cancer: discovery of tumor-specific frequencies and assessment of a novel therapeutic approach. *J Exp Clin Cancer Res.* **2009**;28:51. DOI: [10.1186/1756-9966-28-51](https://doi.org/10.1186/1756-9966-28-51)
- [13] Costa FP, de Oliveira AC, Meirelles R, et al. Treatment of advanced hepatocellular carcinoma with very low levels of amplitude-modulated electromagnetic fields. *British J Cancer.* **2011**;105:640–648.
- [14] Zimmerman JW, Jimenez H, Pennison MJ, et al. Targeted treatment of cancer with radiofrequency electromagnetic fields amplitude-modulated at tumor-specific frequencies. *Chin J Cancer.* **2013**;32:573–581.
- [15] Blackman CF. Treating cancer with amplitude -modulated electromagnetic fields: a potential paradigm shift, again? *Br J Cancer.* **2012**;106(2):241–242.
- [16] Crocetti S, Beyer C, Schade G, et al. Low Intensity and frequency pulsed electromagnetic fields selectively impair breast cancer cell viability. *PLoS One.* **2013**;8(9):e72944. DOI:[10.1371/journal.pone.0072944](https://doi.org/10.1371/journal.pone.0072944)
- [17] Pasi F, Fassina L, Mognaschi ME, et al. Pulsed electromagnetic field with temozolomide can elicit an epigenetic pro-apoptotic effect on glioblastoma T98G cells. *Anticancer Res.* **2016**;36:5821–5826.
- [18] Bielory L. Complementary and alternative medicine population based studies: a growing focus on allergy and asthma. *Allergy.* **2002**;57:655–658.
- [19] Sylver N. The rifle handbook. Phoenix, AZ: Desert Gate Productions LLC; **2011**.
- [20] Ward M, Ward A, Johansson O. Does the mosquito have more of a role in certain cancers than is currently appreciated? The mosquito cocktail hypothesis. *Med Hypotheses.* **2016**;86:85–91.
- [21] Ng TT, Willner DL, Lim YW, et al. Broad surveys of DNA viral diversity obtained through viral metagenomics of mosquitoes. *PloS One.* **2011**;6:e20579. DOI:[10.1371/journal.pone.0020579](https://doi.org/10.1371/journal.pone.0020579)
- [22] Chang TT, Chang TY, Chen CC, et al. Existence of Hepatitis C virus in *Culex quinquefasciatus* after ingestion of infected blood: experimental approach to evaluating transmission by mosquitoes. *J Clin Microbiol.* **2001**;39(9):3353–3355.
- [23] Huang S, Li YY, Wu J, et al. *Mycoplasma* infections and different human carcinomas. *World J Gastroenterol.* **2001**;7(2):266–269.
- [24] Minard G, Mavingui P, Moro CV. Diversity and function of bacterial microbiota in the mosquito holobiont. *Parasit Vectors.* **2013**;6:146. DOI: [10.1186/1756-3305-6-146.s](https://doi.org/10.1186/1756-3305-6-146.s)
- [25] Nakamura-Wakatsuki T, Kato Y, Miura T, et al. Eruptive *Molluscum contagiosum* in a patient with rheumatoid arthritis and lung cancer. *Rheumatol Int.* **2011**;31:1117–1118.
- [26] Anargyrou K, Petrikos GL, Suller MT, et al. Pulmonary *Balantidium coli* infection in a leukemic patient. *Am J Hemat.* **2003**;73(3):180–183.
- [27] Vasilakopoulou A, Dinasongona K, Samarkali A, et al. *Balantidium coli* pneumonia in an immunocompromised patient. *Scand J Infect Dis.* **2003**;35(2):144–146.
- [28] Yazar S, Altuntas F, Sahin I, et al. Dysentery caused by *Balantidium coli* in a patient with non-Hodgkins lymphoma from Turkey. *World J Gastroenterol.* **2004**;10:458–459.
- [29] Sasaki H, Igaki H, Ishizuka T, Kogoma Y, Sugimura T, Terada M. Presence of Streptococcus DNA sequence in surgical specimens of gastric cancer. *Jpn J Cancer Res.* **1995**;86:791–794.
- [30] Garcea RL, Imperiale MJ. Simian Virus 40 infection of humans. *J Virol.* **2003**;77:5039–5045.
