Tilapia Lake Virus: A Threat to the Global Tilapia Industry
Case Study 10

Research goal	Identification, diagnostics and containment of a tilapia lake virus (TiLV)
Beneficiaries	Tilapia farmers and consumers, (one of the most farmed fish species globally and an important source of protein in some third world countries) Global organizations promoting aquaculture disease containment (World Organization. for Animal Health, OiE) and global food security (Food and Agriculture Org., FAO)
Activities conducted in order to achieve the objectives	Isolation of a viral source of global tilapia disease, demonstration that TiLV is the etiological agent of the disease, creation of a genetic library and genomic sequencing of TiLV genome, characterization of TiLV as a novel orthomyxo-like virus, creation of diagnostic tools and vaccination prototypes.
Funding	BARD awards: IS-4583-13 and IS-4903-16C, $455,000
Publications	4 publications, 3 of them in the top impact factor quartile (Q1)
Students involved	1 post-doctorate, 2 graduate students, 1 pre-graduate trainee. Current positions: 1 is in academia, 1 has progressed to a post doc and 2 are conducting graduate studies
Stakeholders' collaboration	Worldwide collaboration with fish farmers, importers (US) and national veterinary services.
Environmental impact	Freshwater tilapia is one of the most effective FCR (Food Conversion Ratio) protein sources. Disease could shift production to less efficient protein sources.
Social impact	Outbreaks of TiLV threaten the livelihoods and food security of millions of people dependent on tilapia farming in developing countries. Identification of the virus and the diagnostic assay enables containment of the disease.
Commercial engagement	Licensed to a large international company for vaccine development
Patents	2 patents granted. 1 joint to US and the 2 Israeli institutions, 1 joint to the 2 Israeli institutions
Practical agricultural applications	Long term: attenuated and inactivated vaccines are now in development stages. Short term: a PCR based diagnostic tool was developed and governments have initiated several containment measures such as sourcing live tilapia only from tested populations, quarantine and post-arrival testing of imported live tilapia.
Economic Impact	Net present value of the BARD's investment is $46 million, thereof $1 million already attained The Internal rate of return is 83% Benefit cost ratio is 91, thereof 3 already attained

1 Objective: To Identify the Cause of a New Tilapia Disease

Tilapia death in Israeli farms motivated the researchers to elucidate the cause of these losses. Preliminary experiments yielded negative results for known parasites, bacteria, viral pathogens or toxins, leading to the researchers' collaboration to identify the novel pathogen of the disease.

2 Research Activities

A 1-year BARD award (IS4583-13) was granted to W. Ian Lipkin (Columbia), Eran Bacharach (TAU) and Avi Eldar (Isr. Vet. Institute) to conduct a preliminary study on the causal agent of the tilapia disease. Following successful isolation and identification of a novel viral agent, named Tilapia Lake Virus (TiLV), the research was extended to a second BARD award (IS-4903-16C) in which a TiLV diagnostic tool and vaccination prototypes have been developed. See Appendix A for details. The research had five steps:

1. Identification of the disease agent as a virus.
2. Validation of TiLV as the disease agent. Healthy tilapia fish were exposed to the identified virus to confirm that the extracted virus was the cause of the disease.
3. Genomic sequencing of the newly identified TiLV. The full genomic sequencing was retrieved using high-throughput sequencing on the purified virus. TiLV was identified as a segmented, negative-sense RNA orthomyxo-like virus.
4. Development of assay for TiLV detection, thereafter termed as "diagnostic tool".
5. Development of prototypes of vaccines based on the observed protective immune response of tilapia that survived an initial TiLV infection.

3 Academic Impact

3.1 Publications

Four peer-reviewed journal publications have been published based on this single BARD award.

