Publishable summary

Summary description of the project context and the main objectives

The overall objective of ANTIDotE is to identify and characterize tick proteins involved in 'tick immunity' and transmission of tick-borne pathogens (TBPs) and to use this knowledge to develop anti-tick vaccines to prevent multiple human tick-borne diseases (TBDs), such as Lyme borreliosis (LB), tick-borne encephalitis virus (TBEV) infection and human babesiosis. In addition, through an integrated and multidisciplinary approach involving public health institutes, health organizations and industrial companies we will examine how to develop anti-tick vaccines and how to implement these in public health systems. Because of its design, which combines state of the art basic science with translational research, ANTIDotE will also deliver essential knowledge on the biological mechanisms involved in the pathogenesis of TBDs. The key objectives are:

First Objective: To identify *I. ricinus* tick salivary gland proteins (TSGPs) that play a role in the transmission of TBPs from the tick to the host, but also TSGPs that are recognized by anti-tick antibodies. Therefore, gene expression profiles will be analyzed of salivary glands (SGs) of uninfected and TBP-infected feeding *I. ricinus* ticks. We will also identify TSGPs that are recognized by mammalian anti-tick immune responses by an *I. ricinus* TSGP Yeast Surface Display (YSD).

Second Objective: Identified TSGPs (described above) will be characterized with the help of RNAi in ticks. Alongside, the functional activity of specific TSGPs (in relation to host defense mechanisms) will be investigated. As part of this objective we will provide in depth insights in the mechanisms involved in transmission of TBPs and will reveal candidates for anti-tick vaccine to prevent TBP transmission. For this objective in vivo animal models for the TBDs under study will be established and improved.

Third Objective: Aims to confirm which TSGPs induce 'tick immunity' in in vivo animal models and the artificial tick feeding system (ATFS). Furthermore, the humoral and cellular immune responses that these TSGPs elicit will be characterized. Thus, the third objective will reveal candidates for anti-tick vaccine that are capable of interfering with tick feeding.

Fourth Objective: As part of this objective we aim to deliver the proof of concept that a single anti-tick vaccine can prevent transmission of bacterial, protozoal as well as viral TBPs. Vaccine studies with promising candidates, or specific combinations of promising candidates, will be performed to identify an anti-tick vaccine that could interfere with all three TBPs under study. Both TSGPs that are crucial for the transmission of *Borrelia*, *Babesia* and TBEV from the tick to the mammalian host, and TSGPs that are of paramount importance for tick feeding, will be tested.

Fifth Objective: The objective of this part of the project is to deliver plans for exploitation and implementation of our findings and concepts to contribute to downscaling of the burden of TBDs on Central and Eastern Europe and other endemic European societies, as well as to disseminate ANTIDotE results. We will do so by attending and arranging workshops and meetings.

These key objective results in the following project objectives (OB):

OB 1.1 Identification of TSGPs involved in transmission of TBPs [M18]

OB 1.2 Identification of TSGPs recognized by anti-tick immune responses [M18]

OB 2.1 Describe the role of TSGPs in the transmission of TBPs in vivo using RNAi in ticks [M42]

OB 2.2 Describe the function of TSGPs in in vitro and ex vivo functional assay [M60]

OB 3.1 To confirm that TSGPs identified induce 'tick immunity' in mice [M42]

OB 3.2 To characterize humoral and cellular anti-tick immune responses [M48]

OB 3.3 To confirm that TSGPs identified induce 'tick immunity' in cows and to develop a robust ATFS for research on anti-tick vaccines [M48]

OB 4.1 To identify an anti-tick vaccine that protects against multiple TBDs by specifically interfering with TSGPs crucial for transmission of TBDs [M60]

OB 4.2 To identify an anti-tick vaccine that protects against multiple TBDs by interfering with tick feeding [M60]

OB 5.1 To exchange knowledge on LB, TBE, human babesiosis and innovative strategies to prevent TBDs

OB 5.2 To disseminate ANTIDotE results [M42, M60]

OB 5.3 To establish a road map for exploitation of novel anti-tick vaccines [M36]

OB 5.4 To explore ways to implement anti-tick vaccines in health systems [M60]

Description of the work performed since the beginning of the project and the main results achieved so far

The aim of the ANTIDotE project was to find tick salivary gland proteins (TSGPs) that block pathogen transmission and/or interfere with tick feeding. Two main antigen discovery strategies were employed. Firstly, a transcriptomic approach (MACE and RNAseq) was used to identify TSGPs upregulated upon infection with *Borrelia*, TBEV or *Babesia*, resulting in the identification of numerous TSGPs, of which a selection was technically and biologically validated. There are currently five manuscripts on the transcriptomes of *I. ricinus* upon feeding and infection in the pipeline. Secondly, an immunoscreening using a yeast surface display was used to identify and validate TSGPs that are recognized by antibodies of frequently tick-exposed humans, and/or tick immune animals. A selection of the thus identified TSGPs was investigated in downstream WPs. As part of WP2 we have established and improved tick-transmission models for *Borrelia afzelii* (submitted for publication), TBEV and *Babesia microti* (WP2). The infrastructure for RNAi in ticks was established and used to investigate the role of multiple TSGPs. Silencing of selected TSGPs did not affect *Borrelia* transmission, but silencing of one target significantly impaired both tick feeding and TBEV transmission. Our data show that one of the identified TSGPs promoted *Borrelia* survival in vivo by impairing phagocytosis. Finally, we have also generated fluorescent *B. afzelii* strain CB43 to study TSGP-*Borrelia* interactions. Production of TSGPs in *E. coli* and *Drosophila* was performed and a selection used for vaccination studies. Vaccination of mice with TSGPs did not result in a significant anti-tick effect and we shifted our focus to the tick-rabbit model to assess tick immunity (WP3). Also, six bovine vaccination experiments were performed, showing that (specific fractions of) tick tissue extracts resulted in a robust anti-tick immunity. The obtained cow sera were used to set-up an in vitro artificial tick feeding system, which demonstrated the need for combined cellular and humoral immune responses to induce tick immunity. As an additional effort, we identified and/or tested tick-borne pathogen antigens (from *B. microti* and *B. afzelii*) as candidates for transmission-blocking vaccines. In WP4 we identified one TSGP that, when used to actively immunize mice, protected against Lyme borreliosis and two TSGPs that, when tested as antigens for an anti-tick vaccine, partially protected mice from lethal TBEV-infection.

