New tick and insect cell line resources for vector-borne disease research from the Tick Cell Biobank

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Summary

Background: Arthropod cell lines play an important role in research on the control of vector-borne pathogens of veterinary, medical and agricultural importance. The Tick Cell Biobank (TCB) at the University of Liverpool is the world's only dedicated collection of cell lines derived from tick and insect vectors of viral, bacterial, protozoan and helminth pathogens. ***Aim***: The TCB in Liverpool and TCB Outposts in Malaysia and Brazil were established to facilitate access for researchers worldwide to tick and insect cell line resources, and training in their maintenance, application and development. ***Methods***: The TCB receives, stores and distributes arthropod cell lines to scientists on request, and generates new cell lines from tick and insect vectors. To facilitate successful uptake of the cell lines, the TCB and its Outposts provide training in arthropod cell culture, and ongoing advice and support to recipients. Cell lines are supplied subject to Material Transfer Agreements. ***Results***: The TCB now houses over 90 cell lines derived from ixodid and argasid ticks, mosquitoes, biting midges, sand flies, tsetse flies, triatomine bugs and honey bees. This paper describes new and recently-developed/acquired resources including cell lines derived from the ticks *Argas reflexus*, *Hyalomma lusitanicum*, *Hyalomma marginatum* and *Rhipicephalus bursa*, and the insects *Anopheles stephensi*, *Apis mellifera*, *Culicoides sonorensis*, *Glossina morsitans*, *Phlebotomus argentipes* and *Triatoma infestans*. ***Conclusions:*** The majority of vector species represented in the collection are of tropical or sub-tropical origin. Through distribution of these existing and new cell lines, the TCB and TCB Outposts will continue to underpin global research on arthropod vectors and the livestock and human pathogens that they transmit.

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■ INTRODUCTION

Over the past six decades, arthropod cell lines have played an increasingly important role in research on the biology and control of vectors and vector-borne pathogens of veterinary, medical and agricultural importance (Walker *et al*., 2014; Bell-Sakyi *et al*., 2007, 2018; Ghosh *et al*., 2020; Goodman *et al*., 2021; Salata *et al*., 2021; He *et al*., 2023). The Tick Cell Biobank (TCB) is the world's only dedicated collection of cell lines derived from tick and insect vectors of viral, bacterial, protozoan and helminth pathogens (Bell-Sakyi *et al*., 2018). As well as storing and distributing cell lines to scientists worldwide, the TCB provides training in arthropod cell line maintenance, application and development, and ongoing advice and support to recipients. TCB Outposts in Malaysia and Brazil facilitate distribution of cell lines and provide training to lower- and middle-income country scientists in Asia and South America (Yean *et al*., 2024).

Part of the TCB's remit is to generate new cell lines from ticks and insects, with an emphasis on neglected vectors of livestock and/or human diseases. This process can be lengthy and challenging, requiring patience and dedication. It can take between a few months and three years for insects, and between one and eight years for ticks (Singh, 1967; Peleg, 1969; Varma *et al*., 1975; Bell-Sakyi, 1991; Munderloh *et al*., 1994; Bell-Sakyi *et al*., 2009, 2020, 2021, 2022, 2024; Goblirsch *et al*., 2013). Here a summary is presented of the new tick and insect cell lines generated by the TCB since the last report seven years ago (Bell-Sakyi *et al*., 2018), as well as cell lines deposited by external collaborators.

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■ MATERIAL AND METHODS

In nearly all cases, arthropod eggs were used as the starting material for generating new cell lines (Bell-Sakyi *et al*., 2020, 2021, 2022; Penrice-Randal *et al*., 2022), with the exception of the tsetse fly, *Glossina morsitans morsitans*, from which adult female ovarian tissues and larvae were used (Bell-Sakyi *et al*., 2024). These were kindly provided by the collaborators acknowledged in the publications listed in Table I or, for previously unpublished cell lines, listed in Table II. Primary cell cultures were generated following published procedures with some adaptations (Singh, 1967; Schneider, 1979; Tesh & Modi, 1983; Bell-Sakyi 1991; Wechsler *et al*., 1991; Bell-Sakyi *et al*., 2020; 2021; 2022; 2024). Engorged female ticks were surface-sterilized prior to oviposition and their eggs were processed to generate primary cell cultures when the rectal sacs and Malpighian tubules of the developing embryos became visible. Insect eggs were surface-sterilized with 0.1% benzalkonium chloride and 70% ethanol upon receipt. Subsequent processing to generate primary cell cultures was carried out using either embryos (Bell-Sakyi *et al*., 2020; 2021; Penrice-Randal *et al*., 2022), or larvae hatched from surface-sterilized eggs following incubation in complete culture medium (Bell-Sakyi *et al*., 2021).

Four complete culture media or combinations thereof were used for maintaining tick cells: L-15, H-Lac, L-15/MEM and L-15B, supplemented as described previously (Bell-Sakyi *et al*., 2022). Complete L-15, H-Lac and L-15B were used with insect cells; in addition, Schneider's Insect Medium (Sigma catalogue number S0146) supplemented with 20% fetal bovine serum and 2 mM L-glutamine was used with *Anopheles stephensi* eggs and *Phlebotomus argentipes* cells.

Primary cell cultures were incubated at 28°C with weekly replacement of part of the culture medium. The first subculture was attempted when significant cell growth was observed. This could occur within weeks for some insects, for example *Culex pipiens* embryo-derived cells (Bell-Sakyi *et al*., 2021), or only after several months or years for other insects, for example *G. m. morsitans* (Bell-Sakyi *et al*., 2024), and for all ticks (Bell-Sakyi *et al*., 2022). Cell lines were considered as established when they could be passaged at regular intervals and successfully cryopreserved and resuscitated following previously-described methods (Bell-Sakyi, 1991; Bell-Sakyi *et al*., 2000).

