ABSTRACT-OBJECTIVES: Brucellosis remains one of the major zoonotic infections creating significant multidimensional medical, public health, social and economic problems to more than 120 countries, especially the developing ones, throughout the world. Moreover, most *Brucella* spp. are listed as biosecurity/ bioterror pathogen by the CDC. Among the 13 identified species, *B. melitensis* poses the highest risk for humans in most countries. This is due to contact with sick livestock or ingestion of raw milk, cream, and soft cheese from infected sheep and goats, which constitute the main reservoir of infection. Facing this zoonosis challenge imparts major attention on national authorities to shoulder responsibility towards building successful prevention and control programs. In any country or geographical zone, the effective establishment and implementation of such programs require tackling several factors including: 1) Awareness and public health education about the disease; 2) Diagnostic capacity and facilities for adequate and reliable identification of cases, avoiding misdiagnosis and underreporting in both public and animal health sectors; 3) Vaccination strategies and programs; 4) Intersectoral and interagency coordination; 5) Intersectoral epidemiological surveillance systems; 6) Political commitment and financial support and 7) Liaison with international organizations such as FAO, WHO and WOAH (OIE), that can provide valuable technical support, expertise sharing, capacity building and funds mobilization. These factors were encountered and addressed through an important technical level’s contribution led by the WHO-Mediterranean Zoonoses Control Centre during the implementation of two “Pilot Brucellosis Epidemiological Surveillance Projects in the Public Health and Animal Health Sectors in Syria and Jordan”. These endeavors revealed a dire need for making available information adapted to reality and addressing appropriate approaches for *Brucella* prevention and control. The present document is based on the long experience of the authors in this field and has been warranted to address the aforementioned issues and factors in a clear, concise, well cross-checked, practical and “easy-to-handle” guidance, applicable before and during brucellosis control activities.

Keywords: zoonoses; brucellosis; epidemiology; prevention/ control; diagnosis; one health

important medical and social impact, it creates a heavy economic burden both at the family and national levels.

Often, this disease, lacking pathognomonic symptoms, can be easily confused with other febrile conditions especially in tropical countries where it might be misdiagnosed as drug-resistant malaria and typhoid fever.

In many developed countries, this disease has been controlled or eliminated. This has been achieved through mass media awareness campaigns, systematic epidemiological surveillance, mass vaccinations, test-and-slaughter policies, compensation of farmers, and other incentives. Such applied strategies in the animal sector lead to a successful reduction of cases in humans. In most developing countries such as the South Mediterranean and the Middle East (SMME), brucellosis often remains unidentified and under-reported. Moreover, diagnosis occurs mostly at an advanced stage entailing prolonged and chronic illness and disability [1-5].

**EPIDEMIOLOGY**

Brucellosis prevalence and incidence in animals and humans vary among countries in the MME regions. First, those of the Northern Mediterranean littoral (NMC) where efficient control programs have been concluded (France) or are in evident progress, e.g. Spain, Portugal, Italy and Greece, showed reflection on the decrease of human cases. Second, countries of the Southern and Eastern littoral together with those of the Middle East (SMME) such as Jordan, Oman, Syria and Turkey, where nationwide free vaccination programs have been implemented for the owners, have had different results in terms of progress and success. In other countries in the same area, e.g. Egypt, vaccination is usually not compulsory and owners of infected slaughtered animals/flocks are not usually compensated. Such epidemiological differences among the two regions can be ascribed to the efficiency of control, and the level of social awareness in association with public health education activities.

Vaccination efforts in animals are also an important factor in controlling the disease in both animals and humans. Many countries succeeded in reducing brucellosis prevalence in cattle, but little success was met with sheep and goats’ vaccination. This is due, among other factors, to serious difficulties encountered by the veterinary services in identifying, vaccinating, and monitoring infected flocks as well as in controlling their movements at national and transboundary levels. Such a situation triggered the FAO/UN to undertake in 2012-2014 the initiative for the development of a “Stepwise Approach for Progressive Control of Brucellosis in Livestock-Principles, Strategies, Stages and Tools”, aiming at the progressive control of brucellosis in accordance to areas and/or zones [6-8].