The ANTIDotE partners were actively engaged with stakeholders from medical and veterinary sciences, policy advisors, governmental institutions, patient interest groups and the pharmaceutical industry. We disseminated knowledge on (inter)national conferences, in peer-reviewed scientific journals and through the ANTIDotE website and (social) media. Indeed, 33 related ANTIDotE and ANTIDotE-related articles have currently been published in peer-reviewed scientific books or journals (of which the majority open access and including 2 PhD theses). These also included a technical report on preventive strategies to prevent TBDs, a review on anti-tick vaccines and a book chapter on the prevention of Lyme borreliosis and a manuscript on the cost-effectiveness analyses of an anti-tick vaccine in a Central Eastern European country where Lyme borreliosis and tick-borne encephalitis are endemic. Finally, the ANTIDotE project contributed to another four anticipated PhD theses and three manuscript have been submitted/accepted for publication.

All in all, we significantly contributed to the understanding of, and steps towards the downscaling of, TBDs in Europe. All our initially planned discovery methods yielded vaccine candidates and we pursued multiple additional antigen discovery approaches and we have characterized and produced selected candidates, of which some protected against LB or TBEV. We will continue our research and investigate whether combining multiple tick and pathogen antigens could lead to the development of a single vaccine to prevent bacterial, protozoal and viral tick-borne diseases. The antigens identified, described and characterized, as well as the assays, materials and methods developed as part of the ANTIDotE project are an excellent starting point.

Description of the expected final results and their potential impacts and use

The incidences of Lyme borreliosis (LB) and TBEV are on the rise in several European countries and diseases caused by other pathogens are emerging. Environmental, socio-economic and demographic factors synergistically increase the risk of acquiring tick-borne diseases (TBDs). Therefore, and also because the societal fear for ticks and TBDs seems to be ever growing, the old adage 'prevention is better than cure' certainly holds true for TBDs. Currently there are no human or animal vaccines against I. ricinus available. Our project aimed to show proof of concept that anti-*I. ricinus* vaccines can protect against multiple TBDs.

The ANTIDotE network (consortium and advisors) consisted of a large community of biomedical scientists, public health officials and private sector stakeholders. The ANTIDotE consortium put much effort in the dissemination of our results and we aimed to translate ANTIDotE's innovative results as much as possible into tangible impact on health services. Moreover, ANTIDotE actively involved health organizations, health policy experts and end-users to further increase its impact. Importantly, ANTIDotE's outcomes spanned from discovery science increasing fundamental biomedical knowledge, to translation of this new understandings into proof of concept studies, to valorization plans contributing to sustainable advances in the prevention of multiple TBDs.

ANTIDotE delivered new understandings on the biological mechanisms underlying tick feeding, anti-tick immune responses and transmission of the causative agents of TBE, LB and human babesiosis. These are all *I. ricinus* tick-transmitted infectious diseases with potential serious acute and chronic sequelae and disproportionately affect Europe. The ANTIDotE project was geared towards translating new knowledge into identification of novel anti-tick vaccines and providing proof of concept that such vaccines can prevent transmission of multiple human tick-borne pathogens. Furthermore, ANTIDotE also integrated basic science with industrial innovation and other (public) health disciplines to generate a road map for product development and to explore how anti-tick vaccines could be implemented into health systems as part of novel preventive strategies to prevent TBDs in endemic European countries.

We maximized our impact by:

1. Establishment of new tools and technologies to study ticks and TBDs

2. Novel fundamental knowledge of ticks and TBDs and identification of candidates for anti-tick vaccines (WP1)

3. Indepth insights in the function of tick salivary gland proteins and mammalian anti-tick immune responses (WP2 and WP3)

4. Proof of concept that anti-*I. ricinus* vaccines can prevent tick feeding/transmission of multiple tick-borne pathogens (WP4)

5. Exploitation and implementation plans for anti-*I. ricinus* vaccines in existing health services (WP5)

6. Dissemination of our results by a sustainable network, with currently 33 peer-reviewed articles published, including important contributions to a free book on ticks and TBDs, a published technical report on the implementation of potential anti-tick vaccines in (inter)national strategies for the prevention and control of TBDs in Europe and two PhD theses. In addition, we disseminated our results by the media (magazines, newspapers, non-scientific journals, internet, radio and television), an open website, three workshops and an open end-conference. The ANTIDotE project also contributed to the anticipated PhD thesis of four additional PhD students that will defend their dissertations in the near future. Finally, at the time of writing of the final report 3 manuscripts have been submitted/accepted for publications. The vast majority of the ANTIDotE output was published open access.

Our concerted multidisciplinary integrated European approach led to multiple breakthroughs in the field of ticks and TBDs and beyond in many ways and impacted a) the research community worldwide, b) health systems, societies, economies and individual patients in European countries where TBDs are endemic, and c) industry. The ANTIDotE outcomes contribute to a knowledge-based society and paved the way for the development of innovative ways to prevent TBDs by industry and health systems in Europe.