DNA extracted from early subcultures was screened to confirm species origin of the cell lines using PCRs targeting tick 16S rRNA, insect cytochrome oxidase I (COI) or triatomine cytochrome B (*cytB*) genes as described previously (Black & Piesman, 1994; Folmer *et al*., 1994; Monteiro *et al*., 2013). Sequences obtained from previously unpublished cell lines were deposited in Genbank with the following accession numbers: for tick 16S rRNA – PV260365 (ARE/LULS71), PV260366 (HLE/LULS63), PV260367 (RBE/LULS62), PV260368 (RBE/LULS67) and PV260369 (HME/LULS73); for insect COI – PV270193 (ASL/LULS76), PV270194 (CSL/LULS64) and PV270192 (PAL/LULS74); for triatomine *cytB* – PV269790 (RPE/LUCH66), PV269791 (TIE/LULS65) and PV269792 (TIE/LULS69). A pan-bacterial 16S rRNA PCR was used to screen for contaminating bacteria (Weisburg *et al*., 1991). All cell lines were tested annually for the presence of mycoplasma as described previously (Bell-Sakyi *et al*., 2021) and consistently found to be negative.

■ RESULTS

The new cell lines generated in the TCB since 2018 are listed in Tables I and II. They include two argasid and 11 ixodid tick cell lines, and 18 new insect cell lines derived from biting midges (n=3), mosquitoes (n=4), sand flies (n=4), triatomine bugs (n=6) and tsetse flies (n=1). All the tick cell lines were derived from embryos, while ten of the insect cell lines were derived from embryos, seven from larvae

Table I: Already-published tick and insect cell lines generated in the Tick Cell Biobank since 2018 /// *Lignées cellulaires de tiques et d'insectes déjà publiées et générées dans la Tick Cell Biobank depuis 2018.*

Arthropod species	Arthropod group/Instar of origin	Cell line name	Year of origin/ current passage level	Reference
Argas reflexus	Tick/embryo	ARE/LULS41	2015/p12	Bell-Sakyi *et al.,* 2022
Culex pipiens	Mosquito/embryo	CPE/LULS50	2019/p88	Bell-Sakyi *et al.,* 2021
	Mosquito/larva	CPL/LULS56	2019/p72	
Culicoides nubeculosus	Midge/embryo	CNE/LULS44	2018/p29	Bell-Sakyi *et al.,* 2020
		CNE/LULS47	2018/p20	
Dermacentor reticulatus	Tick/embryo	DRE/LULS60	2019/p22	Bell-Sakyi *et al.,* 2022
Glossina morsitans	Tsetse fly/adult	GMA/LULS61	2018/p31	Bell-Sakyi *et al.,* 2024
Hyalomma lusitanicum	Tick/embryo	HLE/LULS42	2014/p10	Bell-Sakyi *et al.,* 2022
		HLE/LULS43	2014/p13	
		HLE/LULS48	2014/p15	
Hyalomma scupense	Tick/embryo	HSE/LULS51	2016/p17	Bell-Sakyi *et al.,* 2022
		HSE/LULS59	2016/p15	
Lutzomyia longipalpis	Sand fly/embryo	LLE/LULS45	2018/p44	Bell-Sakyi *et al.,* 2021
	Sand fly/larva	LLL/LULS52	2020/p96	
Phlebotomus papatasi	Sand fly/larva	PPL/LULS49	2019/p42	Bell-Sakyi *et al.,* 2021
Rhipicephalus bursa	Tick/embryo	RBE/LULS58	2018/p8	Bell-Sakyi *et al.,* 2022
Rhodnius prolixus	Triatomine bug/embryo	RPE/LULS53	2019/p36	Penrice-Randal *et al.,* 2022
		RPE/LULS57	2019/p29	
Triatoma infestans	Triatomine bug/embryo	TIE/LULS54	2020/p18	Penrice-Randal *et al.,* 2022

and one from adult tissues. Table I summarizes the new cell lines that have already been published separately; details of the methods used and descriptions of the cell lines are included in the cited references. Details of previously unpublished cell lines are presented in Table II. The methods used to generate them are as follows for each arthropod taxon.

New tick cell lines

The *Argas reflexus* cell line ARE/LULS71 was generated from ~100 6-week-old eggs laid by a single tick that had been fed two years previously and incubated for most of the time at 15°C, with occasional short periods of exposure to room temperature. The eggs were processed and the primary culture handled as described for cell line ARE/LULS41 (Bell-Sakyi *et al*., 2022). ARE/LULS71 cells are grown in L-15 medium at 28°C and were successfully cryopreserved at passage 11. The phenotype is predominantly floating clumps of large, round cells with some very large, vacuolated cells. Following subculture, some of the cells are loosely attached with long cytoplasmic protrusions (Figure 1A). The tick 16S rRNA sequence obtained from DNA extracted from ARE/LULS71 cells at passage 2 was 99.76% similar (100% query coverage) to sequences from *A. reflexus* ticks from Poland (AF001401) and an unspecified origin (L34322). No bacterial sequences were amplified from the DNA.