3.2 Capacity Building

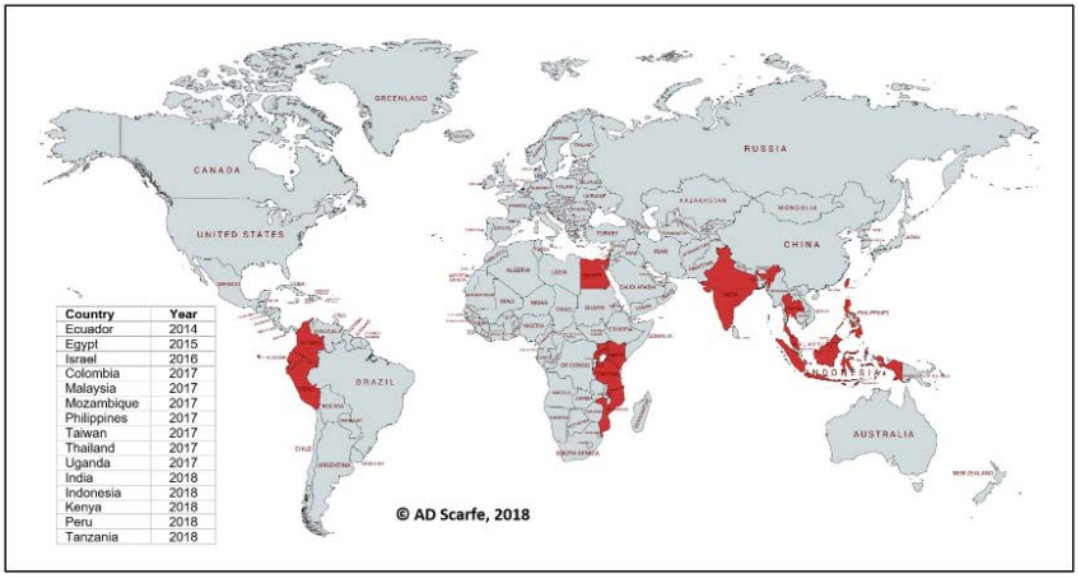
- A post-doctoral researcher at Columbia who is soon to become an assistant professor at Columbia
- One Ph.D. student came from Cameroon to work in Israel on the tilapia project and currently conducts post-doctoral research in the UK.
- One laboratory assistant during the BARD research now has a permanent position at Kimron Vet. Inst.

4 Stakeholder's Collaboration

A nested RT-PCR assay was developed, providing the tilapia aquaculture industry with an important tool for the detection and containment of this pathogen. The novel TiLV diagnostic tool was applied in fish farms worldwide in order to find the cause of devastating, previously undetermined, tilapia disease outbreaks. Figure 1 shows the now mapped prevalence of TiLV globally.

Private tilapia farmers and breeders, government veterinary services and tilapia importers from all over the world have been in contact with the BARD research group for exchange of information and to better understand diagnostic approaches.

Figure 1: Geographical distribution of TiLV



Country	Year
Ecuador	2014
Egypt	2015
Israel	2016
Colombia	2017
Malaysia	2017
Mozambique	2017
Philippines	2017
Taiwan	2017
Thailand	2017
Uganda	2017
India	2018
Indonesia	2018
Kenya	2018
Peru	2018
Tanzania	2018

TiLV Country Outbreaks (as of July 2018) – Credit. Dr. David Scarfe

Several professional documents have been published in relation to this emerging disease in an effort to notify stakeholders. In 2017 the World Organization for Animal Health (OiE) released a "disease card"[1] for TiLV designating it as an emerging disease and urging for prompt reporting to minimize damage and enable effective management. Also, in 2017, the FAO (Food and Agriculture Organization) released a Global Information and Early Warning System (GIEWs) special alert 338 on TiLV[2]. NACA[3] (Network of Aquaculture

[1] http://www.oie.int/fileadmin/Home/eng/Internationa_Standard_Setting/docs/pdf/Aquatic_Commission/A_TiLV_disease_card.pdf
[2] http://www.fao.org/3/a-i7326e.pdf
[3] https://enaca.org/?id=864&title=tilapia-lake-virus-disease-advisory

Centres in Asia-Pacific) and WorldFish/CGIAR[4] have provided disease advisories, factsheets and held regional conventions[5] pertaining to minimize the impact of the disease.