Table I includes brucellosis cases in humans officially reported cases of human brucellosis in selected Mediterranean and Middle East countries over ten years.

**TABLE I**

<table>
<thead>
<tr>
<th>COUNTRY</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
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<th>2012</th>
<th>2013</th>
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<td>5056</td>
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<td>4447</td>
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<td>339</td>
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<td>97</td>
<td>100</td>
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<td>159</td>
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<td>21</td>
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<td>11</td>
<td>21</td>
<td>9</td>
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<tr>
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<tr>
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<td>285</td>
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<tr>
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<td>9818</td>
<td>9324</td>
<td>7658</td>
<td>7177</td>
<td>6759</td>
<td>7225</td>
<td>4475</td>
<td>89673</td>
</tr>
</tbody>
</table>
| **GRAND TOTAL** | **383305** | **OIE data base: zoonoses in humans and EFSA-EU–EFSA Journal 2015; 13: 4329.** **Data not available**

Available at: www.oie.int/wahis2/wash/action7en.php. / www.efsa.europa.eu/efsajournal
reported in most Mediterranean, and Middle East (MME) countries during a decade, while Figure 1 reflects the correlation of the infection diagnosed per millions of inhabitants per country. The improved epidemiological situation among Northern Mediterranean countries (NMC) is due to decades of efficient control programs campaigns, and public health education. Among the SMMER countries, however, the number of cases declared on a yearly basis, in no way reflects the real epidemiological situation. This could be based upon information of ambiguous significance and origin associated with the collapse of prevention/control programs due to conflicts (e.g. Syria) or stagnant situation related to administration weaknesses. Regardless of the reasons, the existence of such cases should constitute the impulse for establishing longstanding strategic control plans. In Jordan, the number of cases reported during the same period generally remained steady [9-19].

Besides the fact that brucellosis is eclipsed as a neglected tropical disease in many countries in the Middle East and the developing world, several other hampering conditions should be considered by national competent authorities in the endeavor towards the prevention and control of this disease. These include entrenched behaviors fostered by ignorance, sociocultural and sociopolitical behaviors together with lack of intersectoral collaboration and coordination, weak national infrastructures, lack of public health education and public awareness. Such and other relevant determinants should be considered by national competent authorities as well as by international organizations as of critical importance towards development progress [20-23].

**Epidemiological surveillance**

Epidemiological surveillance (ES) is a tool used to detect and monitor epidemics and public health emergencies, and constitutes an essential information system for action towards prevention and control. This is needed to estimate the magnitude of the problem in the human population (morbidity, mortality, case fatality, disability, trends of the infection), identify high risk population(s) and risk factors (e.g. fresh cheese, raw milk, contact with animals, etc.), and improve the detection level and treatment of patients. In animals, the detection of outbreaks, as well as the monitoring of changes in *Brucella* species/biovars in a given population and/or area are necessary for the success of the activity.

The aforementioned should go together with establishing a vertical and horizontal communication and planning within and in-between public health and animal health sectors. This should go together with developing a functional database for regular intercommunications among all sectors and agencies that are directly involved in brucellosis prevention and control [24-26].

In the Middle East, a noteworthy example of such endeavor is the “WHO-MZCC Pilot Brucellosis Epidemiological Surveillance Projects in Syria and Jordan 2003-2006”. Requests from the Syrian and Jordan Ministries of Health and Agriculture were addressed to the WHO-MZCC in 2002 and 2004, respectively, for technical assistance in the improvement of their human and animal brucellosis epidemiological surveillance systems. Both projects had perspectives to integrate, in this specific field, the public health and animal health sectors in order to promote the use of data and information system standards to advance the development of an efficient, in-

tegrated and interoperable brucellosis surveillance system at national, provincial and local levels. The reorganisation of the brucellosis diagnosis central laboratories in both countries, together with manpower training, and purchase of necessary equipment and other materials were included among the undertaken measures. At the conclusion of the projects, the national authorities reached the main target, i.e. the horizontal and vertical intercommunication of the information gathered and analyzed by the newly implemented surveillance system [23].