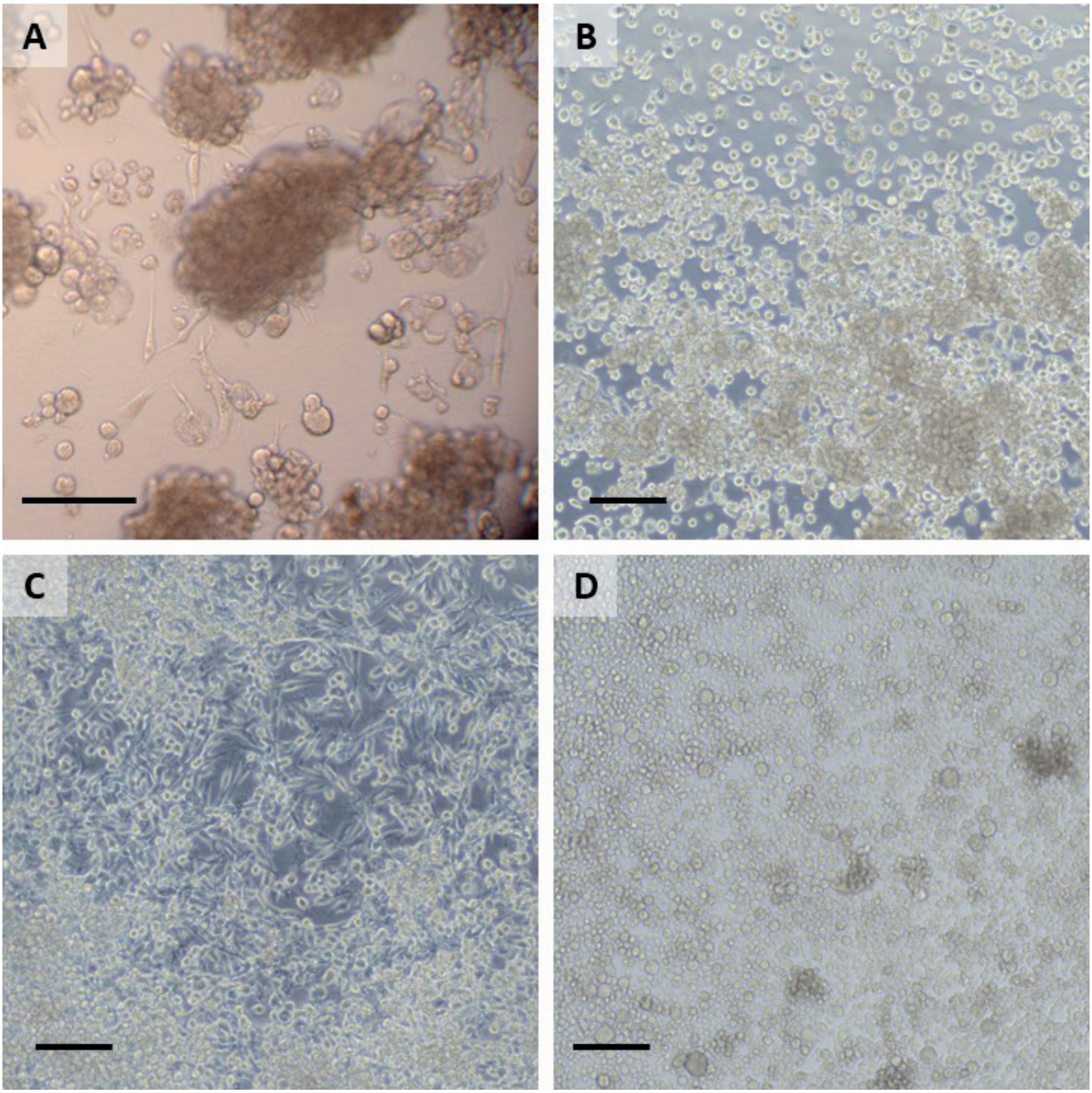

Figure 1: Inverted microscope images of new embryo-derived tick cell lines. A. *Argas reflexus* ARE/LULS71 at passage 22. B. *Hyalomma lusitanicum* HLE/LULS63 at passage 23. C. *Rhipicephalus bursa* RBE/LULS62 at passage 25. D. *Hyalomma marginatum* HME/LULS73 at passage 9. Scale bars = 200 µm /// *Images au microscope inversé de nouvelles lignées cellulaires de tiques dérivées d'embryons. A.* Argas reflexus *ARE/LULS71 au passage 22. B.* Hyalomma lusitanicum *HLE/LULS63 au passage 23. C.* Rhipicephalus bursa *RBE/LULS62 au passage 25. D.* Hyalomma marginatum *HME/LULS73 au passage 9. Échelle = 200 µm*

The fourth surviving *Hyalomma lusitanicum* embryo-derived primary culture described previously (Bell-Sakyi *et al*., 2022) eventually gave rise to the cell line HLE/LULS63, grown at 28°C in L-15/H-Lac medium. It was successfully cryopreserved at passage 4, 8 years and 5 months after initiation. The phenotype of HLE/LULS63 is quite similar to those of the previously-reported *H. lusitanicum* cell lines (Bell-Sakyi *et al*., 2022), comprising round, vacuolated, predominantly floating cells with a tendency to clump (Figure 1B). DNA extracted from HLE/LULS63 cells at passage 1 yielded a tick 16S rRNA sequence with 99.74% and 99.25% similarities (99% query cover) to the sequences obtained from the *H. lusitanicum* cell lines HLE/LULS43 (ON366982) and HLE/LULS48 (ON366981) respectively, and 99.24% similar (98% query cover) to sequences from *H. lusitanicum* ticks from Portugal (KU130444 and LC508315). The absence of contaminating bacteria was confirmed.

Similarly, two of the *Rhipicephalus bursa* embryo-derived primary cultures reported previously (Bell-Sakyi *et al*., 2022) yielded cell lines: RBE/LULS62 was initiated in 2016 and is now grown in L-15/H-Lac medium at 28 and 32°C, while RBE/LULS67 was initiated in 2019 and is grown in H-Lac medium at 28°C. Both lines were successfully cryopreserved, at passage levels 3 and 2, respectively. RBE/LULS62 grows as a layer of attached, lightly-vacuolated cells with a tendency to form scattered clumps within the monolayer (Figure 1C), while the phenotype of RBE/LULS67 is highly variable with a mixture of small, round, hemocyte-like cells and large tissue-like clumps, and cannot yet be considered fully established. Both cell lines were confirmed as *R. bursa* at passage 1, with tick 16S rRNA sequence identities of 100% (100% query coverage) to a sequence from a *R. bursa* tick from Turkey (OL347854) and 100% (99% query cover) to the *R. bursa* cell line RBE/LULS58 (ON366979). Both lines were free of contaminating bacteria. Two additional primary cultures set up at the same time as RBE/LULS67 have been taken through several subcultures, but have not yet been successfully cryopreserved.