- [31] White MK, Gordon J, Reiss K, et al. Human polyomaviruses and brain tumors. *Brain Res Rev.* **2005**;50(1):69–85.
- [32] Rivera Z, Strianese O, Bertino P, et al. The relationship between simian virus 40 and mesothelioma. *Curr Opin Pulm Med.* **2008**;14:316–321.
- [33] Atkin SJL, Griffin BE, Dilworth SM. Polyoma virus and Simian virus 40 as cancer models: history and perspectives. *Sem Canc Biol.* **2009**;19:211–217.
- [34] Mazzoni E, Benassi MS, Corallini A, et al. Significant association between human osteosarcoma and simian virus 40. *Cancer.* **2015**;121:708–715.
- [35] Tognon M, Luppi MD, Corallini A, et al. Immunologic evidence of a strong association between Non-Hodgkin lymphoma and Simian Virus 40. *Cancer.* **2015**;121:2618–2626.
- [36] Mazzoni E, Guerra G, Casali MV, et al. Antibodies against mizotopes of simian virus 40 large t antigen, the oncoprotein, in serum samples from elderly healthy subjects. *J Cell Physiol.* **2017**;232:176–181.
- [37] Carlton JM, Adams JH, Silva JC, et al. Comparative genomics of the neglected human malaria parasite *Plasmodium vivax*. *Nature.* **2008**;455:757–763.
- [38] Genton B, D'Acremont V, Rare L, et al. *Plasmodium vivax* and mixed infections are associated with severe malaria in children: a prospective cohort study from Papua New Guinea. *PLoS Med.* **2008**;5(6):e127. DOI:[10.1371/journal.pmed.0050127](https://doi.org/10.1371/journal.pmed.0050127)
- [39] Mueller I, Galinski MR, Baird JK, et al. Key gaps in the knowledge of *Plasmodium vivax*, a neglected human malaria parasite. *Lancet.* **2009**;9:555–566.
- [40] Baird KJ, Maguire JD, Price RN. Diagnosis and treatment of *Plasmodium vivax* malaria. *Adv Parasitol.* **2012**;80:203–270.
- [41] Thomas F, Laffery KD, Brodeur J, Elguero E, et al. Incidence of adult brain cancers is higher in countries where the protozoan parasite *Toxoplasma gondii* is common. *Biol Lett.* **2011**;8:101–103.
- [42] Hinze-Seich D, Daubener W, Eggert L, et al. A controlled prospective study of *Toxoplasma gondii* infection in individuals with schizophrenia: beyond seroprevalence. *Schizophr Bull.* **2007**;33:782–788.
- [43] Hinze-Seich D, Daubener W, Erdag S, et al. The diagnosis of a personality disorder increases the likelihood for seropositivity to *Toxoplasma gondii* in psychiatric patients. *Folia Parasitol.* **2010**;57:129–135.
- [44] Pearce BD, Kruszon-Moran D, Jones JL. The relationship between *Toxoplasma gondii* infection and mood disorders in the third national health and nutrition survey. *Biol Psych.* **2012**;72:290–295.

- [45] Hamdani N, Daban-Huard C, Lainef M, et al. Relationship between *Toxoplasma gondii* infection and bipolar disorder in a French sample. *J Affect Disord*. 2013;148:444–448.
- [46] Musselman DL, Miller AH, Porter MR, et al. Higher than normal plasma interleukin-6 concentrations in cancer patients with depression: preliminary findings. *Am J Psych*. 2001;158:1252–1257.
- [47] Spiegel D, Giese-Davis J. Depression and cancer: mechanisms and disease progression. *Biol Psych*. 2003;54:269–282.
- [48] Satin JR, Linden W, Phillips MJ. Depression as a predictor of disease progression and mortality in cancer patients. *Cancer*. 2009;115:5349–5361.
- [49] Pinquart M, Duberstein PR. Depression and cancer mortality: a meta-analysis. *Psych Med*. 2010;40:1797–1810.
- [50] Benelli G. Research in mosquito control: current challenges for a brighter future. *Parasitol Res*. 2015;114:2801–2805.
- [51] Benelli G, Mehlhorn H. Declining malaria, rising dengue and Zika virus: insights for mosquito vector control. *Parasitol Res*. 2016;115:1747–1754.