LABORATORY DIAGNOSIS OF BRUCELLOSIS

Human brucellosis can present with a variety of manifestations, making the diagnosis unreachable on mere clinical grounds. Laboratory support is therefore essential. A brief overview of the main laboratory diagnostic procedures used for the detection of infections in humans and animals are described.

Laboratory diagnosis in humans

Isolation of Brucella spp. from patients’ clinical specimens by culture provides the definitive diagnosis. Culture, however, takes a long time, and is associated with a low or variable recovery rate (40-80%) warranting serologic tests to be performed, almost always, in combination. Among different serology tests, the most commonly used are: Slide Agglutination test, Standard Tube Agglutination test (SAT), Rose Bengal test (RBT), 2-Mercaptoethanol test (2ME-in few countries), indirect Coombs test, indirect Enzyme-linked Immunosorbert Assay (iELISA) and Brucellacapt test.

All these tests can provide positive results in patients with acute or subacute brucellosis, where IgM is present. However, complicated cases such as chronic, neurological, and focal brucellosis, can be missed by slide tests and SAT. In fact, in these cases IgG, incomplete, non-agglutinating or blocking antibodies are likely to be present, which requires the use of specific and highly sensitive tests, namely indirect Coombs, Brucellacapt or ELISA, that are able to detect these antibodies and help in the diagnosis.

Therefore, and for practical reasons, it is advisable to deploy a combination of SAT with either indirect Coombs, Brucellacapt or ELISA to avoid missing a diagnosis. Of note, the need to interpret serology results with the history and clinical status of the patient is of high importance, since antibodies may persist despite of treatment and cure, and other illnesses could fall within the differential diagnosis.

RBT is appropriate in small and understaffed laboratories, as it possesses high sensitivity. However, while it is easy to observe a positive RBT agglutination in acute stages, training is required to interpret the thinner agglutination reaction that appears in long evolution cases [27-31].

Laboratory diagnosis in animals

In animals, isolation of the agent in culture is also the only conclusive technique. However, serology is the most common and practical tool used. Serological tests must be interpreted in accordance with the disease history and vaccination status to avoid false positive reactions of the herd/flock.

Serological diagnosis in bovines includes RBT or other Buffered Plate Agglutination tests, Complement Fixation test (CFT), indirect ELISA (iELISA) and Fluorescence Polarization Assay (FPA). They are suitable for screening herds and individual animals. The milk-ring test can be used on bulk milk samples for screening and monitoring dairy cattle. The iELISA should also be useful in bulk milk but the conditions of use remain to be established. The SAT should be avoided due to reduced sensitivity and specificity [32].

In sheep and goats the RBT and CFT should always be used in combination and are recommended for screening flocks and individual animals, though both have low specificity when sera from Rev.1 subcutaneously vaccinated sheep and goats are tested. This drawback is significantly reduced if conjunctival (i.e. ocular instillation) vaccination is adopted due to the reduced level and the shorter duration of the resulting serological response. Between iELISA and competitive ELISA tests, the former one is more sensitive, even more so than both RBT and CFT. None however, can differentiate between infected animals and recently vaccinated ones. Moreover, there is still a need for further experience to be accumulated in the field in order to fully validate the use of ELISA’s in sheep and goats, as the cut-offs suggested by manufacturers need to be reevaluated under the specific epidemiological conditions of most SMMER’s countries.

Other non-widely used tests for financial reasons are: Gel Precipitation test, Immune Capture test and FPA. Brucellin skin allergic test is useful for screening unvaccinated herds and flocks [33-36].

PREVENTION AND CONTROL IN SMMER

Effective prevention and control programs necessitate certain essential requirements and key elements to ensure long-term management of complex projects including: human resources development through capacity building, training of public health, and animal health staff in all aspects of brucellosis, public health education towards community awareness and participation, motivation of breeders’ collaboration, promotion of a cross-sectoral co-
ordinated cooperation of all competent sectors, especially the public health and animal health sectors.