The *Hyalomma marginatum* cell line HME/LULS73 was derived from one of four primary cultures set up from a single egg batch in different culture media (two in L-15 and one each in L-15/L-15B and L-15/H-Lac) at 28°C in June 2023. Cells in the two primary cultures in L-15 started to multiply significantly after only two months; the primary culture in L-15/L-15B exhibited limited cell growth after four months, while no cells grew in the primary culture in L-15/H-lac. The two growing cultures were first passaged after five months, and changed into L-15/L-15B medium thereafter. Only one of the two growing cultures could be regularly subcultured to date, giving rise to the HME/LULS73 cell line, which was successfully cryopreserved at passage 2. A further 12 primary cultures were set up contemporaneously from six other *H. marginatum* egg batches. Six of them have survived at the time of writing, but none have yet exhibited significant cell growth. HME/LULS73 currently comprises predominantly small round cells, some resembling hemocytes, which grow as a dense, three-dimensional layer of predominantly attached, sometimes clumping cells (Figure 1D). DNA extracted from the primary culture at 7 months confirmed species origin as *H. marginatum*, with tick 16S rRNA sequence identities of 100% (100% query coverage) to multiple deposits in GenBank, including sequences from ticks from Portugal (OL454857), Corsica (MH663978), Turkey (OL347853) and Tunisia (PP423063). No bacterial sequences were amplified from DNA extracted at passage 9.

New insect cell lines

The triatomine bug primary cultures that gave rise to the *Rhodnius prolixus* cell line RPE/LUCH66 and the *Triatoma infestans* cell lines TIE/LULS65 and TIE/LULS69 were reported previously (Penrice-Randal *et al*., 2022). RPE/LUCH66 was initiated in L-15 medium, first subcultured at 26 months and successfully cryopreserved at passage 4, 19 months later. The cell line grows in flat-sided tubes at 28°C. To date, attempts to adapt the cells to grow in T25 flasks have not succeeded. RPE/LUCH66 cells grow as a sheet of predominantly large, elongated, firmly-attached fibroblast-like cells with some clumping (Figure 2A). Species origin was confirmed by triatomine *cytB* PCR at passage 5 as *R. prolixus* with 99.67% identity (100% query cover) to the published *R. prolixus* sequences AF421339, MH704763 and EF011721. TIE/LULS65 was initiated from the same pool of eggs as TIE/LULS54 (Penrice-Randal *et al*., 2022), in H-Lac medium, which was later changed to L-15/H-Lac. This line was first passaged at 9 weeks and successfully cryopreserved at passage 3. The TIE/LULS65 cells are a mixture of small round cells and elongated, fibroblast-like cells forming a loosely-attached sheet. TIE/LULS69 was initiated from a separate pool of eggs from the same batch as the other two *T. infestans* cell lines, in L-15 medium, first passaged at 39 months and successfully cryopreserved at passage 6, 7 months later. TIE/LULS69 comprises round cells of varying sizes that form loosely-attached or floating clumps; occasionally groups of cells in a clump produce a red pigment of unknown origin, which may persist for several months or disappear within a week or two (Figure 2B). This red pigment has never been observed in the other two *T. infestans* lines, but first appeared in cells of the parent and low passage cultures of TIE/LULS69 at 38 months after initiation. Species origin of both *T. infestans* lines was confirmed by triatomine *cytB* PCR, with 100% similarity (100% query cover) to published *T. infestans* sequences MH763653 and KY654076 at passage 12 (TIE/LULS65) and 6 (TIE/LULS69), respectively. All three new triatomine bug cell lines were free of contaminating bacteria.

The new mosquito, midge and sand fly cell lines were all generated from larvae hatched aseptically from surface-sterilized eggs, following the methods described previously for mosquito and sand fly cell lines (Bell-Sakyi *et al*., 2021). Two *A. stephensi* cell lines, ASL/LUCH75 (Figure 2C) and ASL/LULS76 were generated as follows. Approximately 100 *A. stephensi* eggs laid within the previous 12 h were surface-sterilized and incubated in Schneider's Insect Medium at 28°C. Around 20 hatched larvae were processed seven or two days later, respectively, to generate two primary cultures in flat-sided tubes in L-15 medium. Large multicellular vesicles began to develop in the primary cultures after three weeks. These continued to grow, accompanied by patches of attached cells of diverse phenotypes. The first subcultures were carried out 7–8 months after initiation, and cells were successfully cryopreserved after 14 months at passage 3 and 7, respectively. Both lines comprise a mixture of small, firmly-attached fibroblast-like and epithelial-like cells and large, round or oval-shaped floating vesicles formed by cells of differing morphology. At the time of writing, in ASL/LULS76 the attached cells are more granular, and the floating vesicles are more pleomorphic and combine to form larger accumulations than in ASL/LUCH75. From passage 5, ASL/LUCH75 cells were found to grow more consistently in L-15/H-Lac medium and a subline was subsequently maintained in this medium. Species origin of ASL/LULS76 was confirmed by insect COI PCR as *A. stephensi* at passage 4 with 100% identity (100% query cover) to sequences from mosquitoes collected in Pakistan (KF406680), India (MN329060) and the United Arab Emirates (MK170098) among others. Both cell lines were free of contaminating bacteria.