A taxonomic proposal has been submitted by the BARD researchers to the International Committee on Taxonomy of Viruses (ICTV) for a new, unassigned genus *Tilapinevirus* that include the new species *Tilapia tilapinevirus*[6].

Two whole-genome sequences from Israel and Taiwan and additional partial sequences originating from several countries have been deposited in the GenBank database.

5 Commercial Engagement

Patent US20170360918A, which protects for "inducing an immune response against TiLV", has been licensed to one of the large international companies for their development of an inactivated virus against TiLV.

5.1 Patents

- *Novel Tilapia Virus and Uses Thereof*, W. Ian Lipkin, Thomas Briese, Nischay Mishra, Eran Bacharach and Avi Eldar; US20170360918A1, Granted 22-1-2019 to Tel Aviv University, Kimron Veterinary Institute and Columbia University.
- *Tilapia lake virus vaccines*, Eran Bacharach and Avi Eldar; US10071152B2, Granted: 11-9-2018 to Tel Aviv University and Kimron Veterinary Institute

6 Practical Agricultural Applications

Since TiLV has a large potential impact on global food security and nutrition, the FOA conducted an expert knowledge elicitation (EKE) risk assessment published in December 2018[7]. The assessment considers that movement restriction of live fish would be the most effective in managing the international spread of TiLV. Measures may include: the prohibition of live tilapia imports; sourcing live tilapia only from populations tested and certified to be TiLV-free; and quarantine and post-arrival testing of imported live tilapia. One African country has completely banned imports of tilapia into the country[8].

The OIE electronic ad hoc Group on Tilapia lake virus (TiLV) was established in November 2017 to evaluate and validate methods for detection of TiLV and recommend assays to be further developed. The next progress report is due in February 2019.

[4] https://fish.cgiar.org/publications/tilapia-lake-virus-tilv-what-know-and-do-bangla-version
[5] https://enaca.org/?id=969
[6] https://talk.ictvonline.org/ICTV/proposals/2016.016a-dM.A.v2.Tilapinevirus.pdf
[7] Food and Agriculture Organization of the United Nations (FAO). 2018. Tilapia Lake Virus Expert Knowledge Elicitation Risk Assessment (December 2018). FAO Animal Health Risk Analysis Assessment, Issue No. 7.
[8] https://thefishsite.com/articles/ghana-bans-tilapia-and-ornamental-fish-imports

Pathological and histological identification of TiLV enables public information campaigns to advise aqua-culturists, many of them smallholders, of TiLV's clinical signs.

As the research outcomes are so new, there is still an urgent need for further knowledge regarding many aspects of TiLV and its implications. It is expected that international collaborations between authorities, scientists, stakeholders and the private sector will narrow the knowledge gap and lead to improved disease containment.

Industry players are in development stages of a TiLV vaccine. As TiLV infects juveniles the target vaccine must be applicable to young fish fry, meaning the vaccine must be administered through mass application methods such as dip immersion or oral vaccines rather than via intra-peritoneal injection.

7 Social Impact

The discovery of the emerging virus has alerted stakeholders worldwide as to the potential global damages of this prominent source of low-cost protein. The number of countries where the agent has been detected is likely to increase rapidly as a result of increased awareness, surveillance and availability of diagnostic methods[9]. Containment of TiLV, which threaten the livelihoods and food security of millions of people in the developing world, would contribute to achievement of the UN 2030 Agenda towards food security and nutrition of developing countries populations, who are dependent on fish protein in their diets and for their livelihood and employment.[10]

8 Economic Impact

8.1 Investment Cost

BARD contributed $455,000 in research funds between 2013 to 2018. NIH contributed much of the laboratory instrumentation at the Center for Infection and Immunity at Columbia where the high-throughput sequencing was conducted[11]. Following consultation with experts, we estimate that the vaccine development efforts by the private sector will require $2 million between 2018 – 2021.