The countries of the South Mediterranean and Middle East regions (SMMER) share many common epidemiological, socioeconomic, structural and other characteristics such as breeding of small ruminants, food and behavioral habits, mentalities, beliefs and education. Therefore, effective long-term prevention and control programs planned to be implemented in the SMMER should respect and take into consideration, with certain exceptions, the aforementioned characteristics. Moreover, the strategic approach for a successful program should address several factors encompassed in the text to follow [10, 37-44].

Design of control plans
The planned strategy should take into consideration several factors including understanding of local and regional variations in animal husbandry practices, epidemiological patterns of the disease, the level of infrastructure support, cross-sectoral brucellosis epidemiological surveillance and coordination, social customs, and community awareness, among others.

Since there is no available efficient brucellosis vaccine for humans, prevention of the infection relies on its control in the animal reservoir. Protecting public health calls for the adoption of long-term programs and strategies related to both the animal and human populations and encompassing national competent agencies and services. In the developing countries such as the SMMER, among other obstacles, data about the real prevalence of brucellosis remains scarce; hence control strategies should be grounded on the following perspectives:

- Establishment of an efficiently-operating intersectoral epidemiological surveillance system.
- Introduction, as much as possible, of extensive and effective vaccination programs for susceptible livestock (bovine, sheep, goats and, where appropriate, buffaloes and camels).
- The strategy of vaccination should take into consideration the mostly nomadic, and transboundary type of sheep and goat-rearing prevailing in the SMMER.
- Animal identification, where feasible, is strongly recommended, otherwise, differentiation between vaccinated and non-vaccinated animals will be almost impossible.
- The only suitable vaccines available at present, despite their known drawbacks, are S19 in bovine and Rev1 in sheep and goats.
- The RB 51 vaccine is recommended for cattle only. However, although it doesn’t confer post-vaccination antibody titers in RBT and CFT, it interferes in ELISAs assays. Furthermore, it seems to be associated with paradoxical outcomes in terms of safety, causing abortions, as well as low level of conferred immunity. Consequently, its use as a strategic immunization tool remains under discussion [37]. Nevertheless, RB51 complementary use to S19 vaccine has been reported in some studies [40,42].

Vaccination process
- During the first year of sheep and goat vaccination programme, mass conjunctival vaccination (young and adult animals including males) should be added, towards more rapid control results. For the years to follow, lambs and kids kept for restocking (i.e., at 3-4 months of age), could be the only ones to be vaccinated. However, under high prevalence and extensive rearing, such strategy needs regular veterinary supervision and assistance which is very difficult or even impossible to be available everywhere.
- Herd/flock immunity remains more solid when vaccination is associated with the elimination of infected animals. However, such a strategy presupposes effective veterinary services contribution and regular laboratory support.
- For S19 (cattle), vaccine-induced abortions are reduced when the ocular route is adopted. This is not the case for Rev.1 in small ruminants, a well-known abortifacient vaccine. However, in both cases the ocular route reduces the post-vaccine serological response. Thus, although the ocular route is of choice for mass vaccination, the mid-pregnancy period should always be avoided. The late calving/lambing/kidding and lactation seasons are to be selected when mass adult vaccination is performed. However, despite its reduced frequency when vaccination is applied by ocular route, the vaccine strain excretion in the milk has to be considered. Concerning safety in males/bulls, while vaccination with Rev1 is safe, subcutaneous vaccination with S19 is not recommended and its safety given by ocular route is still unknown.
- Another kind of mass vaccination strategy is based on alternate years of vaccination, and is only recommended for extensive animal rearing and poor socio-economic conditions. It can be evaluated for adoption in accordance with countries or zones breeding conditions, epidemiological statistics, level of difficulties for authorities, and breeders’ collaboration, among other factors [37-38, 43,45].

Additional control strategies
- The test-and-slaughter strategy, which might gradually lead towards the elimination of infections and establishment of modern cattle farms, cannot be
generally sanctioned for adoption in the SMMER. This is due to a vague epidemiological picture, the need for proficient laboratories, compulsory slaughtering of infected animals, and the lack of adequate funding.