The *Culicoides sonorensis* cell line CSL/LULS64 was generated as follows. *C. sonorensis* eggs laid during the previous 24 h were surface-sterilized and held in Hanks balanced salt solution overnight. On the following day, primary cell cultures were prepared from crushed eggs as described previously for *Culicoides nubeculosus* (Bell-Sakyi *et al*., 2020), but no embryonic tissues were obtained. However, some uncrushed eggs subsequently hatched into larvae and two primary cultures were set up from macerated larvae on days 5 (7 larvae in L-15)

and 8 (4 larvae in L-15B). Very small patches of growing cells were seen from 5 weeks after initiation, and the two primary cultures were combined into a single flat-sided tube after 4.5 months. Appreciable, though very slow, cell growth commenced 1.5 months later. The first subculture was carried out almost one year after initiation, at which time the culture comprised small, attached, spindle-shaped cells and large, attached or floating multicellular vesicles. CSL/LULS64 cells at passage 2 were successfully cryopreserved a month later. The phenotype of the cell line, similar to those reported previously for two *C. nubeculosus* cell lines (Bell-Sakyi *et al*., 2020), has remained fairly constant up to the current passage level. Species origin was confirmed at passage 14 with insect COI sequence identities of 99.83% (100% query coverage) to colony-derived *C. sonorensis*, originally from Texas, USA (BK065013; Ahmed *et al*., 2025) and wild-caught *C. sonorensis* from Kansas, USA (OL604738). No bacterial sequences were detected.

The *P. argentipes* cell line PAL/LULS74 was generated as follows. Around 1000 eggs laid at least two days previously were surface-sterilized and crushed as described previously for *Phlebotomus*

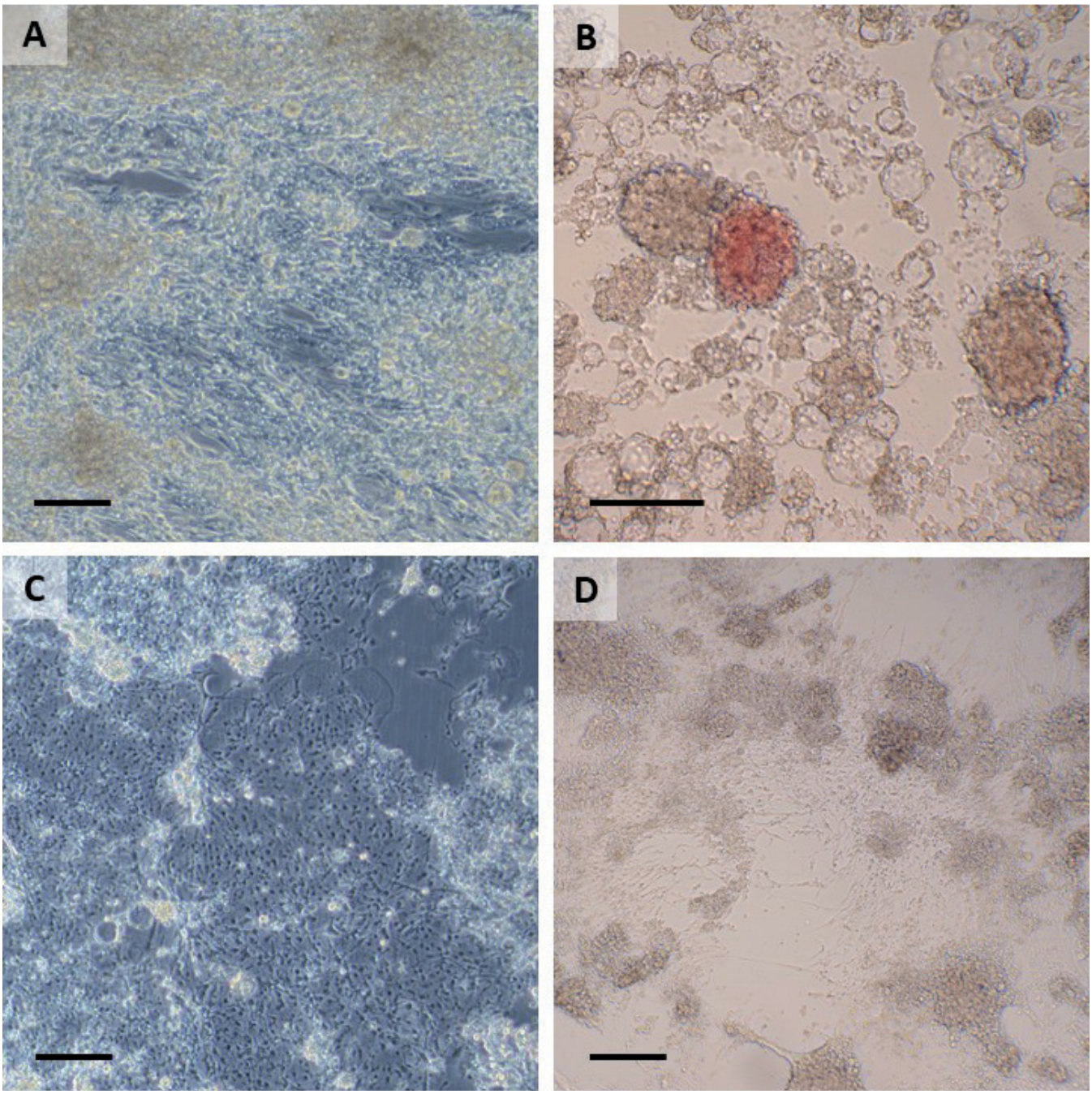

Figure 2: Inverted microscope images of new insect cell lines. A. Embryo-derived *Rhodnius prolixus* RPE/LUCH66 at passage 8. B. Embryo-derived *Triatoma infestans* TIE/LULS69 at passage 10, showing clump of cells expressing red pigment. C. Larva-derived *Anopheles stephensi* ASL/LUCH75 at passage 5. D. Larva-derived *Phlebotomus argentipes* PAL/LULS74 at passage 9. Scale bars = 200 µm /// *Images au microscope inversé de nouvelles lignées cellulaires d'insectes. A.* Rhodnius prolixus *RPE/LUCH66 dérivé de l'embryon au passage 8. B.* Triatoma infestans *TIE/LULS69, dérivé d'un embryon, au passage 10, montrant un amas de cellules exprimant un pigment rouge. C.* Anopheles stephensi *ASL/LUCH75 au passage 5, dérivé de larves. D.* Phlebotomus argentipes *PAL/LULS74 au passage 9. Echelle = 200 µm*