[9] Jansen, M. D. (2018). Tilapia lake virus:a threat to the global tilapia industry?, 1–15, https://doi.org/10.1111/raq.12254

[10] https://enaca.org/?id=864&title=tilapia-lake-virus-disease-advisory),.
http://www.oie.int/ fileadmin/ Home/eng/Internationa_Standard_Setting/docs/pdf/
https://www.worldfishcenter.org/content/tilapialake-virus-tilv-what-know-and-do
https://fish.cgiar.org/publications/tilapia-lake-virus-tilv-literature-review

[11] Personal communication with Ian Lipkin, Director of the Center for Infection and Immunity, Columbia.

8.2 The Benefits

To assess the benefits of the research outcomes we assess the global production of Tilapia, the economic burden of disease and the potential effect of the vaccination which is in development.

Global Production

Tilapia is the common name applied to three genera of fish in the Cichlidae family and includes: Oreochromis, Sarotherodon, and Tilapia. 2017 global Tilapia aquaculture production was 6.5 million tons and during the last years, it has increased at an average annual rate of 5%[12]. The top three producers of Tilapia are China, Indonesia and Egypt. Brazil, Bangladesh, Vietnam and the Philippines are other leading producers. The US annual production is betewen 9,000 – 14,000 tons[13], and Israel's annual production is around 8,000 tons[14].

Disease costs

Mortality rates from TiLV vary between sites and the reasons for the large differences are as yet unknown. Israeli farmers reported a 20% - 30% mortality rate attributed to TiLV, in addition to the background rate of mortality from other causes, following infection by the virus in 2012. Some of these farmers discontinued tilapia production following these outbreaks, leading to production collapse[15]. Mortality levels between 20% and 90% have been observed in affected farms in Thailand, 10-80% in Ecuador, and 5–15% in Egypt, with tilapia fingerlings and juveniles being more vulnerable than larger fish.[16] Epidemiology studies are necessary to understand these differences.

Estimates from Egypt indicate a production loss of 98,000 metric tons, at a value of around US$100 million, due to the 'summer mortality' syndrome in 2015. The average mortality rate was estimated as 9.2%. Tissue samples from three of the seven farms affected by 'summer mortality' tested positively for TiLV.[17]

Disease Management through Vaccination

The BARD research and outcomes made it possible for countries to identify the cause of tilapia morbidity and mortality, and to manage TiLV with biosecurity policies. The host immune response against primary infection as found in this study indicates that an effective

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[12] (a) http://www.fao.org/fi/static-media/MeetingDocuments/TiLV/dec2018/p13.pdf
(b)http://www.fao.org/3/i9540en/I9540EN.pdf (c) http://www.fao.org/3/i9942t/I9942T.pdf
(d) http://www.fao.org/news/story/en/item/888884/icode/

[13]https://www.researchgate.net/publication/303520232_TILAPIA_AQUACULTURE_2016_AND_WHERE_WILL_WE_BE_IN_2026/download
http://www.tilapia-farming.com/resources/united-states-market/u-s-aquaculture-production/

[14] information from the Israeli ministry of agriculture

[15] Personal communication

[16] http://www.fao.org/fi/static-media/MeetingDocuments/TiLV/d28.pdf

[17] https://dspace.stir.ac.uk/bitstream/1893/25140/1/TiLV%20manuscript%20rev%20final.pdf.

and affordable vaccine, now in development stages by at least one private company, can be a powerful tool for TiLV control programs.

A research published in 2019 developed a budget model to appraise the economics of vaccination against *Streptococcus agalactiae* in Nile tilapia farmed in net cages in Brazil.[18] *S. agalactiae* causes mortality and major economic losses in Nile tilapia worldwide and private companies (e.g. MSD animal health and Pharmaq) have developed vaccines to prevent the disease. Two main benefits are attributed to the vaccine; reduction of revenue loss and improved FCR (Feed Conversion Ratio).

The model showed that the financial benefits of vaccination are equal or greater than the costs in the case of a 5% disease mortality rate and a 4% improvement in FCR. If mortality rate is 10% then a 2% improvement in FCR is enough to justify vaccination costs, and if mortality rate is 20%, then vaccination is worthwhile even if no FCR improvement occurs.