- Expansion of milk pasteurization should become a social and economic strategic target, encouraged by governments-private sectors partnerships and industrial incentives.
- Recruiting different means of mass media for running persistent, extensive and structured public health education campaigns addressing for instance, milk boiling and avoidance of raw milk or fresh white cheese consumption. Such campaigns targeting different population groups including animal breeders, abattoirs workers, housewives, and school children are also helpful in the control of brucellosis.
- It is evident that the level of success of a brucellosis control strategy among animals depends on the best possible consideration of different interconnecting factors and prerequisites fulfillment. The final targets to be reached are the preservation of public health and alleviation of social and economic burdens.

Implementing control plans
Successful implementation of control plans, programs and campaigns necessitates appropriate preparedness plans and organization mechanisms. Of paramount importance is the intersectoral collaboration and coordination within and among animal and public health sectors, as well as seeking the collaboration with the FAO. The integral parts for a proper implementation of such plans, can be summarized as follows:

- Public health and animal health sectors should be empowered with sufficient technical and financial resources as well as an appropriate legal background.
- Animal health personnel should be well trained on the cold chain maintenance and safe use of vaccines.
- Vaccines should originate from the same source, and accepted following quality certification of the seed batch strain, including genetic and biochemical characteristics, by an approved international reference laboratory.
- Vaccine batches to be delivered are accepted following control of the accompanying official quality certification of successful control tests performed, describing each one of them and the obtained results. It is reminded that quality control of the Rev1 vaccine strain is critical due to its tendency to dissociate into a useless R form, which decreases its immunogenicity and increases residual virulence.
- Public health and animal health diagnostic laboratories should be well-equipped and staffed with trained personnel. The diagnostic capabilities of the laboratory staff should be checked at intervals through inter-laboratory proficiency. The OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals is an international guide for animal health diagnostic laboratories.
- The quality of diagnostic antigens should be always checked and certified every time a new lot is received/used. Their availability should always be ensured. Technical and financial support to the diagnostic laboratories should be a priority.
- Public health and animal health authorities should coordinate among themselves and exchange data, information and feedback reports vertically and horizontally.
- The progress of the brucellosis control program should be evaluated annually and corrective actions should be adopted where appropriate, or alternative strategies may be considered. A credible indicator for its success is associated with reduction of cases among humans, and abortions among correctly vaccinated flocks.
- Integration with other animal health programs facilitates the development of brucellosis control program, considering its long duration and cost.
- Transboundary livestock’s movements necessitate international collaboration and commitments to ensure sustainability of efficient brucellosis and other zoonoses control programs.
- Legislation promulgation and/or amendment should be endorsed where appropriate.

There is no doubt that under the present situation of conflicts, population displacements, and disruption of public health and animal health activities in certain countries such as Iraq and Syria, implementation of prevention and control activity of any communicable disease in humans and/or animals, remains compromised until tranquility and peace prevail [21,46-48].

General management practice
To support brucellosis control activities, a general management practice needs to be implemented as a rule whenever practically possible, even in the absence of a vaccination programme. This should include the following terms and aspects [49]:

- Avoid all contacts between infected and Brucella-free animals.
- Eliminate all infected animals without delay.
- Remove all abortion materials in a hygienic way as soon as possible and aborting females should be isolated until a serological diagnosis is confirmed.
- Whenever replacing infected animals with Brucella-
free ones, the latter should be kept in quarantine for at least one month to assess their infectious status by serological tests. However, if the brucellosis-free animals are to be introduced among high risk or infected flock animals, they should be first vaccinated.

It is evident that the aforementioned measures, despite their importance, are practically unfeasible under extensive/nomadic rearing conditions, particularly in sheep and goats. However, they constitute a valuable supportive approach in modern cattle farms where management has the possibility to comply with more strictly-controlled practices.