papatasi (Bell-Sakyi *et al*., 2021) and divided between three culture tubes. Of these, two were lost to fungal contamination; 24 larvae hatched from uncrushed eggs were removed from the third tube and macerated to prepare a single primary culture in H-Lac/L-15B medium. Eight days later, all larval pieces were collected, centrifuged, incubated with trypsin (500 µg/ml in PBS) for 5 min at 37°C, resuspended in their original medium and returned to the original tube at 28°C to encourage adherence. A few adherent cells began to appear after a further 5 weeks, but the culture comprised mainly small floating clumps of tissue for the following 20 months. At 23 months, the floating clumps were re-trypsinized and put into a fresh culture tube with Schneider's Insect Medium, where they combined into a single clump. Thereafter, this clump gradually increased in size, sometimes floating and sometimes adhering to the surface of the tube, with a few very small, round and spindle-shaped cells migrating outwards. After a failed attempt to subculture part of the clump at 29 months, the first successful passage was achieved after 34 months *in vitro*. Thereafter, the cells began to grow rapidly, with successful cryopreservation at passage 5, when the PAL/LULS74 cell line was 39 months old. The cells are now maintained in either Schneider's Insect Medium or L-15/H-Lac/L-15B medium. They comprise a mixture of very small, rounded cells forming dense clumps, and loosely-attached, elongated, spindle-shaped cells (Figure 2D). PCR assays on DNA extracted at passage 3 confirmed species origin as *P. argentipes*, with a 658 bp COI sequence 99.39% (MT472524) and 99.24% (PP495526) identical (100% query cover) to sequences from sand flies collected in India, and absence of contaminating bacteria.

Cell lines deposited by external collaborators

In addition to the new tick and insect cell lines generated in-house, the TCB has received deposits of lines developed elsewhere (Table III). These include four widely-used mosquito cell lines derived from *Aedes aegypti* (Aag2), *Aedes albopictus* (U4.4 and C6/36) and *Culex quinquefasciatus* (Hsu), the *Apis mellifera* cell line AME-711, two new tick cell lines derived from Brazilian *Amblyomma sculptum* (ASE-14) and *Rhipicephalus microplus* (RBME-6), and the widely-used North American *R. microplus* cell line BME26. PCR assays carried out following receipt confirmed species origin and absence of contaminating bacteria in all the deposited cell lines (data not shown).

■ DISCUSSION

Since its inception in 2009, the TCB has expanded from its initial focus on exclusively tick cell lines to encompass generation, storage and supply of cell lines representing a much wider range of harmful and beneficial arthropods. The tick cell line collection has grown from the initial 41 lines derived from 13 ixodid and two argasid species to 67 lines derived from 21 ixodid and three argasid species. The insect cell line collection now comprises 28 cell lines derived from 14 insect species, including disease vectors (mosquitoes, biting midges, sand flies, tsetse flies and triatomine bugs), in addition to the world's only honey bee cell line. Most of these cell lines are currently available for distribution. Over the past 15 years, tick and insect cell lines have been supplied by the TCB and TCB Outposts to more than 100 institutes in 37 countries on six continents, and over 150 scientists have been trained in their maintenance, application and/or generation.

While the TCB has welcomed deposits of tick and insect cell lines from external sources (Table III), the majority of additions to the collection have been generated in-house. The focus has been on important disease vectors either from which cell lines were developed many years ago but have since been lost, such as *T. infestans* and

Table II: Previously unpublished tick and insect cell lines generated in the Tick Cell Biobank since 2018 /// *Lignées cellulaires de tiques et d'insectes inédites générées dans la Tick Cell Biobank depuis 2018*

Arthropod species/ country of origin	Arthropod group/ instar of origin	Cell line name	Year of origin/age at first subculture/current passage level	Acknowledgements
Anopheles stephensi/ India	Mosquito/larva	ASL/LUCH75	2023/9 months/p9	Eggs provided by Vish Dhokiya, Tara Joseph and Grant Hughes, Liverpool School of Tropical Medicine, UK
		ASL/LULS76	2023/10 months/p15	
Argas reflexus/ Germany	Tick/embryo	ARE/LULS71	2017/44 months/p24	Fed female ticks provided by Jan Hendrik Forth, Friedrich-Loeffler Institut Riems, Germany
Culicoides sonorensis/ USA	Midge/larva	CSL/LULS64	2021/12 months/p36	Eggs of the *C. sonorensis* Pirbright s-3 strain provided by The Pirbright Institute, UK
Hyalomma lusitanicum/ Spain	Tick/embryo	HLE/LULS63	2014/46 months/p24	Engorged female tick provided by Sonia Olmeda Garcia, Universidad Complutense de Madrid, Spain
Hyalomma marginatum/ France	Tick/embryo	HME/LULS73	2023/5 months/p11	Engorged female ticks provided by Frederic Stachurski and Maxime Duhayon, CIRAD, France
Phlebotomus argentipes/ India	Sand fly/larva	PAL/LULS74	2021/34 months/p14	Eggs provided by Petr Wolf and Nikola Polanska, Charles University, Czech Republic
Rhipicephalus bursa/ Spain	Tick/embryo	RBE/LULS62	2016/74 months/p26	Engorged female ticks provided by Ana Palomar, CIBIR, Logrono, Spain
		RBE/LULS67	2019/45 months/p10	
Rhodnius prolixus/ Venezuela	Triatomine bug/ embryo	RPE/LUCH66	2019/26 months/p10	Eggs provided by Miranda Whitten, Swansea University, UK
Triatoma infestans/ Paraguay	Triatomine bug/ embryo	TIE/LULS65	2020/2 months/p21	Eggs provided by Luke Brandner-Garrod, London School of Hygiene and Tropical Medicine, UK
		TIE/LULS69	2020/39 months/p26	