The calculations are according to information from vaccine resellers, and are based on a vaccine dose cost of $0.032/fingerling and vaccine labor cost of $0.01/fingerling. A survival rate of 60% is assumed for disease-free cages. The revenue per fish was assumed to be $1.5.

Based on these results we make a general claim that vaccination contributes to a farm's economy when its cost is up to 4.7% of the expected revenue (($0.032+$0.01)/60% survival/$1.5 = 4.7%). This result is robust when mortality rate is 20%, and if mortality rate is 5% then FCR improvement is required in order for vaccination to be profitable. An FAO published field study showed 11%-12% improvement in FCR as a result of vaccination.[19] In order to assess the benefit of the BARD project discovery we make several assumptions:

- We estimate that ~ 10% of the world Tilapia production will follow vaccination protocols once a TiLV vaccination is available. This is based on measured adoption rates of tilapia Streptococcal vaccinations in China, S. E. Asia and Sth American (LATAM) countries[20], estimates of adoption growth in Indonesia and estimates of adoption in Egypt. Table 1 shows the fractional 2018 tilapia production by region[21] and the estimated regional and global TiLV vaccination adoption rate.
- For worldwide average farmers, the prevention of TiLV will reduce mortality rate by several % – 90%. We assume that 10% of the world production will follow

[18] https://doi.org/10.1016/j.prevetmed.2018.12.003 , Economic appraisal of vaccination against Streptoccocus agalactiae in Nile tilapia farms in Brazil, Delphino et al., Preventive Veterinary Medicine, (2019), 162

[19] http://www.fao.org/fi/static-media/MeetingDocuments/WorkshopAMR17/presentations/21.pdf

[20] Based on global adoption of the Tilapia Streptococcosis Vaccines, Dec 2017, http://www.fao.org/fi/static-media/MeetingDocuments/WorkshopAMR17/presentations/21.pdf

[21] https://thefishsite.com/articles/brazils-tilapia-boom

vaccination protocols[22]. Therefore, for the average global tilapia production, we estimate the prevention value is ~ 0.5 % (5%×10%) of the expected global revenue. See sensitivity tests to changes in vaccination adoption in section 8.4.

Table 1: Tilapia vaccination adoption rate estimates by region and the globally averaged anticipated adoption rate.

Country/Region	% global production	Vaccinations Adoption rate (2022-2028)
China	34%	0%
S. E. Asia	41%	8% (2022) - 14% (2028)
Africa (Egypt)	16%	20%
LATAM	9%	27%
Globally averaged adoption rate		**8.9% - 11.4%**

- Global tilapia aquaculture production is 5.4 million tons per annum[12].
- Average global expected farm revenue is $1.06/kg[23].
- The global efforts at containment, management and risk control reflects the importance of the discovery to industry, governments and global parties. This is difficult to assess in economic terms, but the FAO in a 2018 report[24] note the large financial expenditure of producers, governments and academia on biosecurity policies, diagnosis, surveillance, containment, training, research, trade disputes, and compensation for TiLV, as an example of one of the "largest aquaculture-related epizootics". To reflect these efforts in economic terms, prior to release of the vaccine in 2022, we attribute 10% of the expected benefit from the vaccine to the ongoing 2017 measures.
- We anticipate, based on consultation with involved parties, that the vaccine will be available by 2022. We attribute benefit for the years 2022 – 2028, based on the adoption range of 10% (2028).
- The FAO predicts that global aquaculture production annual growth rate will decrease from 5.7% between 2003–2016 to 2.1% between 2017–2030[25]. We assume that the benefit attributed to the discovery of TiLV will grow at this 2.1% rate for the years 2017 – 2028. We do not calculate any benefit after 2028.