Cost-benefit assessment and benefits from the role of international organizations

When planning a control program, it is essential to assess its expected cost-benefits, being financial and/or social. This includes looking into the facilities available and the appropriate time to start its implementation or its expansion. In this context, different factors and aspects should be considered as priorities together with the availability of resources, examples include:

- Prevalence and incidence of brucellosis in humans and livestock per country or zone and their socio-economic impact.
- Priority of brucellosis in the area in relation to other public health problems.
- Number of livestock in the area and trends of animal population whether increasing, decreasing, or stable.
- Type of susceptible livestock breeding (nomadic, transhumant, stationary, or mixed).
- Socio-economic conditions, traditional behaviors and awareness level of the population in general, especially in high risk groups.
- Public health and animal health services efficiency level, including horizontal intercommunication and coordination levels.
- The expected increase in the livestock productivity, together with public health and socio-economic development.

For every community or country, determining the costs and the benefits expected from different strategies facilitates selecting the one combining the highest efficiency in association with its feasibility. It is also important to determine from the beginning which costs should be covered by public funds and which should be supported by private ones.

Besides, tracing human brucellosis cases identified in hospitals and private clinics, is an important passive surveillance measure. This is helpful towards estimating the extension of the problem in certain zones of the country. It also allows an indirect assessment of the progress of animal vaccination programs over the years, and an additional cost/benefit evaluation factor [1,5,8,45,49-54].

The international organizations

International organizations such as FAO, OIE and WHO, support developing countries through technical and other types of assistance to improve and upgrade the efficiency of their zoonotic and FBDs prevention and control programs. Support measures include expertise sharing, training, capacity building, structural operation, intersectoral collaboration and coordination promotion, public health education, and funds mobilization.

However, in order for this assistance to become really effective for long-term programs, there is an imminent need for these countries to upgrade their infrastructure (central and district services, laboratory support, intersectoral collaboration/coordination level, political commitment). In this context, concerned countries should have recourse to international organizations for advice and support. A good example is the recently promoted FAO project entitled “A stepwise approach for progressive control of brucellosis in animals and humans”.

Major international organizations such as FAO/UN, WHO, OIE, along with different academic and professional bodies, recognize the interdisciplinary collaboration/integration under the “One Health” concept, as a unique approach to be promoted worldwide.

The “Tripartite Concept Note” issued in April 2010 by FAO, OIE and WHO, stresses on “sharing responsibilities and coordinating global activities to address health risks at the animal-human-ecosystems interface” [6-8, 55-61].

SOCIAL AND ECONOMIC BENEFITS FROM BRUCELLOSIS CONTROL

Following the appropriate implementation of an efficient control program, the most relevant anticipated benefits for a country relate to economic and social benefits [5,50,52,54].

Economic benefits

- Increase in the farm animal production (meat, milk, wool, birth rate, etc.).
- Increase in quantity and quality of animal products.
- Increase in the number of working days per year and per person.
- Decrease in the diagnosis, hospitalization and therapy costs.
- Decrease in the availability of hospital facilities for other health problems.
- Improvement of the country image and better rewards to the national economy.
Social benefits

- Promotion of the physical, psychological and social status of the population.
- Operation and quality development of the animal and public health services as well as administration of the primary health care and hygiene practices.
- Expansion of the awareness of health among rural workers, breeders and the general population.
- Decreased abandonment of farming and other relevant activities by people involved or at risk.
- Reduction in other health and socioeconomic problems, such as food contamination by zoonotic pathogens.

CONCLUSIONS

Brucellosis prevention and control could represent a characteristic prototype of the “One Health” concept to be largely adopted, particularly where the elimination of brucellosis remains far fetched especially among SMME countries in which the disease remains endemic. This concept encompasses coordinating efforts not only among public and animal health authorities, but also among private and national agencies in countries and their leaders as well as liaison coordination with international organizations. Thus, accepting and adopting the provided approaches, as prerequisites and measures of horizontal and vertical collaboration, will surely lead to a successful and efficient brucellosis prevention and control program in any country that needs it.

However, under the present deteriorating situation, conflicts, populations displacements, disruption of public and animal health activities and programs in certain countries of the SMMER, the implementation of any particular activity towards preventing and controlling communicable diseases such as brucellosis remains elusive. It is only when peace is realized, that such control activities can be gradually reestablished for the welfare of the countries.

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