Table III: Cell lines deposited in the Tick Cell Biobank by external collaborators since 2018 /// *Lignées cellulaires déposées dans la Tick Cell Biobank par des collaborateurs externes depuis 2018*

Arthropod species	Arthropod group/instar of origin	Cell line name	Reference
Aedes aegypti	Mosquito/neonate larva	Aag2	Peleg (1969); Lan and Fallon (1990)
Aedes albopictus	Mosquito/neonate larva	U4.4	Singh (1967); Condreay and Brown (1986)
		C6/36	Singh (1967); Igarashi (1978)
Amblyomma sculptum	Tick/embryo	ASE-14	Lima-Duarte *et al.* (2022)
Apis mellifera	Honey bee/embryo	AME-711	Goblirsch *et al.* (2013)
Culex quinquefasciatus	Mosquito/adult	Hsu	Hsu *et al.* (1970)
Ixodes scapularis	Tick/embryo	IDE12	Munderloh *et al.* (1994)
Rhipicephalus microplus	Tick/embryo	BME26	Kurtti *et al.* (1988)
		RBME-6	Lima-Duarte *et al.* (2021)

G. m. morsitans (Pudney & Lanar, 1977; Schneider, 1979; Steiger *et al*., 1977), or from which no cell lines existed, such as *H. marginatum*, *R. bursa* and *C. pipiens*. Subsequent to establishment of our two UK *C. pipiens* cell lines, a third cell line of this species has been generated from embryonic US mosquitoes (Fallon *et al*., 2023). Tick cell lines were successfully generated following standard methods (Varma *et al*., 1975; Bell-Sakyi, 1991; Munderloh *et al*., 1994), although the very long pre-establishment periods experienced in some cases (e.g. 8 years for HLE/LULS63) emphasized the need for patience, as pointed out previously (Bell-Sakyi *et al*., 2007). In contrast, most of the new insect cell lines required the adaptation of existing published protocols or the development of novel protocols tailored to each arthropod species. For example, the approach used for *C. nubeculosus*, in which eggs were transported by overnight mail from The Pirbright Institute midge colony to the TCB and processed more than 48 h after oviposition (Bell-Sakyi *et al*., 2020), was found to be unsuitable for *C. sonorensis*, as the eggs of the latter species started to hatch into larvae within the first 24 h after oviposition. Instead, *C. sonorensis* eggs were surface-sterilized at The Pirbright Institute, transported to the TCB and aseptically-hatched larvae were then used to generate the primary cultures. In some cases, using larvae that hatched naturally from surface-sterilized eggs, rather than embryonic tissues released by crushing the eggs, to generate primary cultures (Bell-Sakyi *et al*., 2020, 2021), also reduced the risk of contamination from fungi, which were apparently incorporated into the structure of the eggshells. This approach was successful for *P. argentipes* and *A. stephensi*, after attempts to generate embryo-derived primary cultures of both species failed due to fungal contamination.

While some of the new insect cell lines developed by, or deposited in, the TCB may not be the only cell lines derived from particular vector species, the TCB is the only culture collection that houses and supplies these cell lines globally. For example, the Cellosaurus website (Cellosaurus, accessed 23.12.24) lists six *A. stephensi* lines, 16 *Culex* spp. lines and five *L. longipalpis* lines, but none of them are listed as available from any culture collection apart from the lines held by the TCB. Similarly, the TCB is the only culture collection that successfully and consistently supplies tick cell lines, and provides effective training and follow-up support to ensure successful transfer and establishment of tick cells in the recipient laboratory.

Honey bees are not traditionally considered as vectors, but are hugely important pollinators globally, and maintaining healthy bee populations is essential for food security (Halvorson *et al*., 2021; Prata and Martins da Costa, 2024). Deposition of the *A. mellifera* cell line AME-711 (Goblirsch *et al*., 2013) in the TCB has enabled it to be distributed more widely outside its country of origin (USA), along with essential training in the maintenance of this relatively fastidious research tool. Experience gained from handling the AME-711 cells has helped the TCB to generate primary cell cultures from UK honey bees, with a view to cell line development. AME-711 and any future *A. mellifera* cell lines provide previously unavailable opportunities for *in vitro* studies of interactions between honey bees and their pathogens, as well as research on the adverse effects of pesticides at the cellular and molecular level.

Other arthropod cell line challenges remain. These include mites, which have so far proved refractory to *in vitro* cultivation despite multiple attempts with four different mite genera, and *Haemaphysalis* spp. ticks, which are of increasing importance globally as vectors of livestock and human pathogens. Although young cell lines were reported from two *Haemaphysalis* spp. nearly 50 years ago (Guru *et al*., 1976), they appear to have been lost, and one recent attempt with this genus did not yield a cell line (Lim *et al*., 2017). On the other hand, the availability of new cell lines from the tick *H. marginatum*, a major vector of Crimean-Congo hemorrhagic fever virus, *G. m. morsitans*, a vector of salivarian trypanosomes, and the *Phlebotomus* and *Lutzomyia* sand fly vectors of *Leishmania* parasites, will facilitate research into these veterinary and/or zoonotic pathogens.

■ CONCLUSION

In conclusion, the resources of the TCB described here, and those reported previously, represent valuable research tools that are available for application in a range of studies on tick and insect vectors and the pathogens they transmit. The TCB's experience in arthropod cell line generation illustrates the importance of maintaining an open mind when applying or adapting previously-used protocols. It is essential to think laterally, be prepared to try anything and, above all, be patient. All the cell lines reported here are available from the TCB, subject to appropriate Material Transfer Agreements.

For further information, contact tickcellbiobankenquiries@liverpool.ac.uk or visit our website at https://www.liverpool.ac.uk/research/facilities/tick-cell-biobank/

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Conflicts of interest

The authors declare that there is no conflict of interest.

Author contributions

CH, JJK, AD, BLM and LBS participated in the conception and design of the study; CH, JJK and LBS carried out the laboratory work including cell line generation, cell line characterization and data interpretation; LBS drafted the manuscript; CH, JJK, AD and BLM critically reviewed the manuscript; AD, BLM and LBS secured the funding for the Tick Cell Biobank.