The result is that global expected revenue from tilapia farming is $5.7 billion/annum. The benefit attributed to discovery of TiLV in 2017 is $2.9 million/annum (0.5% prevention value × $5.7 billion revenue × 10% = $0.0029 billion). The benefit grows according to the

[22] Based on global adoption of the Tilapia Streptococcosis Vaccines, Dec 2017, http://www.fao.org/fi/static-media/MeetingDocuments/WorkshopAMR17/presentations/21.pdf
[23] http://www.fao.org/3/i9942t/I9942T.pdf
[24] Melba G. Bondad-Reantaso, Country Level Implementation: FAO Experience in Aquaculture, Oct 2018
[25] http://www.fao.org/3/i9540en/I9540EN.pdf

above assumptions. As an example, in 2021 it is estimated at $3.1 million and in 2022 after the vaccine is available it increases to $31.6 million. We do not calculate benefit after 2028.

8.3 Economic Results

BARD invested in the initial and hence risky part of the project when the cause of tilapia morbidity and mortality was unknown, and no pathogen had been identified. According to the calculation described in the methodology section we attribute 22% of the benefit to BARD.

- Net present value of the BARD's investment is $46 million, thereof $1 million already attained
- The Internal rate of return is 83%
- Benefit cost ratio is 91, thereof 3 already attained.

The US and Israel economy benefit is calculated according to the insignificant production in these two countries and according to their import, and import prices.

Table 2: Main Results, 2018 Million Dollar-Terms

	The Project	BARD	BARD Attained	Thereof to the US	Thereof to Israel	Other Countries
BARD's Share in the Cost	20%					
Share in the Benefit		22%				
Cost	3	1	1	0.3	0.3	
Benefit	211	46	1			
Net Present Value	208	46	1	2	1	43
Internal Rate of Return	131%	83%	47%	31%	23%	
Benefit Cost Ratio	82	91	3	8	4	

8.4 Sensitivity Analysis

The low and high alternative assumptions used in the sensitivity analysis were brought together to estimate results under pessimistic and optimistic scenarios. Table 2 displays the net present value sensitivity results, between the low result: $12 million, to the high result: $101 million.

Table 3: NPV - Sensitivity Analysis, 2018 Million Dollar-Terms

			BARD's Share in the Benefit		
			Low	Central	High
			12%	22%	32%
Change in Benefit	Low	50%	12	23	33
	Central	100%	25	46	67

	High	150%	37	69	101

9 Appendix A: BARD Awards

Table 3: Details of the BARD awards

Project No	Full Title				
	Investigators	Institutes	Budget	Duration	Start Year
IS-4583-**13**	Identification of the etiological agent of tilapia disease in the Lake of Galilee				
	Bacharach, E. Lipkin, W.I. Eldar, A.	Tel Aviv U Columbia U Isr. Vet. Inst	$190,000	1 year	2013
IS-4903-**16C**	Diagnostics and vaccines for containment of TiLV - a novel RNA virus lethal to Tilapia				
	Bacharach, E. Lipkin, W.I. Eldar, A.	Tel Aviv U Columbia U Isr. Vet. Inst	$265,000	3 years	2016

10 Appendix B: Information providers: Personal communication

- W. Ian Lipkin - Co-PI for BARD grants, Center for Infection and Immunity, Columbia University
- Eran Bacharach – PI for BARD grants, Molecular virology; Tel Aviv University
- Avi Eldar – Co-PI for BARD grants, Fish disease laboratory for Israel's Ministry of Agriculture, Kimron Veterinary Institute
- Yeng Sheng Lee - Sr Specialist, Global Marketing Aqua, MSD Animal Health
- Nadav Davidovich - Israeli Veterinary Services and Animal Health, Ministry of Agriculture, Participant in FAO risk assessment
- Ron Katz - Senior Technology Licensing Officer, Columbia University
- Yitzhak Simon – Head of aquaculture extension service in Israel
- Raanan Ariav – Head of aquaculture department in Phibro, an international inputs company
- Haim Shamir – Aquaculture farm manager of Ein Hamifratz, Israel
- Guy Sarig – Aquaculture farm manager of Lochmei Hagetaot, Israel
- Efraim Gulzer – Aquaculture farm manager of Kfar Masaryk, Israel