Ethics approval statement

Ethics committee approval regarding the use of animals or human samples was not required for this study as no animals or human samples were used.

Data availability

Sequences obtained from previously unpublished cell lines were deposited in Genbank under accession numbers as listed in the text.

Declaration of Generative AI in the writing process

The authors did not use any artificial intelligence-assisted technologies in the writing process.

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Résumé

Hartley C., Khoo J.J., Darby A., Makepeace B.L., Bell-Sakyi L. Nouvelles ressources sur les lignées cellulaires de tiques et d'insectes pour la recherche sur les maladies à transmission vectorielle provenant de la structure internationale Tick Cell Biobank

Contexte : Les lignées cellulaires d'arthropodes jouent un rôle important dans la recherche sur le contrôle des pathogènes à transmission vectorielle d'importance vétérinaire, médicale et agricole. La Tick Cell Biobank (TCB) de l'université de Liverpool est la seule collection au monde de lignées cellulaires dérivées de tiques et d'insectes vecteurs de pathogènes viraux, bactériens, protozoaires et helminthes. ***Objectif*** : Le TCB de Liverpool et ses avant-postes en Malaisie et au Brésil ont été créés pour faciliter l'accès des chercheurs du monde entier aux ressources en lignées cellulaires de tiques et d'insectes, ainsi que la formation à leur entretien, leur application et leur développement. ***Méthodes*** : Le TCB reçoit, stocke et distribue des lignées cellulaires d'arthropodes aux scientifiques qui en font la demande, et génère de nouvelles lignées cellulaires à partir de tiques et d'insectes vecteurs. Pour faciliter l'adoption des lignées cellulaires, le TCB et ses avant-postes assurent une formation à la culture des cellules d'arthropodes et fournissent des conseils et un soutien permanents aux bénéficiaires. Les lignées cellulaires sont fournies dans le cadre d'accords de transfert de matériel. ***Résultats*** : Le TCB abrite désormais plus de 90 lignées cellulaires dérivées de tiques ixodidae et argasidae, de moustiques, de moucherons piqueurs, de phlébotomes, de mouches tsé-tsé, de punaises triatomes et d'abeilles mellifères. Cet article décrit ces ressources nouvelles et récemment développées/acquises, notamment les lignées cellulaires dérivées des tiques *Argas reflexus*, *Hyalomma lusitanicum*, *Hyalomma marginatum* et *Rhipicephalus bursa*, et des insectes *Anopheles stephensi*, *Apis mellifera*, *Culicoides sonorensis*, *Glossina morsitans*, *Phlebotomus argentipes* et *Triatoma infestans*. ***Conclusions :*** La majorité des espèces de vecteurs représentées dans la collection sont d'origine tropicale ou subtropicale. Grâce à la distribution de ces lignées cellulaires existantes et nouvelles, le TCB et ses avant-postes continueront à soutenir la recherche mondiale sur les vecteurs arthropodes et les agents pathogènes pour le bétail et l'homme qu'ils transmettent.

Mots-clés : lignée cellulaire, tique, moustique, moucheron, phlébotome, mouche tsétsé, punaise triatomine

Resumen

Hartley C., Khoo J.J., Darby A., Makepeace B.L., Bell-Sakyi L. Nuevos recursos sobre líneas celulares de garrapatas e insectos para la investigación de enfermedades transmitidas por vectores a partir de la estructura internacional del Biobanco de Células de Garrapatas

Contexto: Las líneas celulares de artrópodos tienen un papel importante en la investigación sobre el control de patógenos de transmisión vectorial con impacto veterinario, médico y agrícola. El Biobanco de Células de Garrapatas (TCB) de la Universidad de Liverpool es la única colección en el mundo de líneas celulares derivadas de garrapatas y de insectos vectores de patógenos virales, bacterianos, protozoarios y helmintos. ***Objetivo***: El TCB de Liverpool y sus puestos avanzados en Malasia y Brasil se crearon para facilitar el acceso de los investigadores de todo el mundo a los recursos de líneas celulares de garrapatas y de insectos, así como para la formación en su mantenimiento, su aplicación y su desarrollo. ***Métodos***: El TCB recibe y almacena líneas celulares de artrópodos y las distribuye a los científicos que las solicitan, también genera nuevas líneas celulares a partir de garrapatas y de insectos vectores. Para facilitar la adopción de líneas celulares, el TCB y sus puestos avanzados garantizan una formación en el cultivo de células de artrópodos y proporcionan consejos y apoyo permanente a los beneficiarios. Las líneas celulares se proporcionan en el marco de acuerdos de transferencia de material. ***Resultados***: El TCB alberga actualmente más de 90 líneas celulares derivadas de garrapatas *Ixodidae* y *Argasidae*, de mosquitos, de mosquitas picadoras, de flebótomos, de moscas tsé-tsé, de triatominos y de abejas melíferas. Este artículo describe estos recursos nuevos y desarrollados/adquiridos recientemente, en especial las líneas celulares derivadas de las garrapatas *Argas reflexus*, *Hyalomma lusitanicum*, *Hyalomma marginatum* y *Rhipicephalus bursa*, así como insectos *Anopheles stephensi*, *Apis mellifera*, *Culicoides sonorensis*, *Glossina morsitans*, *Phlebotomus argentipes* y *Triatoma infestans*. ***Conclusiones***: La mayoría de las especies de vectores representadas en la colección son de origen tropical o subtropical. Gracias a la distribución de estas líneas celulares existentes y nuevas, el TCB y sus puestos avanzados continuarán apoyando la investigación mundial sobre los vectores artrópodos y los agentes patógenos para el ganado y el hombre que transmiten.

Palabras clave: línea celular, garrapata, mosquito, culicoide, flebotomo, mosca tsé-tsé, chinche triatomino